Complement subcomponent C1q secreted by cultured human monocytes has subunit structure identical with that of serum C1q

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An enzyme-linked immunosorbent assay (e.l.i.s.a.) that is capable of quantifying C1q concentrations as low as 2 ng/ml and a sensitive haemolytic assay were used to study the appearance of material that cross-reacts with human serum C1q as well as C1q haemolytic activity in human monocyte culture media. This material was detected in the medium after 10–14 days and continued to be secreted through to day 28 of culture, at which time the cultures were terminated. Material specifically immunoabsorbed with Sepharose–anti-C1q antibody from a culture medium of cells that was metabolically labelled with [³H]proline or [³⁵S]methionine demonstrated a polypeptide pattern identical with that of serum C1q on SDS/polyacrylamide-gel electrophoresis. Under non-reducing conditions two protein bands were detected migrating with the same R_F values as the serum C1q A–B and C–C dimers. On reduction three bands were evident, which migrated identically with the A, B and C chains of serum C1q. The amount of radioactivity in these bands increased with time in culture, consistent with the e.l.i.s.a. and haemolytic C1q assays. These bands were reactive with monospecific anti-C1q antibody after transfer to nitrocellulose.

INTRODUCTION

The multi-faceted role of the serum complement system in effecting host defence, regulating immune responses and contributing to immunopathological events is becoming increasingly apparent (Hugli & Müller-Eberhard, 1978; Cooper & Nemerow, 1983; Brown *et al.*, 1983; Meuth *et al.*, 1983). The occurrence of genetic polymorphism and selective deficiencies of the individual protein components of the complement system has led to the study of the biosynthesis of these components at the protein (Whaley, 1980; Fey & Colten, 1981; Morris *et al.*, 1982) as well as the nucleic acid level (Whitehead *et al.*, 1983; DiScipio *et al.*, 1984). Although the liver has been shown to be the primary site of synthesis for several complement proteins, namely C3, C6, C8 and Factor B (Alpers & Nathan, 1981), components of both the classical and the alternative pathway are synthesized by cells of the human monocyte/macrophage lineage (Whaley, 1980; Colten, 1982; Bensa *et al.*, 1983). Thus these cells have the potential for generating most of the known biological effects of complement, independent of the blood protein source.

C1q is the recognition subunit of C1, the first component of the classical complement system, in that it binds to the Fc region of antibody in immune complexes and to other substances that activate C1 (Ziccardi, 1983). In addition, C1q has been shown to bind to specific receptors on human peripheral-blood leucocytes (Sobel & Bokisch, 1974; Gabay *et al.*, 1979; Tenner & Cooper, 1980, 1981), Raji cells (Ghebrehwet & Müller-Eberhard, 1978; Ghebrehwet & Hamburger, 1982) and selected human fibroblast lines (Bordin *et al.*, 1983, 1984),

triggering cellular responses (Ghebrehwet & Müller-Eberhard, 1978; Korotzer *et al.*, 1980; Tenner & Cooper, 1982). No significant synthesis of C1 has been detected in isolated human liver tissue (Colten *et al.*, 1968), and, although C1r and C1s were detected in the media of HepG2, a human hepatoma-derived cell line, no C1q haemolytic activity could be demonstrated (Morris *et al.*, 1982). C1q has been shown to be synthesized in human intestine (Colten *et al.*, 1968), in cultured human epithelial and mesenchymal cells (Morris *et al.*, 1978a,b) and in human fibroblast cell cultures (Reid & Solomon, 1977). Morris *et al.* (1978a) reported the secretion of C1 haemolytic activity by human monocytes in culture, and subsequently Bensa *et al.* (1983), using immunochemical techniques, have demonstrated C1q secreted by human monocyte-derived cells in culture. Both human and guinea-pig macrophages have also been shown to secrete haemolytically active C1q (Muller *et al.*, 1978).

The present paper presents results from studies in which immunochemical C1q and C1q haemolytic activity secreted by cultured human monocytes were simultaneously quantified. In addition, analysis by SDS/polyacrylamide-gel electrophoresis demonstrated that, in contrast with the C1q secreted by human fibroblasts, the C1q-like material secreted by human monocyte-derived cells in culture is very similar to, if not identical with, serum C1q in subunit structure.

MATERIALS AND METHODS

Purification and radiolabelling of human C1q

C1q was isolated from human serum or plasma by the method of Tenner *et al.* (1981) and radiolabelled with

Abbreviations used: e.l.i.s.a., enzyme-linked immunosorbent assay; PBS, phosphate-buffered saline (0.15 M-NaCl/0.01 M-sodium phosphate buffer, pH 7.4).

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Na¹²⁵I (Amersham International, Arlington Heights, IL, U.S.A.) or NaB³H₄ (New England Nuclear, Chicago, IL, U.S.A.) as described (Tenner *et al.*, 1981). The borol³H hydride method predominantly labels the A chain of C1q.

C1q immunochemical and haemolytic assays

An e.l.i.s.a. was developed and optimized to detect human C1q with the use of a multivalent affinity-purified goat anti-(human C1q) antibody for both coating the plates and detecting bound C1q. The developing antibody was biotinylated with the Enzotest Kit (Enzo Biochem, New York, NY, U.S.A.) in accordance with the manufacturers' instructions. Glucose oxidase/avidin D (Vector Laboratories, Burlington, CA, U.S.A.) was the enzyme system used for colorimetric development (Johnson & Nakamura, 1980). Briefly, affinity-purified goat anti-C1q antibody, at 5 µg/ml and 100 µl per well, was dried down overnight in Immulon II plates (Dynatech, Alexandria, VA, U.S.A.). After a 2 h incubation with RPMI 1640 medium (Microbiological Associates Bioproducts, Walkersville, MD, U.S.A.) containing 0.5% bovine serum albumin (Sigma Chemical Co., St. Louis, MO, U.S.A.) at 37 °C, the plates were washed with PBS medium containing 0.05% Tween 20, and 100 µl of either medium or antigen sample was added and incubated at room temperature for 3 h. The assay was developed by sequential incubation with 100 µl of biotinylated anti-C1q antibody in PBS/Tween for 2 h, 100 µl of glucose oxidase/avidin in PBS containing 1% bovine serum albumin for 30 min, and 200 µl of 2,2'-azinobis-(3-ethylbenzthiazolinesulphonic acid) (Boehringer-Mannheim Biochemicals, Indianapolis, IN, U.S.A.) (Johnston & Nakamura, 1980). The plates were washed between each incubation step. The change in absorbance at 405 nm was determined at various times, and the concentration of C1q in the original sample was calculated from the standard curve that was run with each assay with the use of purified human serum C1q. Values for purified C1q agreed with those determined in normal human serum. The standard curve was linear between 2 and 100 ng of C1q/ml. The haemolytic activity of C1q was determined by the method of Kolb *et al.* (1979). When assaying culture media, 10 µl, 25 µl and 50 µl samples were assayed; a Z value was determined and converted into ng/ml of C1q, with C1q purified from human serum being used as a standard.

Monocyte isolation and culture

Blood was drawn from normal volunteers into sterile EDTA at a final concentration of 20 mM. A 30 ml sample of blood was layered directly over 15 ml of Ficoll-Hypaque ($\rho = 1.09$ g/ml) and spun at 2000 rev./min (Centra-7R centrifuge; IEC, Needham Heights, MA, U.S.A.) for 30 min at room temperature. Alternatively, 30 ml of blood diluted 1:1 with sterile 0.9% NaCl was layered over 15 ml of Lymphopaque (Accurate Chemical Scientific Corp., Westbury, NY U.S.A.) and spun at 1400 rev./min (Centra-7R centrifuge) for 40 min at room temperature. The mononuclear-cell layer at the interface was removed; the platelets were removed by washing five times with a mixture of RPMI 1640 medium and EDTA (with the EDTA in decreasing concentrations of 16 mM, 12 mM, 8 mM and 4 mM) and spinning at 800 rev./min (Centra-7R centrifuge) for 10 min at room temperature. The resultant peripheral-blood mononuclear leucocytes

were washed once with RPMI 1640 medium in the absence of EDTA and resuspended in RPMI 1640 medium containing 0.2% heated (56 °C for 30 min) serum. [In several initial trials, long-term survival of the monocytes cultured in 10% (v/v) foetal-calf serum depended on the presence of 0.2% autologous serum during the adherence step of monocyte preparation rather than the same percentage of foetal-calf serum. Heterologous human serum was not tested.] A 1 ml portion containing 2×10^6 cells (unless otherwise noted) was added per 16 mm-diameter well in a 24-well dish (Costar, Cambridge, MA, U.S.A.). After incubation at 37 °C for 1 h, non-adherent cells were removed by vigorously pipetting the medium. Then 0.5 ml of RPMI 1640 medium containing 20 mM-Hepes buffer, antibiotic (kanamycin or gentamycin at appropriate concentration), 2 mM-L-glutamine, and 10% heated (56 °C for 30 min) foetal-calf serum was immediately added to the monocytes, and the plates were placed at 37 °C in an air/CO₂ (19:1) incubator.

In some experiments 10% autologous serum (heated at 56 °C for 2 h to eliminate all C1q antigen detectable by our e.l.i.s.a.) was used instead of foetal-calf serum. Experience showed that changing the medium every 3.5 days resulted in more stable cultures and higher levels of synthesis.

Culture conditions for radiolabelling cells

Cells were metabolically labelled in RPMI 1640 medium lacking either methionine or proline/hydroxyproline (RPMI 1640 Select-Amine kit; Gibco, Grand Island, NY, U.S.A.) containing 20 mM-Hepes buffer, antibiotic, 1% heated (56 °C for 30 min) foetal-calf serum, 10 µg of ascorbic acid/ml and either 25 µCi of L-[3,4(n)-³H]proline (Amersham International)/ml or 75 µCi of L-[³⁵S]methionine (New England Nuclear)/ml. The media and the cells were collected separately after 48 h. Preliminary experiments demonstrated no difference in the amounts of C1q synthesized in a 2-day period in the presence of 1% foetal-calf serum or of 10% foetal-calf serum at any time between day 12 and day 28 of culture.

Lysis of cells with SDS and Triton

At specified time intervals the medium was removed from the cells, centrifuged for 3 min in a Serofuge, transferred to a new tube and stored at -70 °C. The cells were then washed twice with 1 ml of sterile PBS per well. Next, the cells were lysed with either SDS or Triton. For such lysis, 0.5 ml of 0.1% SDS in PBS was added per well. After removal of the lysing reagent the wells were washed twice with 0.5 ml of PBS, giving a final sample of 1.5 ml of 0.03% SDS in PBS. The samples were frozen immediately at -70 °C. Alternatively, 0.5 ml of 0.05% Triton in PBS was added to each well. The well was washed once with the same reagent, producing a final 1 ml sample of 0.05% Triton in PBS. These samples were also frozen immediately at -70 °C. In some experiments 50 mM-iodoacetamide plus the proteinase inhibitors 1 mM-phenylmethanesulphonyl fluoride, 10 mM-EDTA and 60 mM-6-amino-n-hexanoic acid were added to the lysing reagents immediately before use (Kulczycki *et al.*, 1981).

Immunoprecipitation and preparation of gel samples

Affinity-purified goat anti-(human C1q) antibody was covalently linked to CNBr-activated Sepharose (Pharmacia, Uppsala, Sweden). A 25 µl portion of 50%

Sephacrose-anti-C1q antibody was found to precipitate at least 2 μg of C1q.

A 1 ml portion of medium or cell lysates was rotated at 4 °C for 18 h with 25 μl of 50% Sepharose-anti-C1q antibody beads. Addition of 50 mM-iodoacetamide, 10 mM-EDTA and 60 mM-6-aminohexanoic acid decreased the amount of breakdown of C1q during the immunoprecipitation. Sepharose-anti-C1q antibody beads were pelleted [200 rev./min (Centra-7R centrifuge), 5 min] and washed three times in PBS containing 0.05% Tween 20. After the final wash, 25 μl of Weber-Osborn sample buffer (Weber & Osborn, 1969) was added. After incubation at 37 °C for 90 min with intermittent mixing, the beads were pelleted by centrifugation for 5 min in a Beckman Microfuge (4 °C), and the sample buffer was removed. Glycerol (5 μl) and Bromophenol Blue solution (1 μl) were added before electrophoresis.

Gel electrophoresis and fluorography

The medium and the cell-lysate samples were electrophoresed with the use of the Canelco gel system, a modification of the method of Laemmli (1970), employing a 5% acrylamide/1.25% bisacrylamide stacking gel with a pH 8.9 Tris buffer. The resolving gel was a 5–15% acrylamide/0.14–0.41% bisacrylamide linear gradient

Fig. 1. C1q secretion is enhanced when monocytes are cultured in the presence of autologous serum

C1q secretion assayed by e.l.i.s.a. (a) and total acid phosphatase activity (b) were determined at 7-day intervals in the presence of foetal-calf serum (○—○) or autologous serum (●—●). Total acid phosphatase activity was derived from the sum of the media and Triton-cell-lysate activities. The percentage of total activity that was in the cell lysate was similar under both culture conditions.

Fig. 2. Correlation of C1q and C2 appearance in monocyte culture media

Monocyte cultures were prepared and maintained as described in the Materials and methods section. The medium was changed every 3.5 days. Samples (100 μl) of culture medium were assayed for C2 (●—●) by haemolytic titre with the use of EAC14 cells and for C1q (○—○) by e.l.i.s.a. All points are averages of duplicates.

with the same buffer. SDS (0.1%) was incorporated in the upper-chamber buffer only.

A miniaturized gel system was used with a total resolving gel volume of 6 ml (4.5 cm $\times$ 8.5 cm $\times$ 0.2 cm). After electrophoresis and staining with Coomassie Blue R250, the gel was impregnated with En³Hance (New England Nuclear), the protocol of the manufacturer being followed. Gels were dried down overnight and placed at -70 °C for fluorography with Kodak X Omat AR film. M_r standards were purchased from Bio-Rad Laboratories (Richmond, CA, U.S.A.) and from Amersham International (¹⁴C-labelled protein mixture).

Western-blot analysis

A modification of the Towbin *et al.* (1979) method for electrophoretic transfer to nitrocellulose described by Curtiss & Edgington (1982) was used. Rabbit anti-C1q antibody (Calbiochem, Behring, La Jolla, CA, U.S.A.) followed by ¹²⁵I-labelled-sheep anti-(rabbit IgG) antibody (0.5 $\mu\text{Ci}/\text{ml}$) was employed as the detection system.

Enzyme and protein assays

C2 haemolytic activity was determined with the use of EAC14 cells, as described by Cooper *et al.* (1970). Previously published procedures were used to assay acid phosphatase and β -glucuronidase (Musson *et al.*, 1980), lactate dehydrogenase (Kornberg, 1955) and 5'-nucleotidase (Avruch & Wallach, 1971). Protein was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as a standard.

RESULTS

Biosynthesis of C1q by cultured human monocytes

Immunochemically and haemolytically active C1q was detected in the media of human monocyte cultures only after 7 days of *in vitro*. Out of a total of 19 experiments, C1q was first detected in the culture medium after 7 days in three of the experiments, 10–14 days in 12 experiments, and 17–21 days in four experiments. In all cases in which

Table 1. C1q production by monocytes cultured in the presence of autologous serum or of foetal-calf serum

Experimental details are given in the text. Values reported are from day-21 cultures and represent total C1q secreted between day 18 and day 21 of culture. Abbreviation: N.D., not determined.

Expt. no.	C1q (ng/ml)			
	E.l.i.s.a.		Haemolytic titre	
	Autologous serum	Foetal-calf serum	Autologous serum	Foetal-calf serum
1	152	56	53	14
2	30	18	N.D.	N.D.
3	41	5	28.5	0
4	590	340	265	60

acid phosphatase and β -glucuronidase activities were assayed ($n = 10$), C1q synthesis temporally lagged behind the increase in cellular lysosomal enzyme content. A representative experiment demonstrating these points is presented in Fig. 1. The appearance of C2 haemolytic activity in these cultures also preceded that of C1q (Fig. 2). Interestingly, in nearly all experiments, regardless of the culture conditions, the amount of C1q present as determined by haemolytic titration was less than that detected by our e.l.i.s.a. (for example, see Table 1). Since both assays were standardized with a purified human serum C1q having essentially 100% haemolytic activity, the explanation for this disparity is unknown at this time. Use of 10% foetal-calf serum did not affect the measurements of C1q haemolytic activity.

The C1q haemolytic activity and the e.l.i.s.a. reactivity were both inhibited in a dose-dependent manner by the inclusion of 2,2'-bipyridyl at 1–100 μ M in the culture

medium, with 95–100% inhibition at 100 μ M drug. Inhibition of secreted C1q haemolytic activity by guinea-pig and human peritoneal macrophages has similarly been reported by Muller *et al.* (1978). F(ab')₂ of anti-C1q antibody also blocked these reactivities. Heating at 56 °C for 30 min inhibited all haemolytic activity and over 99% of the e.l.i.s.a. reactivity in all samples tested throughout the culture period. Heating at 56 °C for 2 h destroyed all residual C1q activity detected by this e.l.i.s.a. assay (detailed results not shown).

Absolute concentrations of C1q produced by monocytes cultured varied more than 14-fold in experiments in which different donors and various culture conditions were used. However, in four separate experiments, ascorbic acid (a cofactor for proline hydroxylase) at 2–50 μ g/ml enhanced the amount of C1q produced by monocytes, as assayed by both e.l.i.s.a. and haemolytic titration (Fig. 3). This increase was seen whether the data were expressed

Fig. 3. Ascorbic acid enhancement of monocyte C1q secretion

Monocyte cultures were prepared and maintained as described in the Materials and methods section. Ascorbic acid was present throughout the culture period: (a) none (control); (b) 2 μ g/ml; (c) 10 μ g/ml; (d) 50 μ g/ml. Media were changed every 3.5 days. ●—●, E.l.i.s.a. values; ●---●, results from C1q haemolytic assays.

Fig. 4. Electrophoresis of monocyte culture media immunoabsorbed on Sepharose-anti-C1q antibody

Monocytes were cultured as described in the text except that all culture media contained $10 \mu\text{g}$ of ascorbic acid/ml. At 14, 22 and 27 days of culture, medium containing [^3H]proline was added as described in the Materials and methods section. After 48 h the medium was removed and stored at -70°C . For each gel sample, 1 ml of medium was immunoabsorbed with affinity-purified goat anti-C1q antibody coupled to Sepharose. The beads were stripped with electrophoresis sample buffer and electrophoresed in a 5–15% polyacrylamide-gel gradient with a 5% polyacrylamide stacking gel. The gels were processed for fluorography as described in the Materials and methods section. ^{14}C -labelled standards (lane E) are from Amersham International. C1q concentrations determined by e.l.i.s.a.: days 14–16 (lanes B and F), 25 ng/ml; days 22–24 (lanes C and G), 103 ng/ml; days 27–29 (lanes D and H), 190 ng/ml. Lanes A and I contain purified human C1q radiolabelled with [^3H]borohydride (i.e. A chain detectable only). Lanes F–I are non-reduced, and lanes A–E are reduced [dithiothreitol (DTT) present].

as the absolute concentration of C1q in the medium or as the amount of C1q produced per μg of cell protein per 24 h. Thus the hydroxylation of proline residues may be a limiting factor in the secretion of C1q (see the Discussion section). Furthermore, cells cultured in the presence of autologous serum secreted more C1q (1.7–8.2 times more antigen and over 4 times more haemolytic activity) throughout the culture period than the cells of identical origin cultured in the presence of foetal-calf serum ($n = 7$) (Fig. 1a and Table 1). The concentration of C1q in the medium of day-21 cultures, determined by both e.l.i.s.a. and haemolytic titre from four such experiments, is presented in Table 1.

Polypeptide chain structure

Affinity-purified goat anti-C1q antibody coupled to CNBr-activated Sepharose 4B was used to immunoabsorb culture supernatants after the cultures had been incubated with either [^3H]proline or [^{35}S]methionine for 48 h. On electrophoresis under non-reducing conditions, two major bands (of M_r 54 000 and 40 000) were absorbed from culture medium labelled with [^3H]proline, as shown in the fluorographs in Fig. 4. The position of the larger band coincided with that the A–B dimer of authentic ^3H -labelled serum C1q (Fig. 4), and the smaller band migrated with the R_F of the serum C1q C–C dimer (Table 2). As is characteristic of native serum C1q, these two bands disappeared on reduction, and three new bands were seen migrating as polypeptides of M_r 29 000, 27 000 and 22 000, thus behaving identically with the A, B and C polypeptide chains of serum C1q in this polyacrylamide-gel electrophoresis system (Table 2), as detected with Coomassie Blue staining. Fig. 4 also shows that, with

increasing time in culture, the amount of C1q in the medium increases, supporting the data derived from the e.l.i.s.a. and the haemolytic assay. The ^3H -labelled A chain of serum C1q migrated slightly faster than the analogous monocyte chain. Since the unlabelled A chain from serum C1q and the monocyte 'A' chain migrated identically, the difference in migration of the ^3H -labelled A chain may be due to the periodate oxidation of the terminal sialic acid that occurs during this radiolabelling procedure (Van Lenton & Ashwell, 1971).

Fig. 5 shows the specificity of the immunoabsorption. Monocyte cultures labelled with [^3H]proline were immunoabsorbed with Sepharose-anti-C1q antibody, Sepharose-C3, Sepharose-ovalbumin and Sepharose-C1q. The fluorographs of the material eluted from the Sepharose-anti-C1q antibody and electrophoresed under reducing conditions show the prominence of the three bands migrating at the positions of the A, B and C chains of C1q, whereas the other bands that are present, particularly the high- M_r ($> 200\,000$) band, are clearly non-specific, being absorbed by all Sepharose beads used. When similar gels were subjected to Western-blot analysis, only the bands migrating with the R_F of the serum C1q polypeptide chains were recognized by the anti-C1q antibody probe (results not shown). Surprisingly, bands migrating with the same R_F values as those of the A, B and C chains of C1q were also reproducibly immunoabsorbed by Sepharose-C1q beads. Whether the presence of the C1q bound to Sepharose-C1q represents an exchange reaction, a self-association phenomenon or some other mechanism cannot be deduced from these data.

Table 2 presents a summary of the M_r determinations

Table 2. Apparent M_r of human C1q

Subunit	Monocyte C1q	Serum C1q	^3H -labelled serum C1q
A-B	$54\,900 \pm 1800$ ($n = 8$)	$54\,000$ ($n = 2$) (52750*)	$54\,400 \pm 1000$ ($n = 5$)
C-C	$41\,300 \pm 1300$ ($n = 8$)	$41\,000$ ($n = 2$) (47600*)	
A	$29\,000 \pm 1500$ ($n = 8$)	$29\,300 \pm 1200$ ($n = 3$) (27550*)	$26\,100 \pm 1300$ ($n = 8$)
B	$27\,300 \pm 1300$ ($n = 8$)	$26\,300 \pm 500$ ($n = 3$) (25200*)	
C	$21\,500 \pm 1300$ ($n = 8$)	$21\,600 \pm 1300$ ($n = 3$) (23800*)	

* True M_r values from Reid (1983).

Fig. 5. Evidence of specificity of immunoabsorption

Monocyte cultures were established and radiolabelled as described in the Materials and methods section. Portions (1 ml) of medium from cells labelled with [^3H]proline (days 24–26) were immunoabsorbed with Sepharose–anti-C1q antibody (lane B), Sepharose–ovalbumin (lane C) or Sepharose–C1q (lane D), and, in a separate experiment, with Sepharose–anti-C1q antibody (lane G) and Sepharose–C3 (lane H). The beads were stripped, and the samples were electrophoresed as described in the Materials and methods section. The gels were then processed for fluorography as described in the text except that all samples were run under reducing conditions. Lanes A and E contain ^{14}C -labelled standards, and lane F contains ^3H -labelled C1q.

from repeated electrophoresis of material immunoabsorbed by Sepharose–anti-C1q antibody from the media of five different monocyte cultures as well as the unlabelled and tritiated purified serum C1q run in the same system. The M_r value for monocyte C1q was derived from the R_F values of the bands on fluorographs similar to and including those in Figs. 4 and 5, whereas the M_r values for serum C1q were determined from Coomassie Blue staining of the gels. The metabolically

labelled material, which was immunoprecipitated from the monocyte culture medium, migrates with R_F values identical with those of C1q purified from serum.

DISCUSSION

Early observations in which immunochemical techniques had been used suggested the production of C1q by human macrophages (Stecher *et al.*, 1967; Colten, 1976).

Subsequently, Morris *et al.* (1978a) and Muller *et al.* (1978) demonstrated the secretion of C1 haemolytic activity by long-term (more than 4-week) primary cultures of human monocytes and by peritoneal macrophages respectively. More recently, Bensa *et al.* (1983) used radioimmunoassays to investigate quantitatively the synthesis of the subcomponents of C1, C1q, C1r and C1s, in addition to the synthesis of C1 inhibitor by human monocytes in culture under various conditions. By using an immunochemical assay simultaneously with a sensitive haemolytic assay specific for C1q, we were able to make a quantitative comparison of secreted C1q immunochemical reactivity and C1q haemolytic activity with time in culture. The results from both assays suggest that C1q biosynthesis is linked to a differentiation or activation state of the monocyte/macrophage that is different from that defined by amounts of cellular lysosomal enzymes or by the onset of C2 biosynthesis. Interestingly, the C1q activity detected by haemolytic titration was consistently lower than that measured by our e.l.i.s.a. assay, as seen, for example, in Fig. 3 and Table 1. Since no discrepancy between these assays (standardized with purified human C1q) is apparent when serum or purified human serum C1q is assayed, the basis for this disparity is not known. Assays of media to which the proteinase inhibitors 6-amino-hexanoic acid (60 mM), soya-bean trypsin inhibitor (1 mg/ml) or Trasylol (1:100 dilution) were added during culture also indicated that a greater amount of C1q can be detected immunochemically than with the haemolytic assay. One possible explanation is that the degree of hydroxylation of the monocyte-secreted C1q may differ from that of serum C1q (thus affecting its haemolytic activity), as previously suggested for C1q secreted by epithelial and fibroblast cell lines (Morris & Paz, 1980). In material immunoprecipitated with the anti-C1q antibody from culture supernatants of different cell types, SDS/polyacrylamide-gel-electrophoretic analysis has shown that different polypeptide structures are secreted. Morris *et al.* (1978a) demonstrated that C1q synthesized by primary cultures of human intestinal epithelial cells had a polypeptide structure identical with that of serum C1q on SDS/polyacrylamide-gel electrophoresis. In contrast, C1q produced by human fibroblasts were secreted as higher- M_r polypeptides than the C1q isolated from normal serum (Reid & Solomon, 1977; Skok *et al.*, 1981). More recently, Loos *et al.* (1983) reported that C1q purified from culture supernatants of guinea-pig peritoneal macrophages migrated identically with guinea-pig serum C1q on SDS/polyacrylamide-gel electrophoresis. In the work presented here, we have demonstrated that C1q secreted by human monocytes cultured *in vitro* also migrates with R_F values that are identical with those of C1q purified from human serum under both reducing and non-reducing conditions. Thus, though the site of synthesis of serum C1q has yet to be established, these results provide evidence that the monocyte/macrophage can generate haemolytically active C1q that is structurally equivalent to the serum molecule.

Since C1q contains a region of collagen-like amino acid sequence, investigators have looked for similarities in the control of its biosynthesis with that of collagen. Hydroxylation of proline residues of procollagen is required for the secretion of this molecule. The enzyme responsible for this post-translational modification is Fe^{2+} -dependent and also is enhanced by the presence of

ascorbic acid. Muller *et al.* (1978) demonstrated that the appearance of C1q haemolytic activity in culture media of guinea-pig and human peritoneal macrophages is inhibited by 2,2'-bipyridyl, an Fe^{2+} chelator. In addition, Morris & Paz (1980) have reported that ascorbic acid (50 μ g/ml) enhanced C1q haemolytic activity in human fibroblasts and epithelial-cell cultures, concomitant with increased hydroxylation of the proline residues of C1q. Our experiments extend these findings to the cultured human monocyte/macrophage system. Furthermore, we have demonstrated that the secreted amounts of immunochemically detected C1q as well as haemolytic activity were dramatically decreased in the presence of 2,2'-bipyridyl and enhanced by growth in ascorbic acid. These results support the suggestion that the hydroxylation of proline residues in the collagen-like region of C1q may be crucial for C1q secretion and activity, and thus may be a point of regulation for C1q production.

The data presented here suggest that human blood monocytes do not constitutively synthesize C1q. However, upon leaving the circulation, the monocytes differentiate/mature into macrophages, acquiring various properties including the ability to synthesize many of the complement proteins, dictated at least in part by the substances in the particular extravascular environment (Whaley, 1980; Fey & Colten, 1981; Hartung & Hadding, 1983). Presently, the regulation of monocyte/macrophage complement production appears to be as diverse as the functional properties of this cell type. Bensa *et al.* (1983) have presented data suggesting that lymphokines exert an influence on C1q production *in vitro*. In addition to providing the possibility of assembling a local classical complement pathway, C1q synthesized in tissue may interact with pathological substances (in the presence or in the absence of antibody) and, via the C1q receptor on leucocytes (Tenner & Cooper, 1981, 1982; Sobel & Bokisch, 1975), directly mediate host defence. Thus continued investigation of the C1q-biosynthetic process and its regulation should provide new insights on host local defence mechanisms.

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