Angiotensin I-converting enzyme in human circulating mononuclear cells: genetic polymorphism of expression in T-lymphocytes

Olivier COSTEROUSSE,* Jacqueline ALLEGRINI,* Manuel LOPEZ,† and François ALHENC-GELAS*‡
*Institut National de la Santé et de la Recherche Médicale (INSERM) U367, 17 rue du Fer à Moulin, 75005 Paris, and †INSERM U76, Paris, France

The expression of angiotensin-I converting enzyme (ACE; EC 3.4.15.1) in human circulating mononuclear cells was studied. T-lymphocytes contained the highest level of enzyme, approx. 28 times more per cell than monocytes. No activity was detected in B-lymphocytes. ACE was present mainly in the microsomal fraction, where it was found to be the major membrane-bound bradykinin-inactivating enzyme. An mRNA for ACE was detected and characterized after reverse transcription and amplification by PCR in T-lymphocytes and several T-cell leukaemia cell lines. We have previously observed that the interindividual variability in the levels of ACE in plasma is, in part, genetically determined and influenced by an insertion/deletion polymorphism of the ACE gene. To investigate the mechanisms involved in the regulation of ACE biosynthesis, the ACE levels of T-lymphocytes from 35 healthy subjects having different ACE genotypes were studied. These levels varied widely between individuals but were highly reproducible and influenced by the polymorphism of the ACE gene. T-lymphocyte levels of ACE were significantly higher in subjects who were homozygote for the deletion than in the other subjects. These results show that ACE is expressed in T-lymphocytes and indicate that the level of ACE expression in cells synthesizing the enzyme is genetically determined.

INTRODUCTION

Dipeptidyl carboxypeptidase I (angiotensin I-converting enzyme (ACE), peptidyldipeptidase A, kininase II; EC 3.4.15.1) is a zinc metallopeptidase that plays a major role in blood pressure regulation [1]. In plasma and on the surface of endothelial cells, it converts the inactive decapeptide angiotensin I into the vasopressor- and aldosterone-stimulating octapeptide angiotensin II [2]. It also inactivates bradykinin (BK), a vasodilatory nonapeptide involved in the control of vascular tone and implicated in inflammatory responses [3,4]. Lastly, ACE cleaves substance P [1], a tachykinin peptide possessing numerous physiological activities [5], and may be involved in the metabolism of several other biologically active peptides because of its broad enzymic specificity and wide distribution in the body [1].

ACE has been identified as a membrane-bound enzyme in several types of cells, including vascular endothelial cells, various absorptive epithelial cells, neurones, macrophages, male germinal cells, and is also present in a circulating form in biological fluids such as plasma, amniotic and seminal fluids [6–8]. Although the regulation of ACE synthesis has been extensively studied in endothelial cells *in vitro* [9,10], the factors involved in regulating ACE gene expression *in vivo* remain largely unknown. Measurements of ACE levels in the plasma of healthy humans have revealed a wide interindividual variation that is not explained by differences in candidate hormonal or environmental parameters [11–13]. A study of members of nuclear families has indicated that differences in the plasma ACE levels among individuals are determined in part by a polymorphic gene which has a major effect on the phenotype [13]. The gene responsible for this effect is the ACE gene itself. An insertion/deletion polymorphism located in an intron of the ACE gene was found to be associated with differences in the plasma levels of ACE in a group of healthy subjects [14]. As plasma ACE is believed to originate from endothelial cells and ACE is mostly a membrane-bound ectoenzyme in these cells and in the other cell types where it is synthesized [1,6–8], these observations raise the question whether the level of ACE expression in cells is genetically determined. We have investigated this question and assessed the mechanisms involved in the regulation of ACE biosynthesis in man by studying the expression of the enzyme in peripheral blood mononuclear cells. These cells are readily accessible and ACE is known to be present in human monocytes and macrophages [15]. Our results indicate that in circulating mononuclear cells, ACE is in fact mainly expressed in the T-lymphocyte population. ACE may participate like other ectopeptidases in the regulation of lymphocyte functions. The T-lymphocyte ACE levels of a given subject are highly reproducible when measured on two different occasions, but vary widely between individuals and are associated with the insertion/deletion polymorphism of the ACE gene.

MATERIALS AND METHODS

Preparation and isolation of human circulating mononuclear cells and cell subtypes

Freshly drawn heparinized (30 i.u./ml) human blood (30 ml) was obtained from healthy adult volunteers after an overnight fast and immediately diluted with an equal volume of PBS (120 mM NaCl/2.7 mM KCl/10 mM phosphate buffer, pH 7.4). It was then layered on to Ficoll–Paque (Pharmacia) and centrifuged at 600 *g* for 20 min at room temperature by the method of Bøyum [16]. The mononuclear cells that sedimented at the interface between Ficoll–Paque and diluted plasma were collected and

Abbreviations used: ACE, angiotensin I-converting enzyme; BK, bradykinin; IMDM, Iscove's modified Dulbecco's medium; EBV, Epstein–Barr virus; FCS, fetal calf serum; Hip-His-Leu, *p*-benzoyl-L-glycyl-L-histidyl-L-leucine; RT, reverse transcription; Chaps, 3-[(cholamidopropyl)dimethylammonio]-1-propanesulphonic acid; HS, human serum type AB^+; SSC, 0.15 M NaCl/0.015 M sodium citrate.

‡ To whom correspondence should be addressed.

washed three times with 10 ml of PBS. The cells were then processed for purification of lymphocyte subtypes (see below) or for isolation of monocytes.

Monocytes were isolated by their ability to adhere to plastic surfaces. The washed mononuclear cells were resuspended at a density of 1.2×10^7 cells/ml in Iscove's modified Dulbecco's medium (IMDM, Boehringer–Mannheim) containing penicillin (100 i.u./ml) and streptomycin (100 μg/ml) (Gibco BRL), and 4 mM L-glutamine (Boehringer–Mannheim). Aliquots (3 ml) were placed in plastic Petri dishes (60 mm diam. × 15 mm, Nunc) and incubated for 3 h in a humidified incubator at 37 °C under 5% CO_2/95% air. Non-adhering cells were removed by gentle washing with PBS. Adhering cells were scraped off in 400 μl of PBS containing 8 mM Chaps {3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulphonic acid, Boehringer–Mannheim}, and kept frozen at −20 °C. The preparations contained over 90% monocytes, as indicated by morphological criteria and flow cytofluorometric analysis (see below); the remaining cells were B-lymphocytes.

T- and B-lymphocytes were purified from total mononuclear cells with immunomagnetic beads (Dynabeads M-450, Dynal) coated with monoclonal antibody specific to a surface-antigen marker of each cell type [17]. Cells to be treated were resuspended at $1.0–1.5 \times 10^7$ cells/ml in 4 °C PBS and incubated with Dynabeads M-450 Pan-T CD2 (beads/cells; 7:1) or Dynabeads M-450 Pan-B CD19 (beads/cells; 17:1) for 1 h at 4 °C with gentle stirring to isolate T ($CD2^+$)-lymphocytes or B ($CD19^+$)-lymphocytes respectively. Dynabeads M-450 CD4 and M-450 CD8 were also used to isolate $CD4^+$ and $CD8^+$ T-lymphocytes. The incubated bead–cell complexes were washed three times with 5 ml of PBS at 4 °C and the final pellet kept frozen at −20 °C. For ACE activity measurements, the bead–cell pellets were resuspended in 250 μl of PBS with 8 mM Chaps, and sonicated twice for 30 s at 4 °C. Immunomagnetic beads were separated with a magnet, and the cell homogenate was tested in an enzymic assay. The residual ACE activity on beads after separation was always below 6% of the value measured in the solubilized cells.

Large quantities of purified monocytes or lymphocytes were also isolated by counterflow-centrifugation elutriation [18]. Briefly, leucocytes were collected by leukapheresis from healthy donors after obtaining their informed consent, resuspended in PBS and centrifuged on a Ficoll–Paque density gradient to obtain unfractionated mononuclear cells as described above. These cells were then resuspended in elutriation medium, and introduced into a Beckman JE-5 elutriation chamber and the rotor system of a J6 ME Beckman centrifuge. Monocytes and lymphocytes were separated at different medium flow rates, according to their size and density differences [19]. The purity of the monocyte or lymphocyte preparations obtained was confirmed by flow cytofluorometric analysis (see below) and cell size was determined with a Coulter Channelyzer system (Coulter Electronics). The lymphocyte preparations were about 99% pure, and the monocyte preparations were over 95% pure. Cell viability was 95–97% by Trypan Blue exclusion. Purified cells were pelleted and kept at −20 °C. After thawing, cells were lysed by sonification (2 × 30 s) at 4 °C in PBS containing 8 mM Chaps and kept at 4 °C for 1 h. The cell lysate was then centrifuged at 700 *g* for 10 min, and the supernatant used for enzymic assays.

The cell types in the isolated cell populations were analysed immunologically by flow cytofluorometry, using monoclonal antibodies to human leucocyte differentiation antigens (CD antigens). Surface-marker analysis was performed on cell suspensions in a Facstar Cell Sorter (Becton Dickinson) using standard techniques (Laboratoire Central d'Immunologie Cellulaire et Tissulaire, CH Pitié-Salpétrière, Paris, France). The antibodies used for the characterization were: T11 (CD2), B4 (CD19), My-4 (CD14), NKH1 (CD56), T4 (CD4) and T8 (CD8) which were purchased from Coulter Electronics.

Culture of monocytes, lymphocytes, and established cell lines

Human monocyte-derived macrophages were obtained by *in vitro* differentiation of monocytes. The centrifuged, washed mononuclear cells were resuspended in IMDM containing penicillin, streptomycin and glutamine (as described above) plus 10% (v/v) human serum type AB^+ (HS), and seeded on Costar tissue-culture plates at $0.6–0.8 \times 10^6$ cells/cm^2. The plates were incubated for 3 h in a humidified incubator at 37 °C under 5% CO_2/95% air and non-adhering cells were removed by gentle washing. The adhering monocytes were incubated in medium containing 5% (v/v) HS for a further three days. The cells were then incubated in serum-free medium for 2–4-days, and harvested. Serum-free medium consisted of IMDM containing antibiotics and glutamine, supplemented with human transferrin (30 μg/ml, 30% iron-saturated), BSA (1 mg/ml), soybean lipids (aq. dispersion, 50 μg/ml), and bovine pancreatic insulin (specific activity > 26 units/mg, 1.5 μg/ml) (Boehringer–Mannheim). Cells were washed twice in PBS and harvested by scraping with a rubber policeman in PBS containing 8 mM Chaps. The differentiation of monocytes into macrophages was assessed morphologically. All cultures were prepared in triplicate. Serum-free cell supernatants were also collected, centrifuged for 10 min at 800 *g* and kept at −20 °C.

Lymphocytes isolated by elutriation were suspended in serum-free IMDM with antibiotics and glutamine as described above, plus 1% (v/v) Nutridoma-HU described by the manufacturer as a serum-free defined medium supplement containing human albumin, bovine insulin and human transferrin (Boehringer–Mannheim), and cultured in multiwell Costar tissue-culture plates (0.75×10^6 cells/cm^2). Cells were incubated for 48 h with or without 10^{-6} M dexamethasone, or 10^{-6} M tri-iodothyronin. Cells were also incubated in wells coated with anti-CD3 antibodies (65 ng/cm^2) (a gift from Dr. A. Fischer, INSERM U132, Paris). After incubation, the cells were collected by centrifugation and kept frozen until assayed for ACE activity.

Transformed lymphocyte cell lines were also studied. JURKAT cells derived from a human T-cell leukaemia [20] were obtained from Dr. A. Fischer (INSERM U132), and cultured in suspension in RPMI 1640 medium containing penicillin (100 i.u./ml), streptomycin (100 μg/ml), 2 mM L-glutamine, 1 mM sodium pyruvate and 10% (v/v) fetal calf serum (FCS). For serum-free culture, FCS was replaced by 1% (v/v) Nutridoma-HU (Boehringer–Mannheim). Epstein–Barr virus (EBV)-transformed B-lymphocytes, obtained by the method of Neitzel [21], were kindly provided by J. F. Deleuze (INSERM U56, Paris), and maintained in RPMI 1640 medium containing penicillin (100 i.u./ml), streptomycin (100 μg/ml), fungizone (0.25 μg/ml), 2 mM L-glutamine, 50 μM 2-mercaptoethanol, and 10% (v/v) FCS or 1% (v/v) Nutridoma-HU (for serum-free culture). The acute lymphoblastic leukaemic cell lines Molt-4 [22] and CEM [23] were obtained from the European Collection of Animal Cell Cultures (Salisbury, Wilts., U.K.) and the American Type Culture Collection (Rockville, MD, U.S.A.) respectively, and cultured in the same conditions as JURKAT cells.

Subcellular fractionation of human lymphocytes

Lymphocyte pellets obtained by elutriation were resuspended and homogenized at 4 °C in a 20-fold excess (v/v) of 20 mM sodium phosphate, pH 7.5/0.25 M sucrose/5 mM $MgCl_2$. Subcellular fractions were obtained by differential centrifugation

(at 4 °C) of the crude homogenate at 600 *g* for 10 min to remove cellular debris and unbroken cells, and then at 105000 *g* for 1 h to yield the microsomal and the cytosolic fractions. The microsomal pellet was washed in homogenization buffer, recentrifuged at 105000 *g* for 1 h, and finally resuspended in PBS containing 8 mM Chaps at 4 °C. All fractions were kept to −20 °C prior to sonication (2 × 30 s) and incubated for 1 h at 4 °C. Fractions were then centrifuged at 900 *g* for 10 min and the supernatants used for enzymic assays. All fractions were adjusted to contain 8 mM Chaps prior to enzymic measurements. The lymphocyte preparations used for these fractionations contained 80% T-lymphocytes, the remaining cells were B-lymphocytes and natural killer cells.

Characterization and measurement of ACE activity

ACE activity was determined by hydrolysis of the synthetic substrate *p*-benzoyl-L-glycyl-L-histidyl-L-leucine (Hip-His-Leu, Bachem). The assay conditions were 5 mM substrate, 100 mM potassium phosphate, pH 8.3, 300 mM NaCl, 10 μM $ZnSO_4$ in a volume of 250 μl, incubated at 37 °C, as described by Cushman and Cheung [24]. The reaction was stopped by adding 50 μl of 12% (w/v) H_3PO_4. The amount of hippuric acid liberated from the substrate was analysed by h.p.l.c. on a 10 μm Nucleosil C18 column (4.6 mm × 25 cm) (Société Française Chromato Colonne, France). The mobile phase was acetonitrile/10 mM potassium phosphate buffer, pH 3 (24:76, v/v), and hippuric acid was measured by its absorption at 228 nm. Peaks were quantified by comparing their heights to those of standard solutions of hippuric acid. The limit of detection was about 7.5 pmol of hippuric acid injected on to the column, which corresponds to 0.006% hydrolysis of Hip-His-Leu (at a concentration of 5 mM). One unit of activity was defined as the amount of enzyme catalysing the release of 1 μmol of hippuric acid/min [24]. Substrate consumption was kept ⩽ 5% by using diluted samples, and activity was determined under initial-velocity conditions.

The ability of total lymphocyte-cell homogenates and lymphocyte microsomal fractions to hydrolyse BK was also assayed by incubating the preparations (30–60 μg of proteins) with 0.1–10 ng of BK (Peninsula Laboratories Europe Ltd.) for 30 to 60 min at 37 °C in 500 μl of 50 mM Tris/HCl buffer, pH 8, containing 0.15 M NaCl, 10 μM $ZnSO_4$, and 1 mg/ml lysozyme. The reaction was stopped by adding ice-cold EDTA at a final concentration of 3 mM. The residual BK was measured by radioimmunoassay as described previously, using antibodies to BK that cross-reacted only 0.2% or less with BK fragments [25].

Inhibition of hydrolysis of Hip-His-Leu and BK was measured under the standard conditions described above using enalaprilat (MK422, a gift from Merck Sharp and Dohme) or EDTA. Enalaprilat inhibition of the hydrolysis of Hip-His-Leu or BK was tested by pre-incubating the enzyme for 15 min at 37 °C with or without the inhibitor. Substrate was then added and the incubation carried out as above.

Protein assay

Protein concentrations were measured by the method of Bradford [26] with BSA as the standard.

Reverse transcription of RNA and PCR amplification of cDNA

Total cell RNA was isolated by the method of Chomczynski and Sacchi [27]. Murine Mo virus reverse transcriptase (Perkin Elmer–Cetus) was used to synthesize the single-stranded cDNA using 2 μg of total RNA as template and the synthetic oligomer ASC2352 (5′-GATGTGGCCATCACATTCGTCAGA-3′) as oligonucleotide primer (see below), under the conditions described by the manufacturer (*GeneAmp* RNA PCR kit, Perkin Elmer–Cetus). The cDNA was amplified by PCR using *AmpliTaq* DNA polymerase (Perkin Elmer–Cetus), the oligomer used for cDNA synthesis and oligomer SEC1680 (5′-CACTGCACC-AGTGTGACATCTACC-3′), in a Perkin Elmer–Cetus DNA Thermal Cycler, with the following temperature-cycling conditions: 2 min at 95 °C for one cycle, and 1 min at 95 °C, 2 min at 55 °C, 4 min at 72 °C, for 35 cycles. The oligonucleotide primers were synthesized in an Applied Biosystems DNA synthesizer. SEC1680 corresponds to nucleotide sequence 1680–1703 and ASC2352 to sequence 2352–2329 (complementary strand) of the human endothelial ACE cDNA [28]. A second form of ACE is expressed in the testis, translated from a shorter mRNA under the transcriptional control of a different promoter and corresponding to the 3′ half of the endothelial sequence preceded by a short testicular-specific sequence (see the legend of Figure 3) [29–31]. PCR amplification was also performed with oligomer ASC2352 and an oligonucleotide primer specific for the testicular form of ACE, STC120 (5′-TCCCAACAGGTGAC-AGTCACCCAT-3′), corresponding to the sequence 122–145 of the human testicular ACE cDNA [29].

The PCR amplification products were electrophoresed in 1% (w/v) agarose gel, and revealed by staining with ethidium bromide. They were also analysed by Southern blot hybridization as follows: after electrophoresis, DNAs were transferred by capillary blotting with alkali on to Hybond-N^+ nylon membrane (Amersham International, Amersham, Bucks., U.K.). Hybridization was performed at 50 °C for 12–15 h using the ^{32}P-labelled oligomer SC2238 (5′-AGTACAACAAGATCCTG-TTG-3′), corresponding to nucleotide sequence 2238–2257 of the endothelial ACE cDNA. The membrane was washed under high-stringency conditions (final washing conditions: 15 min at 50 °C in 0.1% SDS/0.1 × SSC (1 × SSC is 0.15 M NaCl, 0.015 M sodium citrate), and autoradiographed for 20 or 60 min at room temperature.

Determination of lymphocyte ACE in individuals having different ACE genotypes

Thirty-five healthy subjects (16 males and 19 females) were selected according to their ACE genotype (11 homozygotes for the insertion II, 13 homozygotes without the insertion DD, and 11 heterozygotes ID). The ACE genotype was determined as previously described [14]. All subjects gave their informed consent. They were all Caucasian, aged 25–50 years, without evidence of current evolving disease. Blood was drawn from the antecubital vein into heparinized tubes between 08:00 and 10:00, after an overnight fast and approx. 10 min of rest. T-lymphocytes were then purified as described above. The plasma ACE levels of all subjects were measured by enzymic assay and direct radioimmunoassay [32]. The reproducibility of lymphocyte ACE levels was tested on 12 subjects of different ACE genotype by collecting blood on two occasions 2–3 weeks apart.

Statistical analysis

The Wilcoxon signed-rank test was used to estimate the stability of ACE levels in T-lymphocytes drawn on two occasions from the same subjects. The influence of the ACE genotype on the level of T-lymphocyte ACE was also studied using non-parametric tests (Kruskal–Wallis test followed by the Mann–Whitney test for comparison between two groups). The plasma and T-lymphocyte ACE levels were compared by linear regression and analysis of variance. The level of statistical significance was taken as $P < 0.05$.

RESULTS

Demonstration of the presence of ACE in circulating mononuclear cells and enzymic characterization

ACE activity was detected in human circulating monocytes separated from total mononuclear cells by either adherence to a plastic surface or elutriation, and it was 25±3 μunits/mg of protein ($n = 5$) (Table 1). The ACE activity in total mononuclear cells (152±60 μunits/mg of protein, $n = 8$) was approx. 6-fold higher than in monocytes, although the monocytes represented only 15–20% of the total mononuclear cells isolated by our methods. The other mononuclear cell types were therefore tested for ACE. T ($CD2^+$)-lymphocytes had the highest levels of ACE activity; the average ACE level per mg of cell protein was 18-fold greater than that of circulating monocytes. B ($CD19^+$)-lymphocytes contained no activity under our experimental conditions (i.e. less than 10 μunits/mg of protein). The low activity in residual cells after T- and B-lymphocyte purification can probably be attributed to residual T-lymphocytes or monocytes (Table 1). The ACE activity in lymphocytes, measured under initial-velocity conditions, remained linear with incubation times ranging from 1 to 7 h, and with the amount of cell-extract proteins ranging from 10 to 150 μg. It was not significantly different when 5, 8, or 10 mM Chaps detergent was used for extraction (results not shown). The population of circulating T-lymphocytes expressing ACE was characterized by purifying $CD4^+$ and $CD8^+$ T-lymphocyte subtypes from mononuclear cells. ACE activity was present in both subtypes ($CD4^+$: 430 and 380 μunits/mg of protein; $CD8^+$: 280 and 250 μunits/mg of protein, measured on two different subjects). Lymphocyte ACE activity was totally inhibited by EDTA (10^{-5} M), and was inhibited in a dose-dependent manner by enalaprilat (MK422), with an IC_{50} of 2.5 nM (Figure 1).

Subcellular fractionation of lymphocytes showed that ACE activity was mainly in the microsomal fraction (Table 2). No activity was detected in the serum-free medium from lymphocytes cultured for 2 days. The ACE activity in these cells was not altered by dexamethasone or tri-iodothyronin, or by cell activation with anti-CD3 antibodies (Figure 2).

ACE expression was also studied during monocyte/macrophage differentiation. Freshly isolated monocytes contained very low levels of ACE activity, but this activity increased up to 100-fold during *in vitro* differentiation (Figure 2). ACE activity was present in both solubilized macrophages (2.8±1.5 munit/mg of protein, $n = 4$) and serum-free culture medium from macrophages (2.0±1.8 munit/mg of protein,

Table 1 ACE activity in circulating mononuclear cells

Various types of mononuclear cells were isolated from the plasma of healthy adult volunteers. ACE activity was quantified by the hydrolysis of Hip-His-Leu at pH 8.3. Results are means±S.D.; $n = 8$, unless otherwise indicated. Residual cells are mononuclear cells after T- and B-cell purification with immunomagnetic beads. Abbreviation: n.d.: not detectable.

Cell type	ACE activity	
	μunits/10^6 cells	μunits/mg of protein
Monocytes	1.7±0.3*	25±3†
T ($CD2^+$)-lymphocytes	47±29	453±137
B ($CD19^+$)-lymphocytes	n.d.	n.d.
Residual cells	0.3±0.1	20±7

* $n = 3$
† $n = 5$

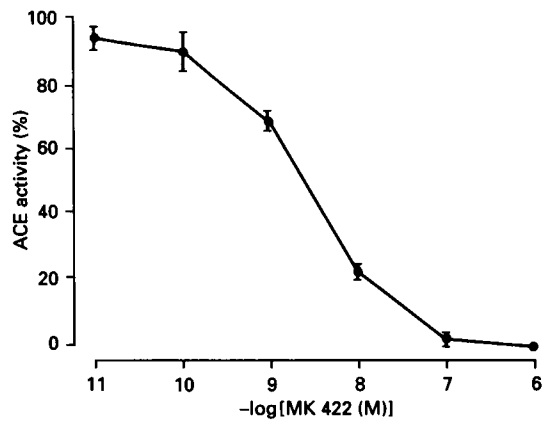

Figure 1 Inhibition of ACE activity in lymphocytes by enalaprilat (MK422)

Lymphocyte homogenate was incubated with MK422 (10^{-11}–10^{-6} M). Results are expressed as percentages of activity measured in the absence of inhibitor ($n = 4$).

Table 2 Subcellular localization of ACE activity in human lymphocytes

Lymphocyte membranes were prepared by homogenizing the cells in a 20-fold excess (v/v) of 20 mM phosphate buffer, pH 7.5, 0.25 M sucrose, 5 mM $MgCl_2$, and centrifuging at 600 *g* for 10 min at 4 °C. The resulting supernatant was centrifuged at 105000 *g* for 1 h. The pellet was washed, and resuspended in PBS with 8 mM Chaps. Each fraction was tested for enzymic activity in the presence of 8 mM Chaps. Results are means±S.D.; $n = 3$ different experiments.

Subcellular fraction	ACE activity (μunits/mg of protein)
600 *g* pellet	210±29
600 *g* supernatant	305±134
105000 *g* supernatant	68±15
105000 *g* pellet	787±266

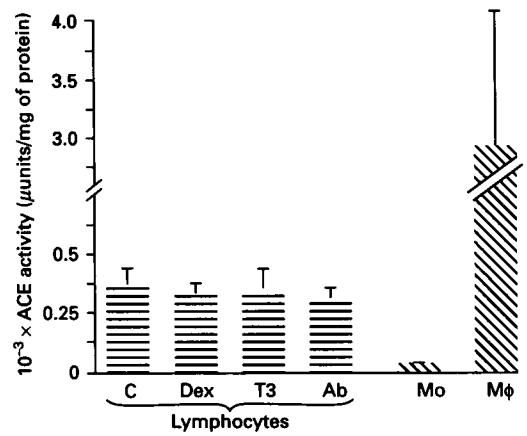

Figure 2 Comparison of the effect of hormones or activation on ACE activity in cultured lymphocytes and monocytes

Dexamethasone (Dex), tri-iodothyronine (T3), and activation by anti-CD3 antibodies (Ab), had no significant effect on lymphocytes compared with controls (C). ACE was also measured in monocytes (Mo) before and after *in vitro* differentiation to macrophages (Mø) by adhesion. The results for macrophages at maximal ACE expression (7 days of culture) are presented. Results are mean±S.D. of three subjects, tested in triplicate experiments.

Table 3 BK hydrolysis by human lymphocytes

The ability of lymphocyte homogenates and microsomal preparations (30–60 μg of protein) to hydrolyse BK was studied by incubating samples with 0.1–10 ng of BK (0.2–2 nM) for 30 min (homogenates) or 60 min (microsomes). Incubation was carried out in a volume of 0.5 ml at pH 8 and 0.15 M NaCl, as described in the Materials and methods section. The effect of enalaprilat (MK422) on kininase activity was tested with 10^{-6} M of inhibitor. Values represent nanograms of BK hydrolysed or percentage inhibition by MK422, from three different experiments, and are means ± S.D.

	Amount of BK added (ng)		
	0.1	1	10
Cellular homogenate			
No inhibitor	0.0385 ± 0.0029	0.358 ± 0.037	3.93 ± 0.25
Enalaprilat (10^{-6} M)	0.0233 ± 0.0015	0.291 ± 0.034	3.46 ± 0.35
Inhibition (%)	39.6 ± 4.0	19.5 ± 2.2	12.2 ± 3.3
Microsomal preparation			
No inhibitor	0.0506 ± 0.0057	0.486 ± 0.051	4.80 ± 0.20
Enalaprilat (10^{-6} M)	0.0016 ± 0.0028	0.020 ± 0.034	0.80 ± 0.75
Inhibition (%)	96.4 ± 6.3	96.3 ± 6.5	83.8 ± 15.1

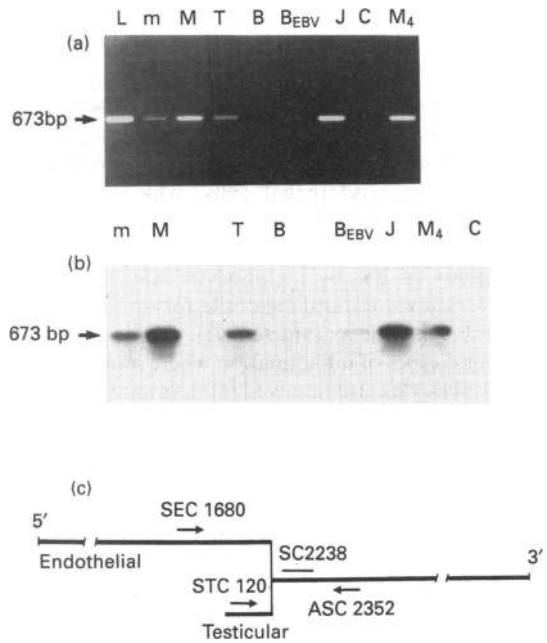

Figure 3 ACE mRNA in human mononuclear cells and lymphoid cells

Figure shows RT-PCR amplification of total RNA (2 μg, unless otherwise mentioned) from human lung (L, 1 μg), monocytes (m), macrophages (M), T-lymphocytes (T), B-lymphocytes (B), EBV-transformed B-lymphocytes (B_{EBV}), and cell lines JURKAT (J), Molt-4 (M_4), and CEM (C). (**a**) DNA products generated between primers ASC2352 and SEC1680 were electrophoresed in 1% (w/v) agarose gel and revealed by staining with ethidium bromide. (**b**) Southern-blot analysis of RT-PCR products: after transfer of DNAs, blot was hybridized using ^{32}P-labelled human SC2238 probe. Below is a schematic representation of the human endothelial and testicular ACE mRNAs with the positions of the oligomers used for RT-PCR amplification and blot hybridization. The nucleotide sequence located downstream of the vertical line is common to the two mRNAs. Arrow heads indicate the 3′ extremity of the primers.

$n = 4$) after 7 days in culture. It was inhibited in a dose-dependent manner by enalaprilat, with IC_{50}s of 3.6 nM (cells) and 2.5 nM (medium) (results not shown).

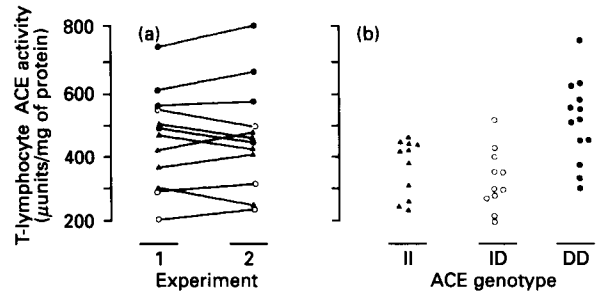

Figure 4 ACE level in human T-lymphocytes

(**a**) Reproducibility was tested by comparing two blood samples taken at 1–3 weeks intervals from 12 subjects having different genotypes. No significant difference was found between the two measurements [see (**b**) for explanation of symbols]. (**b**) Comparison of T-lymphocyte ACE levels in individuals of the II, ID, and DD genotypes. Effect of genotype: $P < 0.002$.

Hydrolysis of BK by lymphocytes

The lymphocyte homogenate and the microsomal fraction hydrolysed BK over the substrate concentration range 0.2 nM–20 nM. Enalaprilat (1 μM) only partly inhibited BK hydrolysis by lymphocyte homogenate, whereas the kininase activity in the microsomal fraction was almost completely inhibited (Table 3).

RNA characterization

cDNA was synthesized from total RNA by reverse transcription (RT) and amplified via PCR using specific primers for the endothelial or the testicular form of the ACE transcript (Figure 3c). RT-PCR amplification of RNA from the various types of human mononuclear cells, performed between primer ASC2352 and the specific endothelial primer SEC1680, revealed one major electrophoretic band of the expected size (673 bp) in human monocytes, macrophages and T-lymphocytes (Figure 3a). This band hybridized with the oligonucleotide probe SC2238 (Figure 3b). Macrophages appeared to have the highest level of ACE mRNA, followed by lymphocytes and monocytes. By contrast, ACE mRNA was not detectable in B-lymphocytes under the same conditions. However, after hybridization and an exposure time of 60 min, a weak signal was revealed (results not shown). The patterns of ACE mRNA expression in monocytes, macrophages, T- and B-lymphocytes were similar after 20, 30, or 35 cycles of PCR amplification, and when 0.5–2 μg of total RNA were tested (results not shown). RT-PCR amplification of RNA performed with primer ASC2352 and primer STC120, which is specific for the testicular form of the ACE transcript, able to generate a major electrophoretic band of 514 bp from human testicular RNA, revealed no amplified DNA fragment even after hybridization with SC2238 and prolonged exposure (results not shown).

Measurements of ACE levels in T-lymphocytes and macrophages from individual subjects

The reproducibility of the T-lymphocyte ACE levels in 12 subjects was explored. T-lymphocyte ACE levels were stable when measured on two different occasions in subjects of different ACE genotype, with mean ACE levels ranging from 230 to 780 μunits/mg of protein ($P = 0.69$) (Figure 4a). By contrast ACE levels measured during differentiation of macrophages *in vitro*, and tested in the same subjects on two different occasions, showed very poor reproducibility. The ACE levels in

macrophages from the same subject varied up to 3-fold between the two experiments (results not shown).

The mean T-lymphocyte ACE level in the 35 subjects studied was 423 ± 133 μunits/mg of protein, with extreme values of 215 and 780 μunits/mg of protein. Comparison among the three groups of subjects defined by the ACE genotype showed that T-lymphocyte ACE levels of the three groups were significantly different ($P < 0.002$, Figure 4b): II, 381 ± 88 ($n = 11$); ID, 341 ± 94 ($n = 11$); and DD, 528 ± 129 μunits/mg of protein ($n = 13$). DD-genotype subjects had higher levels than subjects with the two other genotypes (II versus DD and ID versus DD: $P < 0.01$). There was no significant difference between the II and ID subjects. The plasma immunoreactive ACE levels of the 35 subjects varied from 174 to 640 μg/l, and the plasma levels of the three ACE-genotype groups were significantly different ($P < 0.001$): II, 277 ± 58 ($n = 11$); ID, 362 ± 78 ($n = 11$); and DD, 487 ± 101 ($n = 13$); (II versus DD: $P < 0.001$, ID versus DD: $P < 0.005$, and II versus ID: $P < 0.05$).

The T-lymphocyte and plasma ACE levels of each subject were significantly correlated ($r = 0.424$, $n = 35$, $F = 7.24$, $P < 0.01$).

Identical results were obtained when using plasma ACE activity measurements, which were highly significantly correlated as expected to plasma immunoreactive ACE levels ($P < 0.001$).

Expression of ACE in lymphocyte-derived cell lines

ACE activity was low in EBV-transformed B-lymphocytes. It was 7 μunits/mg of protein when measured after a prolonged incubation time of 7 h. RT-PCR amplification produced only a weak signal after hybridization with SC2238 and a 1 h exposure (results not shown). JURKAT cells, derived from a human T-cell leukaemia, had an ACE activity of 20 ± 6 μunits/mg of protein ($n = 5$). It was not detectable in the serum-free medium collected after 2 days of culture of these cells. JURKAT cells expressed a relatively abundant ACE mRNA as demonstrated by RT-PCR amplification. ACE mRNA was also present in Molt-4, but was not detectable in CEM cells (Figure 3). ACE activity was not detectable in Molt-4 cells (it was < 2 μunits/mg of protein). None of these cell lines appeared to express the testicular form of the ACE transcript, when tested by RT-PCR amplification using the testicular primer STC 120 followed by hybridization with SC2238 and prolonged exposure.

DISCUSSION

ACE is expressed in two types of circulating mononuclear cells, monocytes and T-lymphocytes, but at different levels. T-lymphocytes contain the highest enzyme activity, approx. 28 times more per cell than monocytes. The kinetic parameters for the human endothelial ACE previously determined by the same enzymic assay [33] indicated that the ACE level in peripheral T-lymphocytes was about 2000 molecules/cell, approx. 10–20 times less than the three major cell-surface proteins CD3, CD4, and CD8 [34]. The ACE content of lymphocytes is in the same order of magnitude as in cultured endothelial cells collected from large vessels [10,35]. As T-lymphocytes represent more than 80% of circulating lymphocytes, and the enzyme was not detected in other circulating mononuclear cells or polynuclear cells [36] (except in low quantities in monocytes) lymphocytes appear to be the major cell type expressing ACE in the blood. The ACE concentrations in the two major T-lymphocyte subtypes, CD4 and CD8, were about the same.

The presence of ACE in T-lymphocytes was unexpected and therefore ACE was characterized in T-lymphocytes by its enzymic properties. ACE activity was measured under initial-velocity conditions with the synthetic tripeptide substrate Hip-His-Leu. The reaction was completely inhibited by 10^{-5} M EDTA, a non-specific inhibitor of metalloenzymes, and inhibited in a dose-dependent manner by enalaprilat, a specific ACE inhibitor, with an IC_{50} value of 2.5 nM. These properties are similar to those of purified human kidney or recombinant endothelial ACEs [33]. As ACE is mainly a membrane-bound ectoenzyme in other cells, we assessed the cellular localization of the enzyme in lymphocytes by subcellular fractionation. As in epithelial cells [37], ACE was found mainly in the microsomal fraction of the cell homogenate which contains the cell membranes, where, as in other cells, it is probably anchored by its C-terminal hydrophobic segment [33,38–40]. Other ectoproteins similarly anchored to the lymphocytes by a transmembrane domain located near their C-terminal extremity are those of the antigen–receptor complex and the interleukin 2 receptor [34]. However, unlike endothelial cells, the lymphocytes maintained *in vitro* produced no detectable secretion of the enzyme into the culture medium, although they synthesized a membrane-bound form of the enzyme. Recent studies indicate that the secreted form of ACE is derived from the membrane-bound form, and is probably generated by a post-translational proteolytic cleavage of the membrane anchor [33,38–40]. Although the enzyme responsible for this cleavage has not been identified, it may not be expressed in lymphocytes, in contrast to endothelial cells and macrophages that secrete large quantities of ACE, at least in some species [10,15]. It is also possible that the ACE-cleaving enzyme, although present in lymphocytes, is not accessible to its substrate, or is poorly active because of a low affinity for ACE and the low level of substrate available. This would also account for the very low level of ACE secretion by human endothelial cells, which are known to synthesize less ACE than the endothelial cells of other species [35].

The hydrolysis of BK by lymphocytes was studied because BK is the best-known natural substrate for ACE, i.e. it has the most favourable kinetic parameters [1]. BK is also produced locally in several types of inflammation where mononuclear cells, including lymphocytes, are involved [4]. Lymphocytes hydrolyse BK, even at a low peptide concentration, i.e. well below the K_m for ACE (0.2 μM) and other identified kininases. Experiments with ACE inhibitor indicated that part of the kininase activity of crude cellular homogenate of lymphocytes is attributable to ACE, while the kininase activity in the microsomal fraction is mainly due to ACE. These results confirm that ACE is a membrane-bound enzyme in lymphocytes and indicate that ACE is probably the major membrane-bound kinin-inactivating enzyme in these cells, at least at low peptide concentrations.

The ACE mRNA in T-lymphocytes, monocytes, and macrophages was characterized by RT-PCR amplification. Experiments with specific oligonucleotide primers for either the endothelial or the testicular ACE mRNAS indicated that only the endothelial form of the ACE transcript is present in T-lymphocytes and monocytes. Thus the endothelial but not the testicular promoter of the ACE gene is functional in these cells. The enzyme is constitutively expressed in T-lymphocytes, but not in B-lymphocytes or at a very much lower level than in T-lymphocytes. This is supported by the lack of detectable ACE activity in B-cells, and by the result of RT-PCR amplification where ACE mRNA was scarcely detectable after oligonucleotide hybridization. Our results support the observation that several ectopeptidases, mainly expressed in absorptive epithelial cells and sometimes in endothelial cells, are also present in lymphocytes, although differentially expressed in subtypes of lymphocytes and at particular stages of cell maturation [41]. For

example, endopeptidase 24.11 (enkephalinase) [42,43] and aminopeptidase A [44] are expressed on pre-B-lymphocytes whereas mature lymphocytes are devoid of these enzymes. Dipeptidylpeptidase IV is present, like ACE, on T-lymphocytes, where its expression is developmentally regulated in the mouse [45–47]. ACE transcription may be activated during T-cell differentiation or conversely suppressed in mature B-lymphocytes. The results obtained with T-cell leukaemia cell lines suggest that ACE expression may be activated during T-cell differentiation and maturation. ACE mRNA was indeed found in JURKAT and Molt-4 cells, but not in CEM cells which are classified as less differentiated cells based on cell-surface markers and cellular enzymes [48]. ACE expression was also barely detectable in B-lymphocytes after lymphoblastoid transformation by EBV. However, several pre-B-derived leukaemia cell lines have been found to express an ACE or ACE-like activity [43].

The biological function of ACE in T-lymphocytes, as in macrophages, remains largely unknown. Given the smaller number of circulating T-lymphocytes in peripheral blood compared with the large quantity of endothelial cells lining blood vessels and expressing the enzyme on their surface, circulating T-lymphocytes certainly do not contribute greatly to angiotensin I conversion in the circulation. This reaction takes place mainly on the surface of endothelial cells and in the plasma. The ACE in mononuclear cells may, however, participate in the local production or degradation of regulatory peptides, for example at the site of an inflammatory reaction. It has been reported that angiotensin II is chemotactic for T-lymphocytes [49]. ACE also hydrolyses BK and substance P, which are both implicated in several aspects of the inflammatory and immunological responses, including lymphocyte proliferation and neutrophil chemotaxis [50], phagocytosis [51], and the release of mediators [52]. ACE also hydrolyses the chemotactic peptide *N*-formyl-Met-Leu-Phe [1]. Lastly, ACE might play a role in lymphocyte function, as recently reported for dipeptidylpeptidase IV [45–47].

Ever since Lieberman reported that high levels of serum ACE were associated with the granulomatous disease sarcoidosis, several studies have localized ACE in cells of the monocyte lineage [15]. Our results confirm these reports and show that there is a considerable increase in ACE expression in monocytes during their *in vitro* differentiation to macrophages [15,53], probably mediated at the transcriptional level (Figures 2 and 3). However, this effect was poorly reproducible under the test conditions because of macrophage heterogeneity, and did not allow comparison of macrophage ACE levels among subjects. By contrast, the ACE expression of lymphocytes maintained *in vitro* remained stable, and was not altered by activation of the antigen receptor with anti-CD3 antibodies under our experimental conditions, or treatment with hormones (dexamethasone and triiodothyronine) which have been shown to stimulate ACE synthesis in other cell types [10,53]. This suggests that ACE expression in T-lymphocytes is essentially constitutive and not greatly affected *in vivo* by several candidate environmental or hormonal factors.

The role of genetic factors in the interindividual variability in lymphocyte ACE content was investigated. Our previous studies have demonstrated a familial resemblance in plasma ACE levels, and indicated that a major gene affects the large interindividual variability in plasma ACE levels in man [13]. An insertion/deletion polymorphism located in an intron of the ACE gene was then shown to be associated with differences in the plasma concentration of ACE [14]. Subjects homozygotic for the deletion (DD) have higher plasma ACE levels than do homozygotes for the insertion (II), while heterozygotes (ID) have intermediate levels. These observations raise the question of the relationship of this genetic polymorphism to ACE synthesis in endothelial cells which secrete plasma ACE, and in the other cell types expressing the enzyme. This could be significant as ACE is mostly a membrane-bound ectoenzyme and the cellular enzyme is known to be physiologically more important for peptide metabolism than the plasma enzyme [1]. Circulating T-lymphocytes were used as a relatively accessible source of ACE-expressing cells in individual subjects to investigate the effect of the ACE genotype at the cellular level. As in plasma [12,13], the lymphocyte ACE content varied widely between individuals, but remained remarkably stable when measured repeatedly in the same subject. The ACE genotype influences the lymphocyte-enzyme level. DD-genotype subjects had significantly higher levels than those of the two other genotypes (Figure 4b). The effect of the two short alleles (DD genotype) was to raise mean ACE levels by 75% in plasma and 39% in T-lymphocytes compared with the two long alleles (II genotype). Heterozygotes had an average of 30% more ACE in plasma than did homozygotes for the two long alleles, but their mean lymphocyte ACE content was not different. The smaller genetic effect in lymphocytes than in plasma is probably not due to less precise measurements of ACE content in lymphocytes, as these measurements were as reproducible as these in plasma [12] (Figure 4a). It may be due to one or more of the following factors: (1) the number of subjects tested did not allow recognition of a difference between the II and ID phenotypes; (2) the turnover rate of ACE being lower in lymphocytes than in plasma and a lower level of synthesis occurring than in endothelial cells, as the genetic effect is probably more apparant when the level of synthesis is high, as is found when comparing the plasma ACE levels of children and adults [13]; (3) the effect of undetected genetic or environmental factors. Nevertheless our results indicate that the level of expression of membrane-bound ACE in lymphocytes is associated with the insertion/deletion polymorphism of the ACE gene. A genetic polymorphism has also been observed recently for the level of the cytosolic epoxide hydrolase in peripheral human lymphocytes [54]. The present study strongly suggests that the level of ACE synthesis in cells is genetically determined. The genetic control of ACE synthesis is probably at the transcriptional level, although its precise mechanism remains to be determined. T-lymphocytes can constitute a valuable model to study this question. While the magnitude of the genetic effect may vary from one tissue to another under the influence of local regulatory factors as discussed above, the level of ACE expression is undoubtedly genetically determined in many, if not all, ACE-synthesizing tissues. The physiological consequences of the genetic polymorphism of ACE levels for cardiovascular regulation and other ACE-mediated functions remain to be investigated.

Note added in proof (Received 24 November 1992)

It has recently been observed that the insertion/deletion polymorphism of the ACE gene may be linked to coronary heart disease as subjects of the DD genotype seem to have an increased risk of myocardial infarction [55].

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REFERENCES

1 Erdös, E. G. (1990) Hypertension **16**, 363–370
2 Skeggs, L. T., Kahn, J. R. and Shumway, N. P. (1956) J. Exp. Med. **103**, 295–299
3 Yang, H. Y., Erdös, E. G. and Levin, Y. (1970) Biochim. Biophys. Acta **214**, 374–376
4 Proud, D. and Kaplan, A. P. (1988) Annu. Rev. Immunol. **6**, 49–83
5 Pernow, B. (1985) J. Immunol. **135**, 812s–815s
6 Caldwell, P. R. B., Seegal, B. C., Hsu, K. C., Das, M. and Soffer, R. L. (1976) Science **191**, 1050–1051
7 Defendini, R., Zimmerman, E. A., Weare, J. A., Alhenc-Gelas, F. and Erdös, E. G. (1983) Neuroendocrinology **37**, 32–40
8 Williams, T. A., Hooper, N. M. and Turner, A. J. (1991) J. Neurochem. **57**, 193–199
9 Mendelsohn, F. A. O., Lloyd, C. J., Kachel, C. and Funder, J. W. (1982) J. Clin. Invest. **70**, 684–692
10 Krulewitz, A. H., Baur, W. E. and Fanburg, B. L. (1984) Am. J. Physiol. **247**, C163–C168
11 Lieberman, J. (1975) Am. J. Med. **59**, 365–372
12 Alhenc-Gelas, F., Richard, J., Courbon, D., Warnet, J. M. and Corvol, P. (1991) J. Lab. Clin. Med. **117**, 33–39
13 Cambien, F., Alhenc-Gelas, F., Herberth, B., Andre, J. L., Rakotovao, R., Gonzales, M. F., Allegrini, J. and Bloch, C. (1988) Am. J. Hum. Genet. **43**, 774–780
14 Rigat, B., Hubert, C., Alhenc-Gelas, F., Cambien, F., Corvol, P. and Soubrier, F. (1990) J. Clin. Invest. **86**, 1343–1346
15 Friedland, J., Setton, C. and Silverstein, E. (1978) Biochem. Biophys. Res. Commun. **83**, 843–849
16 Bøyum, A. (1968) Scand. J. Clin. Lab. Invest. **21** (Suppl. 97), 77–89
17 Lea, T., Smeland, E., Funderud, S., Vartdal, F., Davies, C., Beiske, K. and Ugelstad, J. (1986) Scand. J. Immunol. **23**, 509–519
18 Sanderson, R. J., Shepperdson, F. T., Vatter, A. E. and Talmage, D. W. (1977) J. Immunol. **118**, 1409–1414
19 Lopez, M., Fechtenbaum, J., David, B., Martinache, C., Chokri, M., Canepa, S., De Gramont, A., Louvet, C., Gorin, I., Mortel, O. and Bartholeyns, J. (1992) J. Immunotherapy **11**, 209–217
20 Schneider, U., Schwenk, H. U. and Bornkamm, G. (1977) Int. J. Cancer **19**, 621–626
21 Neitzel, H. (1986) Hum. Genet. **73**, 320–326
22 Minowada, J., Ohnuma, T. and Moore, G. E. (1972) J. Natl. Cancer Inst. **49**, 891–895
23 Foley, G. E., Lazarus, H., Farber, S., Uzman, B. G., Boone, B. A. and McCarthy, R. E. (1965) Cancer **18**, 522–529
24 Cushman, D. W. and Cheung, M. S. (1971) Biochem. Pharmacol. **20**, 1637–1648
25 Alhenc-Gelas, F., Marchetti, J., Allegrini, J., Corvol, P. and Menard, J. (1981) Biochim. Biophys. Acta **677**, 477–488
26 Bradford, M. M. (1976) Anal. Biochem. **72**, 248–254
27 Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. **162**, 156–159
28 Soubrier, F., Alhenc-Gelas, F., Hubert, C., Allegrini, J., John, M., Tregear, G. and Corvol, P. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 9386–9390
29 Lattion, A. L., Soubrier, F., Allegrini, J., Hubert, C., Corvol, P. and Alhenc-Gelas, F. (1989) FEBS Lett. **252**, 99–104
30 Kumar, R. S., Kusari, J., Roy, S. N., Soffer, R. L. and Sen, G. C. (1989) J. Biol. Chem. **264**, 16754–16758
31 Ehlers, M. R. W., Fox, E. A., Strydom, D. J. and Riordan, J. F. (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 7741–7745
32 Alhenc-Gelas, F., Weare, J. A., Johnson, R. L. and Erdös, E. G. (1983) J. Lab. Clin. Med. **101**, 83–96
33 Wei, L., Alhenc-Gelas, F., Soubrier, F., Michaud, A., Corvol, P. and Clauser, E. (1991) J. Biol. Chem. **266**, 5540–5546
34 Meuer, S., Acuto, O., Hercend, T., Schlossmann, S. and Reinherz, E. (1984) Annu. Rev. Immunol. **2**, 23–41
35 Johnson, A. R. and Erdös, E. G. (1977) J. Clin. Invest. **59**, 684–695
36 Connelly, J. C., Skidgel, R. A., Schulz, W. W., Johnson, A. R. and Erdös, E. G. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 8737–8741
37 Ward, P. E., Erdös, E. G., Gedney, C. D., Dowben, R. M. and Reynolds, R. C. (1976) Biochem. J. **157**, 643–650
38 Hooper, N. M., Keen, J., Pappin, D. J. C. and Turner, A. J. (1987) Biochem. J. **247**, 85–93
39 Ehlers, M. R. W., Chen, Y. N. P. and Riordan, J. F. (1991) Proc. Natl. Acad. Sci. U.S.A. **88**, 1009–1013
40 Sen, I., Samanta, H., Livingston, W. and Sen, G. C. (1991) J. Biol. Chem. **266**, 21988–21990
41 Kenny, A. J., Stephenson, S. L. and Turner, A. J. (1987) in Mammalian Ectoenzymes (Kenny, A. J. and Turner, A. J., eds.), pp. 169–210. Elsevier Science Publishing, New York
42 Letarte, M., Vera, S., Tran, R., Addis, J. B. L., Onizuka, R. J., Quackenbush, P. J., Jongeneel, C. V. and McInnes, R. R. (1988) J. Exp. Med. **168**, 1247–1253
43 Beaumont, A., Brouet, J. C. and Roques, B. P. (1989) Biochem. Biophys. Res. Commun. **160**, 1323–1329
44 Wu, Q., Li, L., Cooper, M. D., Pierres, M. and Gorvel, J. P. (1991) Proc. Natl. Acad. Sci. U.S.A. **88**, 676–680
45 Hegen, M., Niedobitek, C., Klein, E., Stein, H. and Fleischer, B. (1990) J. Immunol. **144**, 2908–2914
46 Vivier, I., Marguet, D., Naquet, P., Bonicel, J., Black, D., Xiao-Yan Li, C., Bernard, A. M., Gorvel, J. P. and Pierres, M. (1991) J. Immunol. **147**, 447–454
47 Bauvois, B. (1990) Eur. J. Immunol. **20**, 459–468
48 Sangster, R. N., Minowada, J., Suciu-Foca, N., Minden, M. and Mak, T. W. (1986) J. Exp. Med. **163**, 1491–1508
49 Weinstock, J. V., Blum, A. M. and Kassab, J. T. (1987) Cell. Immunol. **107**, 180–187
50 Payan, D. G. (1989) Annu. Rev. Med. **40**, 341–352
51 Bar-Shavit, Z., Goldman, R., Stabinsky, Y., Gottlieb, P., Fridkin, M., Teichberg, V. I. and Blumberg, S. (1980) Biochem. Biophys. Res. Commun. **94**, 1445–1451
52 Lotz, M., Vaughan, J. H. and Carson, D. A. (1988) Science **241**, 1218–1221
53 Vuk-Pavlovic, Z., Kreofsky, T. J. and Rohrbach, M. S. (1989) J. Leukocyte Biol. **45**, 503–509
54 Norris, K. K., DeAngelo, T. M. and Vessell, E. S. (1989) J. Clin. Invest. **84**, 1749–1756
55 Cambien, F., Poirier, O., Lecerf, L., Evans, A., Cambou, J. P., Arveiler, D., Luc, G., Bard, J. M., Bara, L., Ricard, S. et al. (1992) Nature (London) **359**, 641–644

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