Characterization of C1q, C1s and C$\bar{1}$ Inh synthesized by stimulated human monocytes in vitro

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C1q, C1s and C$\bar{1}$ Inh synthesized and secreted by human monocytes were characterized by SDS-PAGE. C1q is formed of three chains A (M_r ~35000), B (M_r ~33000) and C (M_r ~25000) which are associated in two subunits A-B and C-C. It appears identical to C1q purified from plasma. C1s is secreted as a non-activated, monocatenar protein of M_r ~87000 identical to proenzymic C1s from plasma. Secreted C$\bar{1}$ Inh (M_r ~100000) has a slightly higher M_r than purified plasmatic C$\bar{1}$ Inh. Monensin treatment of the cells favours the intracytoplasmic accumulation of products at various glycosylation stages.

Human C1 *Human C$\bar{1}$ Inh* *Macrophage* *Biosynthesis*

1. INTRODUCTION

C1, the first component of complement, circulates in plasma as a calcium-dependent association of two loosely bound subcomplexes, C1q and C1r2-C1s2. Activation of C1, which triggers the classical pathway of complement, reflects a modulation of the intrinsic autocatalytic properties of its dimeric C1r subcomponent [1]. C$\bar{1}$ Inh controls C1 activation [2] and C1 activity [3].

We have shown that cultured human monocytes are able to synthesize the whole C1 subcomponents of C1 and C$\bar{1}$ Inh, when these cells are activated by conditioned media from stimulated lymphocytes [4]. The purpose of this paper is to characterize C1q, C1s and C$\bar{1}$ Inh synthesized and secreted by these cells, the circulating proteins being taken as a reference.

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Abbreviations: FCS, heat-inactivated foetal-calf serum; PBS, phosphate-buffered saline; NP 40, Nonidet P-40; DFP, di-isopropyl phosphofluoridate; IgG, immunoglobulin G; F(ab')2, N-terminal halves of heavy chains of Ig joined by inter-heavy-chain disulphide bond to light chains; SDS-PAGE, SDS-polyacrylamide gel electrophoresis

Nomenclature: The nomenclature of complement components is that recommended by World Health Organization (1968). Activation of a component is indicated by a bar

2. MATERIALS AND METHODS

2.1. *Culture and biosynthetic labeling*

Monocytes were prepared and cultivated as described in [4]. After 8 days the medium was supplemented with 10% (v/v) conditioned medium prepared from concanavalin A stimulated lymphocytes according to [4]. Biosynthetic labeling was conducted after 12–15 days of culture; the cells were washed with RPMI lacking leucine or proline (Selectamine, Flobio) supplemented with 20% FCS and 10% conditioned medium, then incubated for 24 h in the same medium containing 12.5 μCi/ml ^{14}C-labeled L-leucine (300 mCi/mmol, Amersham) or 12.5 μCi/ml ^{14}C-labeled L-proline (250 mCi/mmol, Amersham). Following incubation the medium was removed, centrifuged for 5 min at 10000 × *g*, extensively dialysed against PBS and stored at −20°C. The cells were washed with PBS

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and lysed by 0.5% NP40, 0.5% sodium deoxycholate, 100 mM KCl, 10 mM EDTA, 5 mM DFP, 50 mM Tris-HCl, pH 7.4. The lysates were freeze-thawed twice, centrifuged for 30 min at $10000 \times g$ and stored at $-20°C$.

2.2. *Immunoprecipitation*

Specific IgG to Clq, F(ab')2 fragment of specific IgG to $C\bar{1}s$ or immunoglobulin from antiserum to $C\bar{1}$ Inh were coupled to activated covaspheres Mx (Covalent Technology Corporation) as described by the manufacturer. 1 ml medium or cell lysate was made 5 mM in EDTA and 5 mM in DFP, then rotated for 18 h at room temperature with 20 or 50 μl of the appropriate covaspheres. The covaspheres were collected by centrifugation and washed 5 times with 1 ml 0.15 M NaCl, 0.5% NP40, 0.5 mM EDTA, 0.02% NaN_3 in 0.05 M Tris-HCl, pH 7.4. The covaspheres were then extracted by 0.1 ml 3% SDS for 30 min at 37°C. The extracts were mixed with equal volumes of 8 M urea, 0.2 M Tris-HCl, pH 8.0, then reduced samples were prepared by incubation for 1 h at 37°C with 50 mM DTT followed by alkylation for 20 min at 37°C with 133 mM iodoacetamide and non-reduced samples by incubation for 1 h at 37°C with 20 mM iodoacetamide.

2.3. *SDS-PAGE*

SDS-PAGE was conducted according to Laemmli [5] using 10 or 7.5% acrylamide gels. ^{14}C-labeled M_r markers (Amersham) were included in each slab. After electrophoresis the gels were fixed by soaking in 25% trichloroacetic acid overnight, washed for 1 h with water, impregnated for 30 min with 1.5 M sodium salicylate, dried and exposed to Kodak X-omat AR film at $-70°C$.

3. RESULTS

3.1. *Biosynthesis of Clq*

The particular amino acid composition of the collagenous tail of Clq was taken into account to enhance Clq specific labeling by choosing [^{14}C]-proline as biosynthetic label. Monocytes were cultivated in the presence of [^{14}C]proline then the culture supernatants and the cell lysate were immunoprecipitated with covasphere-bound anti-Clq IgG. The immunoprecipitates were solubilized in 3% SDS and run on SDS-PAGE in reducing and non-reducing conditions.

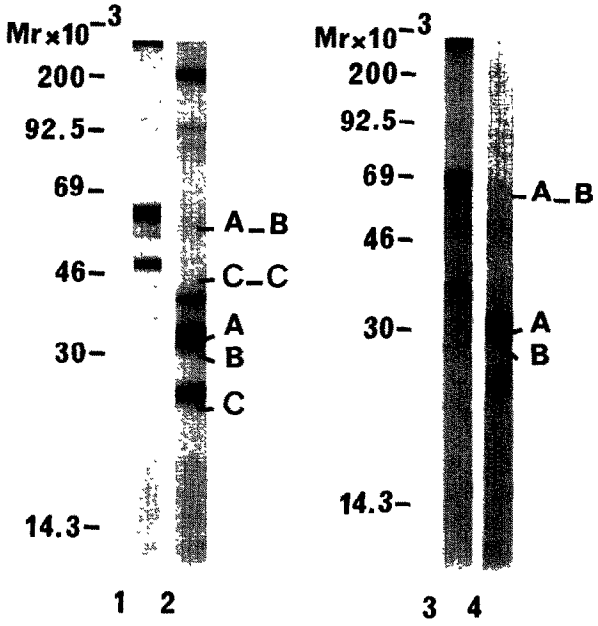

Fig.1. Immunoprecipitation of Clq synthesized by monocytes. Autoradiograph following SDS-PAGE of Clq immunoprecipitated from [^{14}C]proline labeled culture medium (lane 1, non-reduced sample; lane 2, reduced sample) and cell lysate (lane 3, non-reduced sample; lane 4, reduced sample).

When the immunoprecipitation was made on the culture supernatant (fig.1, lanes 1 and 2) the reduced sample shows the 3 bands characteristic of serum Clq, A, B and C at $M_r \sim 35000$, 33000 and 25000; the non-reduced sample shows the A-B and C-C dimers of Clq at $M_r \sim 57000$ and 46000. An extra band of $M_r \sim 52000$ in the non-reduced sample and $M_r \sim 40000$ in the reduced sample was always found; this band could be compared to the reduced bands of $M_r \sim 47000$ and 42000 which have been shown to be synthesized by fibroblast cultures [6]. The reduced sample also shows a band of $M_r \sim 200000$ which does not penetrate into the gel when non-reduced; the identity of this band is not certain but it could correspond to secreted fibronectin which is known to bind to Clq (review [7]).

When the immunoprecipitation was made on the cell lysate (fig.1, lanes 3 and 4) both reduced and non-reduced samples showed the same bands as secreted Clq. However A chain seemed to be predominant over B and C chains; this could be

due to a combination of a partial dissociation of intracellular Clq in its two dimers A-B and C-C and a preferential affinity of polyclonal anti-Clq IgG for epitopes of A chain. There was no evidence of proClq.

3.2. *Biosynthesis of Cls*

Cls biosynthesis was studied with [^{14}C]leucine as label. Covasphere-bound anti-C$\bar{1}$s F(ab')2 was used as immunoprecipitant to avoid eventual Fc binding of synthesized Clq.

Immunoprecipitates from culture supernatant (fig.2, lanes 1 and 2) showed a single band of M_r ~ 87 000 in reduced form. Secreted Cls thus appeared as a monocatenar protein; there was no evidence of A and B chains, indicating that secreted Cls was entirely in its proenzymic form.

When the immunoprecipitation was made on cellular lysates, Cls was hardly detectable over the background; this is probably related to the low intracytoplasmic accumulation of this protein that we had noticed [4]. Monensin was used to enhance intracytoplasmic protein accumulation. Fig.2 (lanes 3 and 4) shows the electrophoretic pattern of immunoprecipitates from lysates of cells labeled in the presence of 2 μM monensin. At least one band identical to secreted Cls appeared both in reduced and non-reduced samples.

3.3. *Biosynthesis of C$\bar{1}$ Inh*

Fig.3, lane 1 shows the electrophoretic pattern of C$\bar{1}$ Inh immunoprecipitated from culture supernatant, labeled with [^{14}C]leucine. Secreted C$\bar{1}$ Inh appears as a monocatenar protein of M_r ~ 100 000, slightly higher than its purified plasmatic counterpart of M_r ~ 95 000 (fig.3, lane 2).

Monensin was used to promote intracytoplasmic accumulation of C$\bar{1}$ Inh which otherwise was hardly detectable. Immunoprecipitates of lysates from monensin-treated cells show a diffuse band between M_r ~ 80 000 and M_r ~ 90 000 (fig.3, lane 3), which could be accounted for by different degrees

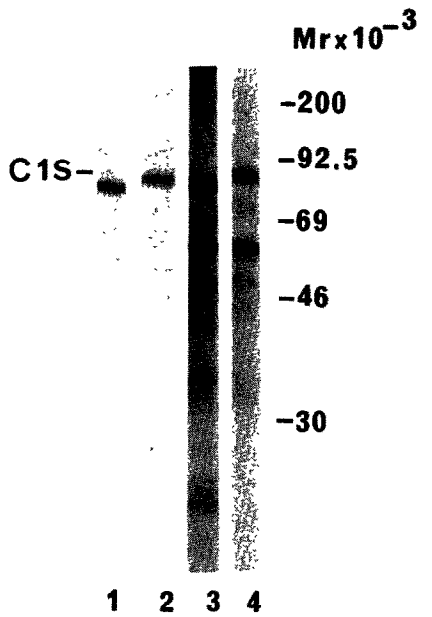

Fig.2. Immunoprecipitation of Cls synthesized by monocytes. Cls was immunoprecipitated from [^{14}C]leucine labeled culture medium and from lysates of cells labeled [^{14}C]leucine in the presence of 2 μM monensin. The immunoprecipitates were analysed by SDS-PAGE and fluorography. Lanes 1 and 2, non-reduced and reduced secreted Cls; lanes 3 and 4, non-reduced and reduced intracytoplasmic Cls.

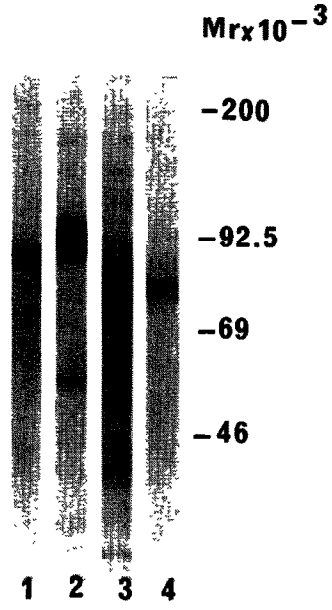

Fig.3. Immunoprecipitation of C$\bar{1}$ Inh synthesized by monocytes. C$\bar{1}$ Inh was immunoprecipitated from [^{14}C]leucine labeled culture medium and from medium and lysates of cells labeled by [^{14}C]leucine in the presence of 2 μM monensin. The immunoprecipitates were anslysed by SDS-PAGE in reducing conditions and fluorography. Lane 1, ^{125}I-labeled purified plasmatic C$\bar{1}$ Inh; lane 2, secreted C$\bar{1}$ Inh; lanes 3 and 4, intracytoplasmic and secreted C$\bar{1}$ Inh from cells cultivated in the presence of monensin.

of $C\bar{1}$ Inh glycosylation. In this case an underglycosylated form of $M_r \sim 80000$ was secreted (fig.3, lane 4) emphasizing that complete glycosylation of $C\bar{1}$ Inh is not a prerequisite for its secretion.

4. DISCUSSION

Our results indicate that Clq is secreted by monocytes in a form identical to plasmatic Clq. Besides, we found that secreted Clq is hemolytically active. There is no evidence for intracytoplasmic or secreted proClq. Identical results were found by Tenner et al. [8]. In contrast Loos [9] had evidence for intracellular proClq in mouse peritoneal macrophages. As regards fibroblastic Clq, Reid and Solomon [6] have shown that it may also be synthesized and secreted in a form of a higher molecular mass than purified plasmatic Clq. But the results of Skok et al. [10] suggest that a cell type other than fibroblasts is responsible for the synthesis of serum Clq and that there are distinct genes for the fibroblast Clq and serum Clq.

Müller et al. [11] suggested that Cls secreted by human or guinea pig peritoneal macrophages should be activated to explain the rapid decrease of C4 hemolytic activity in the cultures. As regards monocytic Cls we have shown that it is secreted in a proenzymic form. This observation could reflect that Cls is secreted in association with Clr as isolated Clr2-Cls2 has been shown to be stable [1]. Besides, this non-activation of Cls does not fit with accepted concepts on Cl activation as association of Clr2-Cls2 with Clq inside Cl complex allows the cleavage of Cls by autoactivated Clr [2]. Our results indicate that monocytes are able to secrete $C\bar{1}$ Inh nearly identical to its purified plasmatic counterpart. Similar results have been found by Yeung Laiwah et al. [12]. Cl, if assembled, could thus be protected from activation by locally produced $C\bar{1}$ Inh.

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