Biosynthesis *in vitro* of complement subcomponents C1q, C1s and C1 inhibitor by resting and stimulated human monocytes

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(Received 15 April 1983/Accepted 28 July 1983)

The capacity of cultured human monocytes to synthesize and to secrete the subcomponents of C1 and C1 inhibitor was examined. Non-stimulated monocytes secreted C1q and C1s from day 5 of culture. C1s reached a plateau immediately at its maximum level, whereas C1q secretion increased progressively until the end of the second week. Between day 12 and day 25, C1q secretion remained nearly constant (1–15 fmol/day per μg of DNA, depending on the donor), whereas C1s secretion decreased and even in some cases stopped. C1r and C1 inhibitor were not secreted in detectable amounts by these resting cells. Stimulation of monocytes by yeasts, immunoglobulin G-opsonized sheep red blood cells or latex beads did not modify consistently C1q and C1s secretion. Activation by conditioned media from mitogen-, antigen- or allogeneic-stimulated lymphocyte cultures increased C1q production from 2 to 7 times and re-activated C1s secretion. Under the same conditions of activation, C1 inhibitor was secreted (up to 300 fmol/day per μg of DNA) and C1r became detectable in culture supernatants. Isolated human monocytes are thus able to synthesize the whole C1 subcomponents; C1, if assembled, could be protected from non-immunological activation by locally produced C1 inhibitor. Activated monocytes appear to be a good tool for studying the assembly of C1 subcomponents and the role of C1 inhibitor in this process.

Abbreviations used: FCS, heat-inactivated foetal-calf serum; NP40, Nonidet P40; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid; phosphate-buffered saline, 0.145 M-NaCl/0.010 M- Na_2HPO_4 , pH 7.2; MLC, mixed lymphocyte culture; FITC, fluorescein isothiocyanate; IgG, immunoglobulin G; Fc γ , C-terminal half of heavy chain dimer from IgG; F(ab') $_2$, N-terminal halves of heavy chains of Ig joined by inter-heavy-chain disulphide bond to light chains; iPr $_2$ P-F, di-isopropyl phosphorofluoridate; SACI, *Staphylococcus aureus* Cowan 1; LPS, bacterial-derived lipopolysaccharide; PPD, protein-purified derivative (prepared from filtrate of culture of *Mycobacterium tuberculosis*); EA, sheep erythrocyte (E) bearing specific antibodies (A); SDS, sodium dodecyl sulphate. The nomenclature of the complement components and subcomponents is that recommended by the World Health Organisation (1968); activation of a component is indicated by an overbar.

The first component of complement, C1, circulates in plasma as a Ca^{2+} -dependent complex. When Ca^{2+} is removed by chelation with EDTA *in vitro*, C1 dissociates into three distinct proteins: C1q, dimeric C1r and dimeric C1s.

Two different approaches have been used to demonstrate the biosynthesis of C1q and C1. Immunological methods have shown that, in human, C1q is synthesized by various tissues such as liver, spleen, intestine, bone marrow, lung, peritoneal macrophages, lymphoid tissues, leucocytes and fibroblasts. Other studies have demonstrated that tissues from monkey and piglet are also able to

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synthesize C1q (reviewed in Loos, 1982). Production of haemolytically active C1 has been demonstrated in the intestine of man (Colten *et al.*, 1968) and guinea pig (Colten *et al.*, 1966), in cultures of human epithelial and mesenchymal cells (Morris *et al.*, 1978) and in human fibroblast cell line cultures (Reid & Solomon, 1977).

As regards mononuclear phagocytes, long-term primary cultures of human monocytes (Morris *et al.*, 1978) as well as human and guinea-pig macrophages (Müller *et al.*, 1978) have been shown to synthesize haemolytically active C1; with peritoneal macrophages, evidence has been provided that C1 subcomponents are synthesized independently from each other (Loos *et al.*, 1981).

It is the purpose of the present paper to examine the capacity of human macrophages in culture to synthesize C1q, dimeric C1r, dimeric C1s and C1Inh in order to discuss their assembly, leading to the C1 complex and the role of C1Inh in this process. The structural characteristics of purified C1 subcomponents and C1Inh have been studied in detail in our laboratory (Arlaud *et al.*, 1977, 1980a; Villiers *et al.*, 1982a; Reboul *et al.*, 1977). Dimeric C1r appears to play a key role in C1 activation owing to its intrinsic autocatalytic properties, which are directly influenced by companion proteins inside C1, C1q and dimeric C1s (Arlaud *et al.*, 1980b) and also by C1Inh, which recently have been shown to play a new role in controlling C1 activation (Ziccardi, 1982). C1-subcomponent biosynthesis by human monocytes may be considered as an unbalanced contribution to circulating C1, the balance between C1q, C1r and C1s being equilibrated by subcomponents synthesized by cells from other origins. In fact, from recent, data weak interactions between C1q and C1r₂-C1s₂ inside circulating C1 have been postulated (Kilchherr *et al.*, 1982; Siegel & Schumaker, 1982); these interactions allow C1 integrity to be restored *in vivo* from subcomponents of different origins, after activation. For the present study monocytes were chosen, since their basal activity can be modulated and thus allow a comparative dynamic estimate of the synthesis of the above proteins. Also, since the presence of C1q receptors has been described on these cells (Tenner & Cooper, 1980), the possible regulating role of these receptors on C1q activity is discussed.

Materials and methods

Materials

Ficoll-Hypaque, penicillin, streptomycin, fungizone, RPMI 1640 and FCS were purchased from Flow Laboratories. DNA, LPS from *Escherichia coli* E55 B5, *Micrococcus lysodeikticus* and latex beads were obtained from Sigma. Concanavalin

A and Ultrogel AcA 34 were from Industrie Biologique Française; PPD and MDP were from Institut Pasteur. Sheep erythrocytes were obtained from Merieux; sheep erythrocyte anti-serum was from Behring and SACI from Bethesda Research Laboratories. The dye H 33258 was from Hoechst. FITC-labelled anti-C1q was purchased from Behring, and anti-[rabbit F(ab')₂] was purchased from Cappel Laboratories, Courbevoie, France. Lactoperoxidase (purified grade) was from Calbiochem. Na¹²⁵I (sp. radioactivity 2 Ci/μmol) was from Amersham International.

Separation and culture of monocytes

Blood from healthy donors (Centre de Transfusion Sanguine, Grenoble, France) was collected in the presence of 15 units of heparin/ml. Blood (1 vol.) was centrifuged at 150g (*r*_{av.} 14.7cm) for 20 min at 20°C. Platelet-rich plasma was separated and the cells suspended in 2 vol. of phosphate-buffered saline. The mononuclear cells were then isolated by centrifugation at 800g (*r*_{av.} 12.7cm) for 20 min at 20°C on Ficoll-Hypaque, density 1.077g/ml. Three successive washes [resuspension of the cells in ice-cold phosphate-buffered saline, followed by centrifugation at 200g (*r*_{av.} 14.7cm) for 15 min at 4°C] were necessary to eliminate all free platelets. The cells were then suspended in RPMI 1640 supplemented with 20mM-Hepes, 1mM-pyruvate, 100 units of penicillin/ml, 100μg of streptomycin/ml, 2.5μg of fungizone/ml and serum [15% (v/v) heat-inactivated autologous serum or 20% (v/v) FCS]. Mononuclear cells [(2–3) × 10⁶ in 1ml] were layered directly on plastic wells (16mm diameter; Costar plates from Flow Laboratories) or on to 12-mm-diameter glass coverslips. The plates were incubated at 37°C for 1h in a humidified atmosphere of CO₂/air (1:19). The non-adherent cells were removed by three vigorous washings with serum-free medium. More than 95% of the cells were viable as assessed by Trypan Blue exclusion, and 75–90% were able to ingest non-opsonized yeasts.

The monocytes were incubated in RPMI 1640 medium supplemented as described above. When the culture was started in autologous-serum-supplemented medium, this medium was changed at day 4 or 5. At day 8 or 9 the cells, after three washes in serum-free medium, were then cultivated in 20%-FCS-supplemented medium.

Sampling of supernatants and cell lysates

Supernatants were collected from individual wells, centrifuged at 400g (*r*_{av.} 14.7cm) for 10 min and stored at –20°C.

Adherent cells from individual wells were washed once with 1 ml of RPMI 1640 serum-free medium and lysed by incubation at 37°C for 15 min in 800 μl

of NP40 buffer [0.25% (v/v) NP40 in 0.15 M-NaCl/0.5 mM-EDTA/0.02% (w/v) NaN_3 /1% (w/v) ovalbumin/0.05 M-Tris/HCl, pH 7.4]. The cells removed by the washing step were harvested by centrifugation and lysed as above by 200 μl of NP40 buffer. The two lysates were pooled, centrifuged at 10000 g (r_{av} , 5 cm) for 10 min and the supernatants were pooled at -20°C .

Preparation of conditioned media

For primary MLC, 10^7 human mononuclear cells, prepared as described above, were suspended in 10 ml of 20%-FCS-supplemented RPMI 1640 medium containing either 10 μg of concanavalin A/ml or 10 μg of PPD/ml or 2×10^6 Daudi cells previously irradiated with 2500 rad. The cells were then incubated in 25 cm² flasks (Falcon) at 37°C under CO_2 /air (1:19) for 2 days or 6 days for concanavalin A- or PPD- and allogeneic-stimulated lymphocytes respectively. The resulting conditioned media were spun free of cells, filtered on 0.22 μm -pore-size Millipore membranes and frozen at -20°C .

Conditioned media were also obtained from monocyte-depleted lymphocytes, prepared by Sephadex G-10 filtration as described by Ly & Mishell (1974) and stimulated by concanavalin A.

Secondary MLC were obtained from 12-day-old primary MLC. After being washed the lymphocytes were incubated at 37°C for 2 days with fresh irradiated Daudi cells. Supernatants were collected, filtered and stored at -20°C .

DNA determination

DNA was determined in cell lysates by H 33258 fluorescence measured at 456 nm (excitation at 356 nm). A lysis buffer consisting of 2 M-NaCl/0.05 M- Na_3PO_4 /5 mM-EDTA, pH 7.4, was chosen to avoid detergents likely to interfere in the fluorescence assay (Higgins *et al.*, 1981). Washed adherent cells covered by this medium (1 ml/well) were scraped off with a rubber policeman and lysed by freezing at -20°C and thawing. Lysate (250 μl) was mixed with 2 μg of H 33258 in 1.75 ml of lysis buffer.

Standards were prepared from purified calf thymus DNA (1–8 μg). An average value of 1.3 μg of DNA corresponds to 10^5 mononuclear cells.

Purification and radiolabelling of complement proteins

C1q, C1r, C1s and C1Inh were purified as described by Arlaud *et al.* (1979a) and Reboul *et al.* (1977) respectively. These proteins were ^{125}I -labelled to a specific radioactivity of 1500–2500 c.p.m./ng by lactoperoxidase catalysis by the method of Heusser *et al.* (1973).

Preparation of specific antibodies against complement proteins

Antisera to purified C1q, C1r, C1Inh and glutaraldehyde-insolubilized C1s were raised in rabbits (Porter, 1955). Immunoglobulins were obtained by Na_2SO_4 precipitation of rabbit sera (Prahll & Porter, 1968). Specific antibodies to C1q and C1s were purified by affinity chromatography (Villiers *et al.*, 1982b).

F(ab')_2 fragments of rabbit Ig were prepared by pepsin digestion in 0.12 M-sodium acetate/0.145 M-NaCl, pH 4.2, at 37°C for 20 h with 3:97 (w/w) pepsin/Ig ratio. After purification by chromatography on Ultrogel AcA 34, F(ab')_2 fragments were ^{125}I -labelled to a specific radioactivity of 1500 c.p.m./ng by lactoperoxidase catalysis (Heusser *et al.*, 1973).

Radioimmunoassay of C1q, C1r, C1s and C1Inh

Portions of culture supernatants were transferred into conical microcentrifuge tubes containing 500 μl of NP40 buffer supplemented with 5 mM- $\text{iPr}_2\text{P-F}$ and 0.01 M-EDTA; then 20 μl of ^{125}I -labelled antigen (2000 c.p.m.), 20 μl of diluted antibodies (titrating 50% of the labelled antigen) and FCS-supplemented RPMI 1640 to 1 ml final volume were added. After overnight incubation at room temperature, 100 μl of a 10% (w/v) suspension of heat-killed and formalin-fixed SACI in NP40 buffer were added and the mixture was incubated further at 4°C for 30 min. After centrifugation (3 min at 1000 g, r_{av} , 5 cm) the ^{125}I radioactivity was measured in the SACI pellets and in the supernatants. Purified complement components were used as standards. When measuring C1q, the concentration of NaCl in NP40 buffer was raised to 0.2 M in order to prevent non-specific binding of C1q to SACI; the medium was adjusted to 80 mM- MgCl_2 just before adding SACI. Moreover, SACI had to be treated by 10% (w/v) 2-mercaptoethanol/3% (w/v) SDS in phosphate-buffered saline at 95°C for 30 min as indicated by the manufacturer.

For the calculations, C1s and C1r were taken as dimers of mol.wt. 170000 (Valet & Cooper, 1974a,b); mol.wts. of 410000 and 100000 were taken respectively for C1q (Reid, 1982) and C1Inh (Reboul *et al.*, 1977).

Measurement of lysozyme and C2 activities

Lysozyme was estimated from the lysis of *M. lysodeikticus* observed at 450 nm (Gordon *et al.*, 1974). One unit of lysozyme corresponds to a decrease of 0.001 A/min.

C2 haemolytic activity was measured as described by Thielens *et al.* (1982), and the results expressed as the number of effective molecules: $Z \times$ number of EA, where $Z = -\ln(1-y)$, y being the proportion

of lysed cells. Purified C2 (Thielens *et al.*, 1982) was used as a standard when necessary (1 pmol corresponds to 2.4×10^9 effective molecules).

Detection of cellular C1q

Membrane-associated C1q was estimated by direct or indirect immunofluorescence on adherent cells cultivated on glass coverslips. In direct assays, the cells, washed once, were incubated for 45 min at 4°C in 200 µl of FITC-labelled rabbit anti-C1q serum diluted 1:20 in RPMI 1640 containing 20 mM-Hepes and 1% (w/v) ovalbumin (RPMI-OVA). Fluorescence was observed after two washes in RPMI 1640. In indirect assays, in the first step the cells were treated with 20 µg of anti-C1q F(ab')₂ in 200 µl of RPMI-OVA as described above; in the second step, after washing, bound F(ab')₂ was revealed by incubation for 45 min at 4°C with FITC-labelled anti-rabbit F(ab')₂ diluted 1:40 in RPMI-OVA.

Membrane-associated C1q was also estimated by immunolabelling with anti-C1q ¹²⁵I-F(ab')₂. Cells were incubated for 30 min at 4°C with 20 µg of unlabelled anti-C1q F(ab')₂ containing 200 ng of ¹²⁵I-labelled fragments (sp. radioactivity 1900 c.p.m./ng) in 200 µl of RPMI-OVA. Controls were run in parallel using non-immune rabbit F(ab')₂. After washing, radioactivity bound to the coverslips was counted.

Total cellular C1q was estimated by immunofluorescence by using the same techniques as described above, but on acetone-fixed cells.

Results

Biosynthesis of C1q and C1s by resting human monocytes

C1q and C1s in supernatants of monocyte cultures. C1q and C1s were measured in supernatants of monocyte cultures up to 24 days of incubation *in vitro*. C1q and C1s were undetectable within the first 5 days in culture. Between day 5 and day 8 the secretion of C1q, measured in eight cultures, varied from 0.25 to 2 fmol/day per µg of DNA, and the secretion of C1s, measured on six cultures, from 0.5 to 1 fmol/day per µg of DNA.

As during the first days the level of secretion of C1q and C1s was low, the kinetics of production of these proteins were estimated on long-term cultures. In these cases the cultures were started in autologous-serum-supplemented medium and the measurements made after transfer of the cells to FCS-supplemented medium. The kinetics of secretion of C1q and C1s during 14 days, after transfer to FCS-supplemented medium at day 10, are reported in Fig. 1. The secretion of C1q parallels the secretion of C2 and lysozyme during the same

Fig. 1. Cumulative synthesis of C1q and C1s by human monocyte cultures

Monocytes were prepared and cultivated in wells as indicated in the Materials and methods section. At day 10 the cells were transferred from 15% autologous serum to 20%-FCS-supplemented medium. Each day the supernatants of two wells were harvested, frozen at -20°C until use and the cells discarded. Medium was renewed at day 14 and day 17. C1q (●) and C1s (▲) were estimated in supernatants as described in the Materials and methods section, and the calculations took into account the amounts of proteins found on day 14 and day 17 respectively.

time (Fig. 2), these two proteins being taken as a reference for the secretory capacity of the cells. The amounts of C1q and C1s secreted during the first 5 days are fairly comparable, but then C1s secretion decreases and even stops after day 20. The decrease in C1s secretion was found to be independent of the change of the medium, as in the experiment reported in Fig. 1 the culture was replenished with fresh medium on days 14 and 17.

Similar patterns were obtained with other cultures, but the amounts of protein secreted were strongly dependent on each individual donor. For 15 different donors, C1q secreted from day 10 to day 24 varied between 0.75 and 3 fmol/day per µg of DNA; for 18 other donors between 5.1 and 16 fmol/day per µg of DNA. During the same period the secretion of C1s was less than, or equal to, 4.6 fmol/day per µg

Fig. 2. Cumulative synthesis of C2 and lysozyme by human monocyte cultures

The monocyte culture is the same as for Fig. 1. C2 (●) and lysozyme (▲) were estimated in culture supernatants as described in the Materials and methods section.

Table 1. Biosynthesis of C1q and C1s by human monocytes

Monocytes were prepared and cultivated in wells as described in the Materials and methods section. At day 10 the cells were transferred from 15% autologous serum to 20%-FCS-supplemented RPMI 1640 medium. At day 14 the medium was removed and replaced by fresh 20%-FCS-supplemented medium. Cells were then grown for 24, 48 or 72 h before collection of the supernatants and lysis of the cells. C1q and C1s were measured in supernatants and cellular lysates as described in the Materials and methods section.

Time in culture (days)	Synthesis (fmol/day per μg of DNA)			
	C1q		C1s	
	Super-natants	Cellular lysates	Super-natants	Cellular lysates
15	10.11	2.77	3.24	9.83
16	10.04	1.39	2.40	3.76
17	7.04	0.81	2.40	2.73

of DNA; the C1q/C1s ratio was higher than 2 for 15 out of 26 cultures, between 1 and 2 for 9 out of 26 cultures and less than 1 for 2 out of 26 cultures. C1r was scarcely or not detectable in the culture

Table 2. Effect of phagocytosis on the secretion of C1q and C1s by human monocytes

Monocytes were prepared and cultivated in wells as indicated in the Materials and methods section. The phagocytic substrates were added between day 10 and day 14 at the respective concentrations of 5 × 10⁶ cells/ml for heat-killed *S. cerevisiae* and EA, and 10⁸ cells/ml for *M. lysodeikticus* and *E. coli* K12. Then C1q and C1s were measured in the culture supernatants, as described in the Materials and methods section, after 24 and 48 h of incubation. The ratio between the amount of protein secreted in the presence of phagocytic substrates and the amount of protein secreted by resting cells of the same donor is given. The number of independent measurements is given in parentheses.

	<i>S. cerevisiae</i>	EA	<i>M. lysodeikticus</i>	<i>E. coli</i> K
C1q	0.46 (13)	1.65 (5)	1.18 (3)	0.87 (3)
C1s	1.61 (13)	2.24 (4)	1.47 (3)	1.20 (3)

supernatants and in all cases except one we did not find measurable amounts of C1Inh.

C1q and C1s in cellular lysates. C1q and C1s were measured simultaneously in supernatants and cellular lysates of five cultures. One of these experiments is illustrated by Table 1. In all cases a high proportion of C1q was secreted; in contrast, C1s appeared to be predominantly distributed inside the cells. C1q and C1s did not accumulate in the cytoplasm even when their synthesis was amplified by stimuli as described below.

Membrane-associated C1q. Membrane-associated C1q was investigated in monocyte cultures from eight individual donors, between day 1 and day 18.

FITC-labelled anti-C1q F(ab')₂ fragments were unable to reveal any C1q. Similar absence of membrane-associated C1q was also observed using ¹²⁵I-labelled anti-C1q F(ab')₂ fragments. It should be stressed that, under similar conditions, cytoplasmic C1q was detected from day 5 of culture in 25–75% of acetone-fixed monocytes by direct or indirect immunofluorescence.

As secreted C1q is likely to bind to the cells and contribute to membrane-associated C1q, the binding of exogenous C1q to monocytes membranes was investigated by immunofluorescence as described above. At isoionic strength, no C1q binding was observed upon incubation of 0.1–10 μg of C1q with 5 × 10⁵ adherent monocytes for 15 min at 4°C. By contrast, when the ionic strength was lowered to 0.06, a bright granular fluorescence was visible on 95% of the monocytes.

Effect of phagocytosis on C1q and C1s secretion

The influence of phagocytosis on C1q and C1s secretion is shown in Table 2. Phagocytosis appeared to have only a slight influence on the

Fig. 3. Influence of conditioned media on the secretion of C1q, C1s and C1Inh by human monocytes. Monocytes were prepared and cultivated in wells as described in the Materials and Methods section. At day 8 the medium was replaced by conditioned-medium-supplemented medium (10%, v/v). Then at the days indicated, the culture supernatants were harvested and replaced by new conditioned-medium-supplemented medium. C1q, C1s and C1Inh were estimated in culture supernatants as described in the Materials and Methods section. (a) □, Control. Conditioned medium prepared as described in the Materials and Methods section from lymphomononuclear cells stimulated by PPD (▨), allogeneic cells (▤) or from secondary MLC (■). (b) □, Control. Conditioned medium, prepared by concanavalin A stimulation of lymphomononuclear cells (▨) or monocyte-depleted lymphocytes (▤).

secretion of these proteins. However, a significant increase in the secretion of C1q and C1s was mediated by EA.

Opposite effects of yeasts on the secretion of the two proteins was generally observed; yeast phagocytosis lowered the secretion of C1q in ten cases out of thirteen (measures made on five different cultures), whereas it increased C1s secretion in five cases and did not modify it in seven other cases.

LPS (2 µg/ml), MDP (100 µM and 10 µM) and 0.3 µm-diameter latex beads (10⁸ beads/ml) did not modify the secretion of C1q and C1s measured after

24 h, 48 h and 72 h of incubation on two cultures of 15 days.

Secretion of C1q, C1s, C1r and C1Inh by stimulated monocytes

The influence of conditioned media was tested on nine cultures. Results concerning two cultures are shown in Figs. 3(a) and 3(b). These Figures feature the influence of conditioned media from MLC (nine cases), lymphomononuclear cells stimulated by PPD (two cases), concanavalin A (one case) and from monocyte-depleted lymphocytes stimulated by concanavalin A (one case). Activation always induced a

stimulation of the secretion of C1q, which increased from 2 to 7 times and varied between 1.2 and 44 fmol/day per μg of DNA; secretion of C1s was re-activated between 1.3 and 11.6 fmol/day per μg of DNA. Low amounts of C1r (up to 3 fmol/day per μg of DNA) were detectable in some supernatants. Secretion of C1Inh by stimulated monocytes varied between 15 and 534 fmol/day per μg of DNA. Figs. 3(a) and 3(b) indicate that the effects of the conditioned media persisted as long as they were present in the culture; the increase in secretion of C1 subcomponents and C1Inh was maintained with time and was even amplified in the case of C1q. However, when the conditioned media were removed, their stimulatory effects disappeared within 48 h (results not shown).

Discussion

C1q and C1s were detected by immunological techniques in cultures of human resting monocytes, whereas C1r was scarcely measurable. In the present case, haemolytic assays of C1q and C1s were hampered by the presence of FCS, which was found to inhibit C1 activity, as described previously (Müller *et al.*, 1978).

Differences in the kinetics of secretion of C1q and C1s were observed: the capacity of monocytes to secrete C1q was quasi-constant when measured from day 10 to day 24 in culture; during this period the kinetics of C1q secretion paralleled those of C2 and lysozyme. C1s secretion proceeded at a constant rate at the beginning of this period, then decreased and even in some cases stopped. Preliminary experiments tend to show that the decline of C1s secretion was not entirely due to a degradation of the protein; about 70% of exogenous C1s added to a culture was recovered after 5 days, whereas, in parallel experiments, 100% of exogenous C1q was recovered.

The major part of the C1q synthesized by monocytes was secreted; no significant intracytoplasmic accumulation of C1q was observed; it was never detected on membranes; this can be readily explained by the inability of C1q, at the ionic strength used for the culture, to bind to C1q membrane receptors, as described by Tenner & Cooper (1980) for mononuclear phagocytes. The absence of membranous C1q on resting human monocytes is in contrast with findings by Loos (1982) on peritoneal macrophages, which tend to show that part of the C1q synthesized serves as a membranous Fc γ -binding protein. This property may be expressed only by certain types of mononuclear phagocytes or may depend on the life history of the cells.

Phagocytic substrates did not increase the capacity of monocytes to secrete C1q and C1s. On the

contrary, activation by conditioned media from mitogen-, antigen- or allogeneic-stimulated lymphocyte cultures increased C1q production from 2 to 7 times and re-activated C1s secretion. Under stimulating conditions C1Inh was secreted and C1r became detectable in culture supernatants. These observations tend to demonstrate that stimulated monocytes are able to synthesize whole C1; C1Inh, produced in parallel, could preserve C1 from local activation, since this component is able to protect C1 from spontaneous or non-immune activation (Ziccardi, 1982). The observation of the unbalanced secretion of C1q, C1r and C1s may be partly influenced by individual variations of their immunological properties: antigenic properties of C1r are known to be dependent on its association state (Ziccardi & Cooper, 1978; Arlaud *et al.*, 1979b).

A net contrast exists between the amounts of C1q, C1r and C1s found in the monocyte culture supernatants and the 1:2:2 C1q/C1r/C1s stoichiometry of these components in circulating C1. This contrast can be explained by the capacity of C1 subcomponents from various tissue origins to associate. The physiological stoichiometry of C1 subcomponents inside circulating C1 could be maintained owing to low reversible interactions between C1q and C1r₂-C1s₂, as reported by Kilchherr *et al.* (1982) and Siegel & Schumaker (1982). Precise data on the structure of secreted C1 subcomponents will allow further progress.

We thank Ms. Nicole Saou for excellent technical assistance. This work was partly supported by the Fondation pour la Recherche Médicale.

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