C1q binding to mitochondria: a possible artefact?

Alfio Comis+ and Simon B. Easterbrook-Smith*

Department of Biochemistry, University of Sydney, Sydney, NSW 2006, Australia

Received 9 April 1985

Mitochondrial preparations, obtained from human tonsils and from rat spleen, liver, heart, and kidney tissues, bound [^{125}I]C1q with affinities of 10^7–10^8 M^{-1}. The binding of C1q was not affected by treatment of the mitochondrial preparations with pronase, trypsin, or phospholipase D, but it was lowered 5–6-fold following treatment of the mitochondria with DNase and RNase. Binding of C1q to mitochondrial preparations was also greatly diminished by limited chemical modification of C1q with cyclohexane-1,2-dione. It is suggested that the reported binding of C1q to mitochondria may have arisen from the protein binding to DNA and/or RNA contaminating the mitochondrial preparations.

C1q *Mitochondria* *Nucleic acid* *Nuclease* *Cyclohexane-1,2-dione*

1. INTRODUCTION

The classical pathway of complement activation is triggered by the interaction of the first complement component (C1) with aggregates of IgG or IgM [1]. This activation leads to the generation of several biological activities that initiate inflammation and facilitate interactions with various effector cells. A substantial number of membrane preparations have been shown to bind C1, with the resultant activation of the classical pathway, in the absence of specific antibodies [2–4]. In these cases, interaction with subcomponent C1q has been demonstrated, implying that complement is activated by a mechanism similar to that of IgG immune complexes.

Antibody-independent activation of C1 in serum following exposure to isolated human heart mitochondrial membranes in vitro has also been reported [5–9], leading to the hypothesis that the release of mitochondrial membranes of portions thereof from traumatized cardiac cells following an acute myocardial infarction, can lead to a direct antibody-independent activation of the complement system, resulting in the initiation of an inflammatory reaction at the site of tissue damage [8].

Below we present evidence that the complement activation by mitochondrial preparations may be caused by contaminating DNA and/or RNA. Interaction of C1q with DNA, leading to complement activation, has already been established [10], but the possibility that this is the cause of the observed binding of C1q to mitochondrial preparations has not been previously examined.

2. MATERIALS AND METHODS

Bovine serum albumin (BSA), polyethylene glycol (M_r 8000, PEG), ribonuclease A (EC 3.1.27.5) and deoxyribonuclease I (EC 3.1.21.1) from bovine pancreas, trypsin (EC 3.4.21.4), pronase E, and phospholipase D (EC 3.1.4.4) were from Sigma. Cyclohexane-1,2-dione was from Pierce, Rockford, USA. Human C1q was isolated from human serum and labelled with ^{125}I using lactoperoxidase [11]. Human mitochondria were prepared from tonsils removed from patients of Petersham Private Hospital, Sydney, Australia,

* To whom correspondence should be addressed
+ Present address: School of Physiology and Pharmacology, University of N.S.W., Kensington, NSW 2033, Australia

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

washed immediately with 0.25 M sucrose in 0.01 M Tris (pH 7.3) and stored at 4°C until processed (usually less than 24 h).

2.1. *Mitochondrial preparations*

Human and rat tissues were trimmed, suspended in 0.25 M sucrose in 0.01 M Tris (pH 7.3) and homogenized for 10 s at 4°C. The crude homogenate was centrifuged at $800 \times g$ for 10 min and the supernatant recentrifuged at $10000 \times g$ for 30 min at 4°C. The mitochondrial pellet was resuspended in the same buffer and recentrifuged before being divided into aliquots and stored at −70°C. Mitochondrial 'ghost' membranes were prepared by suspending thawed mitochondria in distilled water 3 times; the membranes were collected by centrifuging ($10000 \times g$, 5 min, 4°C).

2.2. *Clq binding assay*

The binding of Clq to mitochondrial membranes was determined by incubation of ^{125}I-labelled Clq (0–0.04 mg/ml, 6.2×10^6 cpm/mg) with a mitochondrial suspension (typically 0.09 mg/ml protein) in 0.01 M phosphate, 0.12 M NaCl, 0.5% (w/v) BSA, pH 7.2 for 1 h at 37°C. Ice-cold PEG was quickly added to the mixture to give a final concentration of 2.8% (w/v) and the tubes incubated on ice for 1 h. The insoluble Clq-membrane complexes were collected by centrifuging ($10000 \times g$, 5 min, 4°C) and the radioactivity associated with the pellet and the supernatant determined.

2.3. *Modification of arginyl residues*

Clq (0.17 mg/ml, 6.2×10^6 cpm/mg) in 0.025 M borate, 0.5% (w/v) BSA, pH 9.0, was incubated for 30 min at 37°C in the presence of 0.025 M cyclohexane-1,2-dione. The solution was the adjusted to 0.05 M in arginine and the protein chromatographed over Sephadex G-50, equilibrated with 0.02 M borate, 0.15 M NaCl, 0.5% (w/v) BSA, pH 7.2.

2.4. *Enzymatic digestions*

Aliquots of the mitochondrial preparations (typically containing 0.04 mg protein) were incubated with the appropriate enzyme in a total volume of 0.2 ml at 37°C for 1 h; trypsin digestion (1% w/w protein) was performed for 10 min and the reaction stopped with trypsin inhibitor (2% (w/w) protein) [^{125}I]Clq (0.039 mg/ml) was then added quickly followed by ice-cold PEG (2.8%, w/v) in a total volume of 0.4 ml.

3. RESULTS AND DISCUSSION

A number of reports [5–9] have convincingly demonstrated that cellular and subcellular membranes obtained from various sources can bind and activate the first component of complement Cl; Storrs et al. [6] also showed that Cl interacted with membrane binding sites through the globular head regions of subcomponent Clq.

We also have obtained high affinity binding of Clq to mitochondrial preparations from human tonsils and from rat spleen, liver, heart, and kidney tissues. As observed previously with mitochondrial membranes from other species [5,6], the Scatchard plots of the binding data were biphasic (fig.1) suggesting the presence of at least two classes of Clq binding sites in the mitochondrial preparations.

Linear regression analysis of the two linear sections of the Scatchard plots gave the results shown in table 1. No significant difference in the affinity of Clq for the mitochondrial membranes of different tissues was observed, but human mitochondrial preparations were able to bind more Clq than rat preparations.

Enzymatic treatment of human mitochondria with pronase, trypsin, or phospholipase D did not affect their Clq binding activity (table 2). These results suggest that the Clq binding activity is probably not associated with a membrane surface protein or with the charged moieties of the membrane phospholipids. However, treatment of the preparations with nucleases resulted in a substantial decrease in their Clq binding activity (table 3), implicating contaminating nucleic acids as the Clq receptors. Lysed mitochondria, as well as the cytoplasmic fraction obtained during the preparation of the organelles, were also able to bind Clq and the Clq binding activity of these fractions was also reduced by the nuclease digestions. The binding was reduced after 3 washings of the control-lysed mitochondria ('ghost membranes'), supporting the idea that a contaminant associated with the mitochondria was responsible for the binding. Chemical modification of Clq with the arginine-specific reagent cyclohexane-1,2-dione decreased

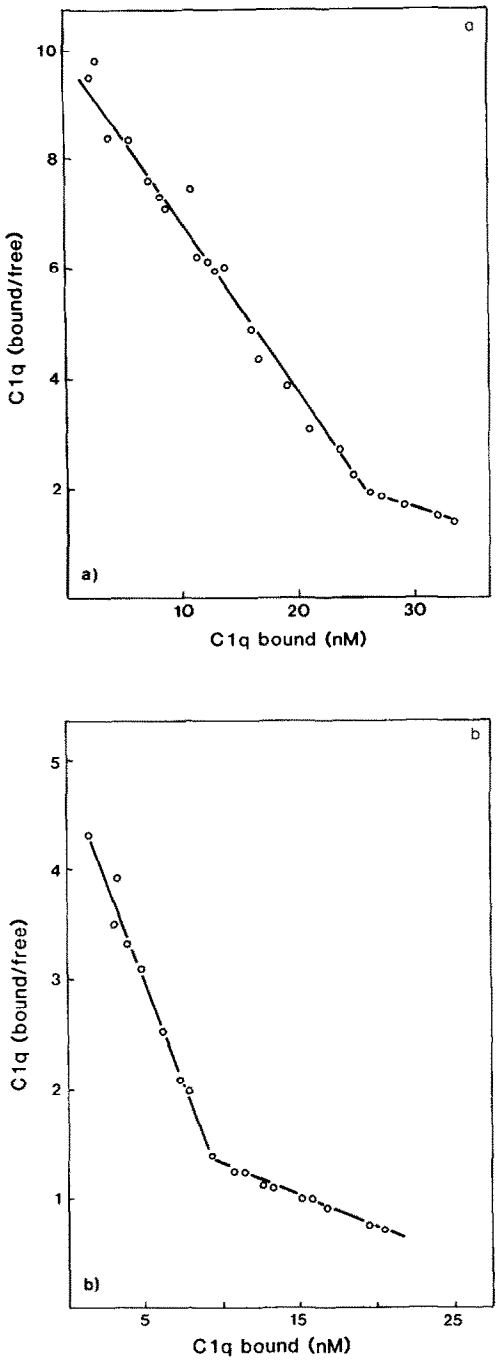

Fig.1. Binding of human Clq to mitochondrial preparations from human tonsils (a) and rat spleen (b). The concentration of ^{125}I-labelled Clq bound to the organelles (0.2 mg protein/ml) was determined, as in section 2, with an input ^{125}I-labelled Clq concentration range of 0–0.04 mg/ml (6.2×10^6 cpm/mg).

Table 1

Kinetic analysis of Clq binding to mitochondrial preparations from human and rat tissues

Tissue	Capacity (nmol/mg protein)	Affinity constants ($\times 10^8$ M^{-1})
Human tonsils	0.36	2.63
	0.20	0.77
Rat spleen	0.16	3.07
	0.19	0.61
Rat kidney	0.06	3.21
	0.10	0.64
Rat heart	0.10	3.33
	0.14	0.58
Rat liver	0.08	3.10
	0.12	0.70

Clq (0–0.04 mg/ml) and the mitochondrial suspensions (0.09 mg protein/ml) were incubated as in section 2 and the results were analysed by linear regression analysis of the two linear sections of the Scatchard plots obtained to give the extrapolated amount of Clq bound at saturating Clq concentrations (capacity) and the apparent affinity constants for the interactions

Table 2

The effect of the enzymatic treatment of mitochondria and of the chemical modification of Clq on the binding of Clq to the organelles

Treatment	Binding (%)
Mitochondria	
Control	100.0
Pronase	99.7
Trypsin	100.8
Phospholipase D	99.2
Clq	
Control	100.0
Cyclohexane-1,2-dione	32.9

Human mitochondrial preparations were incubated with the appropriate enzyme and Clq was modified with cyclohexane-1,2-dione as described in section 2. The binding of Clq (0.039 mg/ml) to the mitochondrial preparations (0.04 mg protein) is expressed as a percentage of that to untreated control preparations

Table 3

The effect of nuclease digestion of the mitochondrial preparations on their Clq binding activity

Preparation	Binding (%)			
	Human tonsils		Rat spleen	
	Control	Digested	Control	Digested
Mitochondrial fraction	69.9	12.2	67.1	11.7
Cytoplasmic fraction	77.1	8.5	70.6	4.6
Lysed mitochondria	74.6	14.3	70.9	5.3
Mitochondrial 'ghosts'	40.2	13.3	39.5	10.9

An aliquot of each preparation (0.04 mg protein) was incubated with DNase (0.006 mg) and RNase (0.03 mg) in a total volume of 0.2 ml of 0.01 M phosphate, 0.15 M NaCl and 0.5% (w/v) BSA, pH 7.2 at 37°C for 1 h. [^{125}I]Clq (0.016 mg/ml) and ice-cold PEG (2.8%, w/v) were then added and incubation continued on ice for 1 h. The ^{125}I-labelled Clq bound to membranes was determined as in section 2, and is expressed as a percentage of the input ^{125}I-labelled Clq

the binding to 1/3 that of a control sample. Chemical modification of about 37 arginyl residues of Clq has been reported to abolish 85% of the DNA-precipitating activity of Clq [10].

Our results would then suggest that the observed binding of Clq to mitochondria and the subsequent complement activation may be due to Clq binding to nucleic acids contaminating the preparations. We suggest that results of Clq binding to mitochondria should be treated with caution pending an assessment of the extent of nucleic acid contamination of the preparations.

A physiological role for the antibody-independent activation of complement by several membrane preparations has been suggested [6]; such membranes, normally localized within the boundary of the plasma membrane, are exposed to plasma Clq after tissue injury. Binding and complement activation would then lead to acute inflammation through the complement anaphylatoxins, chemotactic peptides and opsonins. It is relevant to note that the suggested physiological role for the antibody-independent activation would still be valid if Clq binds to nucleic acid, which is always released after tissue injury.

ACKNOWLEDGEMENTS

This work was supported by the Australian Research Grants Scheme. Clq was prepared from human serum supplied by the Red Cross Blood Bank, Sydney. We are grateful to Dr S.C. Stylis, Dr L. Klein, and Petersham Private Hospital, Sydney, for supplying the human tonsils used in mitochondria preparations.

REFERENCES

[1] Porter, R.R. and Reid, K.B.M. (1979) Adv. Protein Chem. 33, 1-71.
[2] Cooper, N.R., Jensen, F.C., Welsh, R.M. and Oldstone, B.A. (1976) J. Exp. Med. 144, 970-984.
[3] Alving, C.R., Richards, R.L. and Guirguis, A.A. (1977) J. Immunol. 118, 342-347.
[4] Bartholomew, R.M., Esser, A.F. and Muller-Eberhard, H.J. (1978) J. Exp. Med. 147, 844-853.
[5] Storrs, S.B., Kolb, W.P., Pinckard, R.N. and Olson, M.S. (1981) J. Biol. Chem. 256, 10924-10929.
[6] Storrs, S.B., Kolb, W.P. and Olson, M.S. (1983) J. Immunol. 131, 416-422.
[7] Pinckard, R.N., Olson, M.S., Kelley, R.E., De Heer, D.H., Palmer, J.D., O'Rourke, R.A. and Goldfein, S. (1973) J. Immunol. 110, 1376-1382.
[8] Pinckard, R.N., Olson, M.S., Giclas, P.C., Terry, R., Boyer, J.T. and O'Rourke, R.A. (1975) J. Clin. Invest. 56, 740-750.
[9] Giclas, P.C., Pinckard, R.N. and Olson, M.S. (1979) J. Immunol. 122, 146-151.
[10] Van Schravendijk, M.R. and Dwek, R.A. (1982) Mol. Immunol. 19, 1179-1187.
[11] Tenner, A.J., Lesavre, P.H. and Cooper, N.R. (1981) J. Immunol. 127, 648-653.