Complement-subcomponent-C $\bar{1}$ -inhibitor synthesis by human monocytes

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By using a radioimmunoassay, C $\bar{1}$ -inhibitor was found to accumulate in the supernatants of human monocyte cultures. The production of this protein was inhibited reversibly by cycloheximide. When C $\bar{1}$ -inhibitor synthesis was compared with C2 synthesis, it was found that C $\bar{1}$ -inhibitor synthesis continued, whereas synthesis of C2 appeared to cease after about 7 days in culture. Immunoprecipitation of supernatants of monocyte cultures that had been pulsed with [35 S]methionine showed a specific band with an M_r of 105000. Immunoprecipitates of the lysates revealed a band of M_r 83000; this was thought to represent a partially or non-glycosylated precursor of C $\bar{1}$ -inhibitor. C $\bar{1}$ -inhibitor produced by the monocytes was shown, by using a haemolytic assay, to be functionally active. However, the functional activity of C $\bar{1}$ -inhibitor was reduced by only 44% in the presence of cycloheximide, whereas the concentration of this protein in cycloheximide-treated culture supernatants fell by more than 93%. This finding suggests that monocytes secrete a second molecule, which inhibits C $\bar{1}$ activity but is distinct from classical C $\bar{1}$ -inhibitor.

Mononuclear phagocytes are not only highly specialized phagocytic cells, but they also synthesize and secrete a large number of biologically

Abbreviations used: C $\bar{1}$ ^{rat}-EDTA, rat serum diluted 1:15 in iso-osmotic veronal-buffered saline containing EDTA (40 mmol·l⁻¹); VBS, iso-osmotic veronal-buffered saline, pH 7.5, containing sodium barbitone (0.375 g/l), diethylbarbituric acid (0.575 g/l) and NaCl (8.5 g/l); PBS, phosphate-buffered saline, pH 7.4, containing K₂HPO₄ (0.34 g/l), KH₂PO₄ (1.21 g/l) and NaCl (8 g/l); DGVB²⁺, equal volumes of VBS containing CaCl₂ (150 μ mol·l⁻¹) and MgCl₂ (1 mmol·l⁻¹); RPMI-PBS-EDTA, equal volumes of RPMI.1640 and PBS containing EDTA (10 mmol·l⁻¹); PBS-BSA, PBS containing bovine serum albumin (1%, w/v); RPMI-FCS, RPMI.1640 containing 20% (v/v) heat-inactivated (2 h at 56°C) foetal-calf serum; RPMI-ABS, RPMI.1640 containing 10% (v/v) heat-inactivated (2 h at 56°C) human blood-group-AB serum; EA, IgG-antibody-coated sheep erythrocytes; EA(19S)43b, sheep erythrocytes coated with IgM antibody and the activated fourth and third complement components; EAC4, EA bearing the activated fourth complement components; SDS, sodium dodecyl sulphate; PMSF, phenylmethanesulphonyl fluoride; MEM, (Dulbecco's) minimal essential medium; RB, reagent blank. The terminology for complement components is that recommended by the World Health Organisation (1968).

active products (Nathan *et al.*, 1980). Among the proteins that are secreted by these cells are components of the complement system (Colten, 1976; Hartung & Hadding, 1983). So far it has been shown that human mononuclear phagocytes synthesize C $\bar{1}$ subcomponents (Muller *et al.*, 1978), C2 (Einstein *et al.*, 1976) C3 (Strunk *et al.*, 1983) and factors B, D, P, H and I of the alternative pathway (Whaley, 1980). Although the hepatocyte is the major source of complement proteins in plasma (Alper & Rosen, 1968; Hobart *et al.*, 1977), synthesis of complement components by mononuclear phagocytes is probably important in the extravascular spaces such as the peritoneal cavity (Ezekowitz *et al.*, 1984) and at sites of inflammation (De Ceulaer *et al.*, 1980), where complement activation leads to depletion of complement components.

C $\bar{1}$ inhibitor (C $\bar{1}$ -INH) is a glycoprotein that is a stoichiometric inhibitor of C1r and C1s (Donaldson & Evans, 1963; Harpel & Cooper, 1975; Harrison, 1983). The hepatocyte is thought to be the major source of this protein (Johnson *et al.*, 1971). Here we present evidence to show that C $\bar{1}$ -INH is synthesized in, and secreted by, human peripheral blood monocytes in culture.

Materials and methods

Bolton-Hunter reagent (Amersham International), [^{35}S]methionine (New England Nuclear), the dye H33258 (Hoechst Pharmaceuticals), cycloheximide, PMSF, protein A-Sepharose (Sigma), Linbro 24-well tissue-culture plates, tissue-culture flasks (75 cm^2), RPMI 1640 and Ham's F12 tissue-culture medium (Flow Laboratories, Irvine, Ayrshire, Scotland, U.K.), foetal-calf serum, MEM and antibiotic/antimycotic solution containing penicillin, streptomycin and fungizone (Gibco-Biotech, Paisley, Renfrewshire, Scotland, U.K.), human blood-group-AB plasma (West of Scotland Blood Transfusion Service, Law Hospital, Carlisle, Lanarkshire, Scotland, U.K.), donkey anti-rabbit precipitating antiserum and normal rabbit serum (Scottish Antibody Production Unit, Law Hospital, Carlisle, Lanarkshire, Scotland, U.K.) were from the sources cited.

Complement components and antisera

Human C1-INH (Reboul *et al.*, 1977), C2 (Ruddy & Austen, 1967) and guinea-pig C1 (Lachmann & Hobart, 1978) were purified by standard techniques. EAC4 were prepared from EAC1 by using the procedure described by Borsos & Rapp (1967). Rat serum was diluted 1:15 in iso-osmotic veronal-buffered saline, pH 7.4, containing gelatin (0.1%) and EDTA ($40\text{ mmol}\cdot\text{l}^{-1}$) to provide a source of C3-C9 (C^{rat}-EDTA). A monospecific antiserum to C1-INH was produced by immunizing rabbits with the purified antigen, which had been emulsified in Freund's complete adjuvant.

Preparation of monocyte monolayers

Venous blood (60 ml) from normal volunteers was collected in preservative-free heparin. After centrifugation over Ficoll/Hypaque gradients (Boyum, 1968), the mononuclear leucocytes at the interphase were harvested and resuspended in 36 ml of RPMI-FCS. A 12 ml portion of the suspension was added to tissue-culture flasks that had been precoated with the micro-exudate of BHK (baby-hamster kidney) cells (Douglas *et al.*, 1981). After incubation at 37°C for 45 min, the culture medium containing non-adherent cells was decanted, and the monolayer washed three times with warm (37°C) RPMI 1640. Adherent platelets were then removed by a brief exposure (30 s) to RPMI-PBS-EDTA. The adherent monocytes were detached from the flasks by a second incubation with RPMI-PBS-EDTA at 37°C for 15 min. The detached monocytes were washed once in ice-cold RPMI-ABS and then resuspended to $5 \times 10^5/\text{ml}$ in RPMI-ABS.

Portions (1 ml) of cell suspensions were added to the wells of Linbro tissue-culture plates, which

were then incubated at 37°C for 3 h in a humidified atmosphere consisting of CO_2/air (1:19). The supernatants were removed and the monolayers washed with warm RPMI 1640. The monocytes were finally cultured in 1 ml of RPMI-FCS per well. The incubation was continued for 7 days under the conditions described above to allow the monocytes to mature into macrophages (Strunk *et al.*, 1983). On day 7 the culture supernatant was replaced by fresh RPMI-FCS and the culture was continued. Levels of C1-INH and C2 in culture supernatants were monitored after this change of medium, hence this day was designated as '0' in experiments. Each experiment was performed in triplicate wells and the results were expressed as means $\pm$ S.E.M. of the three values.

Characterization of cells in monolayers

All the cells present in monolayers stained positively for non-specific esterase (Horwitz *et al.*, 1977). Over 90% were able to phagocytose latex and serum-treated zymosan particles. The presence of Fc and C3b receptors was shown by the finding that over 95% of the cells were able to form rosettes with sheep erythrocytes coated with IgG antibody (EA) and EA(19S)C43b cells respectively (Bianco & Pytowski, 1981).

Sampling of cultures

To show the appearance of C1-INH and C2 in culture supernatants, 200 μl of supernatant was removed on alternate days and the volume restored by the addition of 200 μl of fresh RPMI-FCS. Samples of supernatants were stored at -70°C until used. After the entire supernatant was removed on the last day of culture the monolayer was lysed in 200 μl of 0.05% SDS. The lysate was then stored at -20°C before assay of DNA content.

Cumulative synthesis of C1-INH and C2

In order to measure the rate of appearance of C1-INH and C2 in the monocyte culture fluid, the entire culture supernatants were changed on alternate days for 14 days.

DNA content of monolayers

The DNA content of the lysates of monolayers was determined by spectrofluorimetry (Cesarone *et al.*, 1979). The volume of the lysate was made up to 5 ml with PBS, and after the addition of 20 μl of H33285 dye the content of DNA was measured by using a Shimadzu fluorimeter with transmission and emission wavelengths of 364 nm and 448 nm respectively. A standard curve of known DNA content was included in all assays. By using this assay, 1 μg of DNA corresponds to 1×10^5 monocytes. Results were expressed as ng of C1-INH or effective molecules of C2 per μg of DNA,

Inhibition of protein synthesis by cycloheximide

Nine monolayers were cultured in RPMI-FCS for 5 days, during which time the culture medium was sampled at regular intervals. On day 5 the entire culture supernatants were removed and normal RPMI-FCS added to three monolayers. RPMI-FCS containing cycloheximide (1.0 $\mu\text{g}/\text{ml}$ and 0.5 $\mu\text{g}/\text{ml}$) was added to the remaining six monolayers. The culture was continued for a further 5 days, with regular sampling of the supernatants, and on day 10 the culture medium was removed completely from all the monolayers. After washing of the monolayers with warm RPMI-FCS, fresh RPMI-FCS not containing cycloheximide was added to the cultures. The culture was continued with samples being taken on alternate days for a further 6 days. The culture was then terminated as described above.

Internal labelling of monocyte C $\bar{\text{T}}$ -INH

Monocyte monolayers were cultured in RPMI-FCS for 5 days. The supernatants were then removed, the monolayers washed with methionine-free MEM and then cultured for a further 3 h in methionine-free medium to which [^{35}S]methionine (500 $\mu\text{Ci}/\text{ml}$) had been added. The supernatants were removed and centrifuged to remove non-adherent cells, whereas the monolayer was washed in warm RPMI and then lysed in Tris buffer (50 $\text{mmol}\cdot\text{l}^{-1}$, pH 7.4) containing NaCl (50 $\text{mmol}\cdot\text{l}^{-1}$), KCl (100 $\text{mmol}\cdot\text{l}^{-1}$), Triton X-100 (1.0%, v/v), sodium deoxycholate (0.5%, w/v), PMSF (0.5 $\text{mmol}\cdot\text{l}^{-1}$) and EDTA (10 $\text{mmol}\cdot\text{l}^{-1}$). The lysate was centrifuged to remove particulate material. The supernatants and lysates were then incubated for 1 h at room temperature in the presence of anti-C $\bar{\text{T}}$ -INH (5 μl of antiserum diluted 1:10 for each 1 ml of supernatant or lysate). C $\bar{\text{T}}$ -INH-anti-C $\bar{\text{T}}$ -INH complexes were precipitated with protein A-Sepharose [20 μl of a 10% (w/v) suspension of beads/ml of supernatant or lysate] during a 30 min incubation at room temperature.

After centrifugation the Sepharose beads were washed four times in PBS containing Triton X-100 (1%, v/v), and then the complexes were eluted by the addition of 50 μl of Tris buffer (63.8 $\text{mmol}\cdot\text{l}^{-1}$, pH 6.8) containing SDS (3%, w/v), 2-mercaptoethanol (5%, v/v) and glycerol (10%, v/v). The eluate was boiled for 2 min, and then subjected to SDS/polyacrylamide-gel electrophoresis in a 7.5% (w/v) polyacrylamide slab gel (Laemmli, 1970), after which the gels were analysed by fluorography (Laskey & Mills, 1975).

Radioimmunoassay for C $\bar{\text{T}}$ -INH

Purified C $\bar{\text{T}}$ -INH was radiolabelled with ^{125}I by using the Bolton-Hunter reagent. The specific

radioactivity of different preparations of ^{125}I -C $\bar{\text{T}}$ -INH ranged from 24 to 32 $\mu\text{Ci}/\mu\text{g}$ of protein. Concentrations of C $\bar{\text{T}}$ -INH in monocyte culture supernatants were monitored by using a double-antibody radioimmunoassay technique (Morgan & Lazarow, 1963). A portion (100 μl) of supernatant was incubated with 100 μl of rabbit anti-C $\bar{\text{T}}$ -INH (diluted 1:4000) in PBS-BSA, 100 μl of ^{125}I -C $\bar{\text{T}}$ -INH (5 ng/ml) and 400 μl of PBS-BSA overnight at 2°C. Antibody-bound ^{125}I -C $\bar{\text{T}}$ -INH was precipitated by the addition of 100 μl of normal rabbit serum (diluted 1:55) in PBS-BSA. After a further overnight incubation at 2°C the tubes were centrifuged [2000 rev./min (1000g) for 30 min at 2°C] and the supernatants aspirated. The radioactivity in the precipitates was counted by using an automatic counter (Packard 800C). A standard curve was constructed with unlabelled purified C $\bar{\text{T}}$ -INH at concentrations ranging from 1 to 320 ng/ml. The assay was sufficiently sensitive to detect 100 pg of C $\bar{\text{T}}$ -INH in 100 μl samples of culture fluid. RPMI-FCS did not influence the assay.

Functional activity of monocyte C $\bar{\text{T}}$ -INH

Monolayer cultures were maintained in RPMI-FCS for several days, after which the supernatants were removed, the monolayers washed in RPMI 1640 and the culture continued for a further 3 days in Ham's F12 medium [supplemented with L-glutamine (2 $\text{mmol}\cdot\text{l}^{-1}$)] in the absence of serum. The culture supernatants were then harvested, centrifuged to remove cellular debris, concentrated 25-fold with an Amicon flow cell and then stored at -70°C until used.

C $\bar{\text{T}}$ -INH activity in concentrated supernatants was assayed by its ability to inhibit fluid-phase C $\bar{\text{T}}$ (Gigli *et al.*, 1968). A 100 μl portion of culture supernatant was incubated with guinea-pig C $\bar{\text{T}}$ (10^7 effective molecules in 100 μl of DGVB $^{2+}$ for 30 min at 30°C. A 100 μl portion of EAC4 ($1 \times 10^8/\text{ml}$ in DGVB $^{2+}$) was added, and the incubation continued for a further 30 min at 30°C. Human C2 (50 units/ml in DGVB $^{2+}$) was added to the resultant EAC14, and after a further incubation period of 2.5 min [T-max time, the time taken for maximum formation of C42 sites (Rapp & Borsos, 1970)] at 30°C, the C3 convertase sites were developed by the addition of 300 μl of C $^{\text{rat}}$ -EDTA. After 1 h at 37°C, 2 ml of 0.15 M-NaCl were added to the tubes, which were then centrifuged to pellet unlysed cells. The degree of lysis was measured spectrophotometrically at 414 nm. Controls included a cell blank consisting of 600 μl of DGVB $^{2+}$ and 100 μl of EAC4, a reagent blank (RB below) comprising of 100 μl of EAC4, 100 μl of C2, 300 μl of C $^{\text{rat}}$ -EDTA and 200 μl of DGVB $^{2+}$, 100%-lysis tube containing 100 μl of C2, 100 μl of DGVB $^{2+}$, 100 μl of EAC4, 100 μl of C2 and 300 μl of C $^{\text{rat}}$.

EDTA, a solo tube containing C $\bar{I}$ (but no C $\bar{I}$ -INH), 100 μ l of DGVB $^{2+}$, 100 μ l of C1, 100 μ l of EAC4, 100 μ l of C2 and 300 μ l of C $\bar{I}$ ^{rat}-EDTA, and a complement-colour control containing 100 μ l of concentrated Ham's F12, 300 μ l of DGVB $^{2+}$ and 300 μ l of C $\bar{I}$ ^{rat}-EDTA. At the end of the incubation period, 2 ml of iso-osmotic saline was added to all the control tubes except the 100%-lysis tube, to which 2 ml of water were added.

The percentage lysis in the solo tube and the tubes under assay was calculated as follows:

$$\text{Percentage lysis} = \frac{A_{\text{Test sample}} - A_{\text{RB}}}{A_{\text{100\%-lysis tube}} - A_{\text{RB}}} \times 100$$

Inhibition of haemolysis due to C $\bar{I}$ -INH activity was expressed in Z' units (Gigli *et al.*, 1968), where:

$$Z' = \ln \frac{A_{\text{C}\bar{\text{I}} \text{ inhibited}}}{A_{\text{C}\bar{\text{I}} \text{ solo}}}$$

C2 concentrations in culture fluid were measured by stoichiometric haemolytic assay (Rapp & Borsos, 1970).

Results

Kinetics of C $\bar{I}$ -INH and C2 production

The concentration of C $\bar{I}$ -INH in monocyte culture supernatants increased linearly from day 0 to day 14 (Fig. 1). By contrast, an increase in the concentration of C2 was linear between days 0 and 3, after which time the rate of increase declined, and by day 7 the concentration of C2 remained steady (Fig. 1). This plateau was not seen when the entire culture supernatant was changed on alternate days (Fig. 1).

When ^{125}I -C $\bar{I}$ -INH was added to monocyte cultures on day 7, the rate of loss of immunoprecipitable activity was no greater than that observed when added to fresh RPMI-FCS (percentage decrease 4 and 8%/24h respectively). When functionally purified C2 was added to monocytes on day 7, the loss of C2 functional activity was not significantly different from that observed when C2 was added to fresh RPMI-FCS (percentage decrease in C2 activity 8 and 5%/24h respectively).

As day-7 culture fluid and purified C $\bar{I}$ -INH in RPMI-FCS at the concentration found in day-7 cultures did not decrease the amount of C2 in a standard haemolytic assay, it is unlikely that the failure of C2 levels to increase after day 7 is due to the presence of C $\bar{I}$ -INH.

In three separate cultures from different individuals the mean production rate for C $\bar{I}$ -INH over an 8-day period was 8312 pg/ μ g of DNA. Assuming an M_r of 150000 for C $\bar{I}$ -INH (Harpel &

Fig. 1. Cumulative (----) and continuous (—) synthesis of (a) C $\bar{I}$ -INH and (b) C2

Human monocytes were cultured as described in RPMI-FCS at a concentration of 5×10^5 cells/ml. For the cumulative-synthesis experiments, the entire supernatants was replaced with fresh RPMI-FCS, whereas for continuous synthesis only 200 μ l samples of the supernatant were removed and replaced with RPMI-FCS every 2–3 days. C $\bar{I}$ -INH was measured by radioimmunoassay and C2 by standard haemolytic assay. Each point represents the mean value $\pm$ S.E.M. for triplicate cultures.

Cooper, 1975), these results indicate that the production rate for C $\bar{I}$ -INH is 596×10^7 molecules/24h per μ g of DNA. As 1 μ g of DNA is equivalent to 10^5 cells (Littman & Ruddy, 1977), our results indicate that 60282 molecules of C $\bar{I}$ -INH are produced by each monocyte every 24h.

Inhibition of C $\bar{I}$ -INH and C2 synthesis by cycloheximide

The addition to cycloheximide to cultures inhibited the production of C $\bar{I}$ -INH (Fig. 2). After the replacement of cycloheximide-containing cul-

Fig. 2. Inhibition of C $\bar{\text{I}}$ -INH synthesis by cycloheximide

Human monocytes were cultured in RPMI-PCS for 5 days before the supernatant was replaced with fresh RPMI-FCS alone (○) or RPMI-FCS containing cycloheximide at a concentration of 0.5 $\mu\text{g}/\text{ml}$ (△) or 1 $\mu\text{g}/\text{ml}$ (□). On day 10 the culture medium was removed completely from all the monolayers and replaced with fresh RPMI-FCS not containing cycloheximide. The cultures were continued for a further 7 days before being terminated. Each point represents the mean value $\pm$ S.E.M. for triplicate cultures.

ture medium by fresh RPMI-FCS, synthesis re-
 cured.

[^{35}S]Methionine labelling of C $\bar{\text{I}}$ -INH

The major band precipitated with anti-C $\bar{\text{I}}$ -INH antiserum from culture supernatants had an M_r of 105000, which is the same as that of purified C $\bar{\text{I}}$ -INH, whereas the major band in the lysates corresponded to M_r of 83000 (Fig. 3). The minor bands were present in control immunoprecipitates.

Functional activity of monocyte C $\bar{\text{I}}$ -INH

C $\bar{\text{I}}$ -inhibitory activity could be detected in the concentrated cycloheximide-treated monocyte culture supernatants, although it was less than that found in the corresponding untreated control supernatants (Fig. 4). In two separate experiments the functional activity of C $\bar{\text{I}}$ -INH was 83 and 94 Z' units in the cycloheximide-treated cultures. The concentrations of C $\bar{\text{I}}$ -INH protein in these cultures were 5.7 and 4.6 $\mu\text{g}/\text{ml}$ and 85 and 102 ng/ml respectively. The high levels of C $\bar{\text{I}}$ -inhibitory material in cycloheximide-treated cultures suggest that a second inhibitory factor is released from cultured monocytes. By using simultaneous equations, the specific functional activity (units/ng of protein) of

Fig. 3. SDS/polyacrylamide gels of immunoprecipitates of [^{35}S]methionine-pulsed monocyte culture supernatants and lysates. A (lysates) and C (supernatants) represent immunoprecipitates produced by rabbit anti-C $\bar{\text{I}}$ -INH, and B (lysates) and D (supernatants) represent control immunoprecipitates produced by rabbit anti-ovalbumin. The patterns of the M_r standards are shown on the right of each pair of gels.

Fig. 4. Measurement of the functional activity of C1-INH by a fluid-phase haemolytic assay in the concentrated supernatants of cycloheximide-treated ($\mu\text{g/ml}$) cultures. Line (a) represents the results of control cultures, whereas line (b) shows the results obtained with cycloheximide-treated cultures. Concentrated culture medium alone ($\bullet$) and concentrated medium containing cycloheximide ($\circ$) did not have significant C1-inhibitory activity. The vertical bars on the right represent the concentrations of C1-INH protein measured by radioimmunoassay in (a) the control and (b) the cycloheximide-treated cultures.

monocyte C1-INH was calculated to be 1.14 and 0.74 in two separate experiments, whereas that of purified plasma C1-INH was 0.45.

Discussion

In the present paper we have shown that cultured human monocyte synthesize C1-INH. The evidence for synthesis is (1) the accumulation of C1-INH in culture supernatants with increasing duration of culture (Fig. 1), (2) appearance of C1-INH in culture fluid, which is inhibited reversibly by cycloheximide (Fig. 2), and (3) incorporation of [^{35}S]methionine into C1-INH (Fig. 3).

The pattern of accumulation of C1-INH in monocyte culture supernatants differed from that of C2 (Fig. 1). After a period of 5–7 days, the level of C2 did not increase further, whereas the concentration of C1-INH continued to rise. The finding that the rates of loss of ^{125}I -C1-INH and C2 from the culture supernatants were the same as their rates when incubated in RPMI-FCS indicates that increased degradation of C2 does not account for this plateau, and that decreased synthesis of C2 must occur. As C1-INH synthesis is not decreased, as witnessed by the continued accumulation of this protein, this effect must be specific for C2. By using guinea-pig macrophages it has been shown that C4 synthesis is inhibited specifically by extra-

cellular C4, but inhibition of C2 synthesis was not produced by extracellular C2 (Auerbach *et al.*, 1984). Thus our findings on human monocytes are at variance with observations made on guinea-pig macrophages, although species differences could explain this discrepancy.

[^{35}S]Methionine was incorporated into material that could be precipitated from monocyte lysates and supernatants by using antiserum to C1-INH. The M_r of intracellular monocyte C1-INH (83000) was lower than the M_r of the secreted form in the culture supernatant (105000). The intracellular molecule probably represents the precursor form of secreted C1-INH (pro-C1-INH). The lower M_r suggests that pro-C1-INH has not been glycosylated. The observation that the M_r of human plasma C1-INH falls to 79000 once the carbohydrate has been removed (Harrison, 1983) supports this conclusion. The M_r of the secreted form of C1-INH (105000) is identical with that of human plasma C1-INH (Harpel & Cooper, 1975).

C1-INH produced by the monocytes appeared to be functionally active. Although the concentrated supernatants from the cycloheximide-treated cultures also exerted some inhibitory C1 activity on the haemolytic assay, this was unrelated to the concentrated culture medium or culture medium containing cycloheximide. The specific functional activity of monocyte C1-INH was higher than that of purified C1-INH, probably because of some loss of activity occurred during the process of purification from plasma. However, it was comparable with the lower range of normal values (mean specific functional activity 1.76 units/ng of C1-INH) previously reported (Gigli *et al.*, 1968).

In summary, our experiments show clearly that human monocytes in culture synthesize C1-INH. In a recent paper it was reported that human monocytes only synthesize C1-INH when they have been activated by lymphokines (Bensa *et al.*, 1983); this finding disagrees with the results of our experiments, which show that monocytes which have not been activated can synthesize C1-INH in quantities in excess of those reported by these workers. Differences in the techniques used for the isolation and culture of monocytes could account for this discrepancy.

The ability of monocytes to synthesize C1-INH in culture could provide an important tool for studies of the control of the synthesis of this protein, and the molecular basis of C1-INH deficiency and its treatment with anabolic steroids.

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