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Solid Phase C1q Microassay for the Rapid Detection of Circulating Immune Complexes

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A C1q solid phase microassay was designed for the rapid detection of circulating immune complexes. Its level of sensitivity is comparable to that of the Raji cell and greater than the C1q binding assay; furthermore, it is faster and low in cost. These conditions make it more practical and applicable in the clinical setting.

Key words: *C1q solid phase microassay — Raji cell radioimmunoassay — circulating immune complexes — C1q binding assay — aggregated human IgG*

Introduction

A large number of methods designed to detect circulating immune complexes (CIC), involving unknown antigens in biological fluids have been described (Lambert et al., 1978; June et al., 1979). Of the methods available, only a few can be considered as suitable for routine clinical investigation. When considering variables such as specificity, sensitivity and possible effect of interfering substances, it has been suggested that several of the available methods should be tested simultaneously in order to reach appropriate conclusions (Zubler and Lambert, 1978; Theofilopoulos and Dixon, 1980; Williams, 1980).

In this paper, we describe a microassay to detect CIC in the clinical setting; the technique developed was based on the C1q solid phase method as originally reported by Hay et al. (1976). The conditions established may provide an easy and reproducible method of monitoring CIC in several human diseases.

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Material and Methods

Population studied

One hundred healthy controls were selected following clinical and laboratory criteria as previously described (Contreras et al., 1982); 25 pathological sera from patients with systemic lupus erythematosus (SLE) (Cohen et al., 1971), or with histologically proven gastric cancer or viral hepatitis were also included.

Reagent standardization

Human C1q was isolated from fresh serum by a modification of the method of Volanakis and Stroud (1972) (Zubler et al., 1976). The purified C1q was radioiodinated as required using lactoperoxidase (Heusser et al., 1973), to a specific activity of 1–2 μCi ^{125}I/μg and stored at −70°C until used.

Aggregated human IgG (AHG)

The AHG was prepared as previously described (Contreras et al., 1982).

Anti-human IgG

The antiserum against human IgG was purchased from Atlantic Antibodies (Scarborough, ME). Radioiodination of the specific antibody was performed according to the procedure of McConahey and Dixon (1966).

Adsorption of C1q to the solid phase

A solution of 6.25 mg/l of C1q in carbonate buffer, 0.05 M, pH 9.6, was prepared, and 200 μl dispensed in flat-bottom polystyrene wells with a capacity of 300 μl (Cooke Laboratory Products, No. M179A, Alexandria, VA) contained in 96-well styrofoam holders (Cooke). After incubation for 3 h at 37°C, the wells were washed 3 times with cold veronal-buffered saline (VBS, pH 7.2, 0.15 mM Ca, 0.05 mM Mg, Mérieux, Charbonnières-les-Bains) containing 0.05% Tween (VBS-T), filled with 100 μl of 0.1% gelatin solution in VBS-T, and incubated for 2 h at room temperature. Following 3 washes with VBS-T, the wells were immediately inverted and drained. The quantitative studies of adsorption were carried out with C1q, radiolabelled as described above.

Microassay solid phase C1q (MAC1q-SP)

The method used was similar to that of Hay et al. (1976) with a prior incubation of sera with EDTA. Twenty-five μl serum samples were incubated with 50 μl of 0.2 M EDTA at pH 8.3 for 30 min at 37°C. Duplicates, 200 μl of a final dilution 1 : 20 of the sample (in VBS-T) sera, were dispensed into the C1q-coated wells, and were incubated for 2 h at room temperature. Following 3 washes with cold VBS-T, ^{125}I-labelled anti-human IgG (1 μg/ml in VBS-T) was dispensed at 200 μl per well. After incubation for 3 h at room temperature, the unbound antibody was removed by 3 washes with cold VBS-T and wells were counted on a gamma counter. Each assay included duplicates of ^{125}I-labelled anti-human IgG for total activity, and C1q-coated wells without serum samples. The amount of radiolabelled antibody was

referred to a standard curve of antibody uptake by C1q-coated wells, incubated with 200 μl of a 1 : 20 final dilution of various amounts (from 35 μg to 15 ng) of AHG prepared in fresh NHS (1 : 10).

The mixture of NHS or pooled NHS-AHG has been treated with EDTA as previously described, and analyzed in the same conditions as the serum samples. Aggregates used in this test were stored at −70°C for no more than 2 months; before use, centrifugation at 7000 rpm for 20 min was carried out in order to remove large, insoluble aggregates. The amount of complex in each serum tested was expressed as micrograms AHG equivalents per millilitre of serum.

Other procedures

Raji cell radioimmunoassay (RIA-RAJI) was performed as described by Theofilopoulos et al. (1976); C1q binding assay (C1q-BA) was carried out using the modification proposed by Zubler et al. (1976). Samples were tested in duplicate and without prior thawing for the MA C1q-SP, the C1q-BA and the RIA-RAJI methods; samples showing values greater than 2 standard deviations (S.D.) above the mean of the 100 controls were considered as containing significantly elevated levels of CIC.

Statistical analysis

Student's *t*-test, and Spearman's rank analysis were applied.

Results

Binding of C1q and of AHG to the solid phase

In order to assess the amount of C1q bound to the solid phase, 3 different concentrations were used: 1.25, 2.5 and 3 μg/200 μl of ^{125}I-labelled C1q/carbonate solution. The total activity from each concentration, including the residual radioactivity, were measured after 3 h incubation at 37°C and 3 washes with VBS-T; the results (Table I) indicated optimal binding at 1.25 μg/200 μl (6.25 mg/l) of C1q, and this concentration was utilized thereafter. The binding of ^{125}I-labelled AHG to the solid phase coated with C1q increased proportionally with AHG concentration (Fig. 1).

TABLE I

MICROASSAY C1q-SP [a]

^{125}I-C1q (μg/200 μl)	% of binding	C1q bound (μg)
1.25	67	0.83
2.5	31	0.77
3.0	26	0.78

[a] ^{125}I-labelled C1q bound to the solid phase.

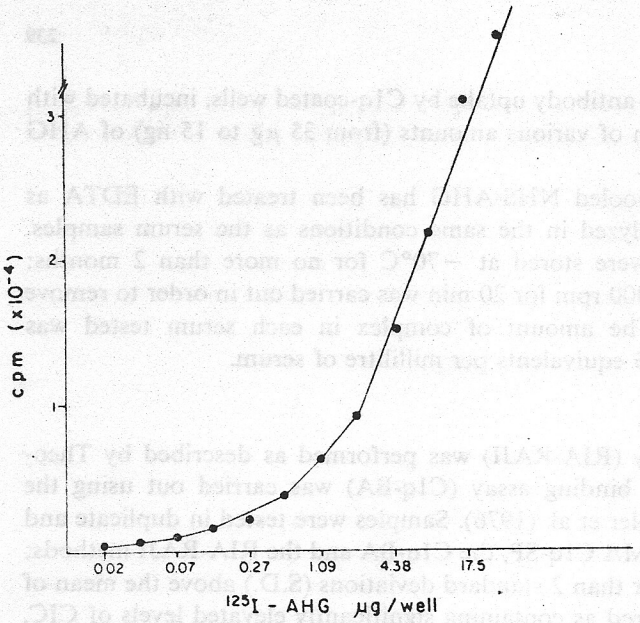

Fig. 1. Binding of ^{125}I-labelled AHG to solid phase coated with C1q.

Reference standard curve

A standard curve of radioactive antibody uptake was prepared by incubation of various amounts of AHG, an optimal NHS concentration (diluted 1 : 10), and ^{125}I-labelled anti-human IgG. As shown in Fig. 2, the uptake of radiolabelled antiserum increased depending on the amount of AHG present in the wells.

Levels of CIC detected by the MAC1q-SP

Levels of CIC were determined in 100 normal individuals as well as in 6 patients with gastric cancer, 14 with SLE and 5 with viral hepatitis (Table II); mean values

TABLE II

LEVELS OF CIC DETECTED BY THE MICROASSAY C1q-SP

Patients	n	µg equiv. AHG/ml (mean ± S.D.)	P [a]
Gastric cancer	6	91 ± 141 [a]	< 0.005
SLE	14	71.6 ± 88.8 [a]	< 0.005
Hepatitis	5	128.8 ± 147.6 [a]	< 0.005
NHS	100	16.1 ± 10.2	

[a] Student's *t*-test (between patients and normals).

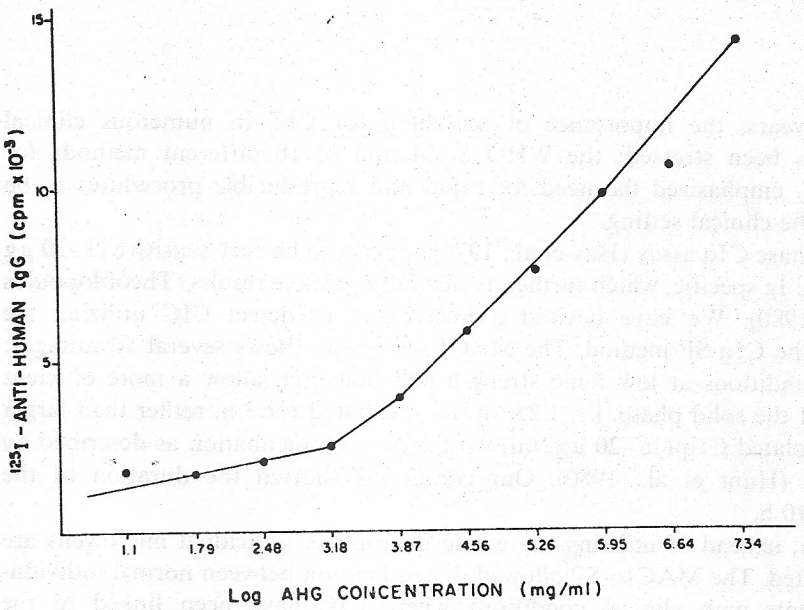

Fig. 2. Uptake of radiolabelled antiserum in relation to the amount of AHG (log n concentration) present in the wells.

for the 3 groups of patients were significantly greater ($P < 0.005$) than the normal controls.

Comparison of CIC detection level between MAC1q-SP, C1q-BA and RIA-RAJI

In order to assess further the detection of CIC by MA-C1q-SP, a comparison was established with results obtained when C1q-BA and RIA-RAJI were employed; the 3 methods detected significantly elevated levels of CIC in the patient groups compared to normal individuals (Table III). Furthermore, a significant correlation between both C1q-BA and MAC1q-SP was found (Spearman rank (r): 0.56,

TABLE III

LEVELS OF CIC DETECTED BY THE MICROASSAY C1q-SP, THE C1q-BA AND THE RIA-RAJI

Patients	n	C1q-BA (%)	μg equiv. AHG/ml (mean ± S.D.)	
			RIA-RAJI	MA-C1q-SP
Gastric cancer	6	8.4±9.2	171.8±133.8	91 ±141
SLE	14	6.1±4.3	156.8±147.5	71.6± 88.8
Hepatitis	5	6.8±4.3	788 ± 26.8	128.8±147.6
NHS	100	2.2±0.9	19.2± 17.8	16.1± 10.2

$P < 0.005$) when CIC levels were investigated among the selected patients; no such correlation existed between the results obtained by MAC1q-SP and RIA-RAJI.

Discussion

In recent years, the importance of searching for CIC in numerous clinical conditions has been stressed; the WHO evaluation of 18 different methods for detecting CIC, emphasized the need for rapid and reproducible procedures to be employed in the clinical setting.

The solid phase C1q assay (Hay et al., 1976) appears to be very sensitive (1–10 μg AHG/ml) and Ig specific, which further avoids false positive results (Theofilopoulos and Dixon, 1980). We have devised a microassay to detect CIC utilizing the principles of the C1q-SP method. The MAC1q-SP assay shows several advantages: the coating conditions at low ionic strength and high pH, allow a more efficient preparation of the solid phase, i.e. 1.25 μg/ml incubated for 3 h, rather than larger amounts of isolated C1q (10–20 μg/ml) with a 20–72 h incubation as described by other authors (Hunt et al., 1980). Our conditions shorten the duration of the procedure to 10 h.

In addition, instead of utilizing the whole microplate, individual microwells are readily evaluated. The MAC1q-SP allowed discrimination between normal individuals and patients with clinical conditions where CIC have been linked to the physiopathology of the disease. Moreover, a significant correlation was found between levels of CIC detected by both the C1q-BA and MAC1q-SP procedures in the diseased groups. The fact that specific antisera may allow the identification of the Ig class involved in the complex offers an additional advantage over the conventional C1q-BA assay.

We suggest that MAC1q-SP is a very useful and rapid screening procedure to detect CIC at the clinical level, and can perhaps be employed in monitoring conventional and new forms of therapy which are currently applied in CIC-mediated diseases.

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