Clin. exp. Immunol. (1982) **48**, 37–42.

Complement-mediated solubilization of immune complexes in systemic lupus erythematosus

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(Accepted for publication 16 October 1981)

SUMMARY

The capacity of complement-mediated solubilization of immune complexes (complex releasing activity: CRA) was studied in 63 sera from eight systemic lupus erythematosus (SLE) patients. CRA in sera of active SLE ($35 \pm 17{\cdot}8\%$) was significantly lower than that of inactive SLE ($64{\cdot}1 \pm 24{\cdot}1\%$, $P < 0{\cdot}001$). In addition, 20 of 23 sera collected during active diseases demonstrated CRA values less than 50% of the control pooled serum. On the other hand, CRA of 29 of 40 sera from inactive disease exceeded the 50% level. CRA in SLE sera correlated with complement component levels and in particular with the CH50. Serial determination of CRA and of levels of circulating immune complexes (CIC), C4 and C3 in two active patients indicated that the correlation between CRA and the complement components was positive, while that between CRA and CIC was negative. These studies provide evidence that CRA may be useful for following the activity of SLE and that CRA reflects the levels of the complement components of both classical and alternative pathways. The possibility that CIC may be solubilized and opsonized by complement and cleared by the reticuloendothelial system was discussed.

INTRODUCTION

It has been shown that immune complexes can be solubilized by the complement cascade (Miller & Nussenzweig, 1975). The complement-mediated solubilization of immune complexes (complex releasing activity: CRA) requires activation of C3 via the alternative pathway, without participation of the late-acting components, C5–C9 (Takahashi *et al.*, 1976). CRA is substantially enhanced by concomitant participation of the classical pathway (Takahashi, *et al.*, 1976). The possibility that CRA may also be operative *in vivo* is conceivable but, to our knowledge, CRA of clinical materials has been little studied. The following studies elucidate CRA in systemic lupus erythematosus (SLE) in relation to its clinical activity and the serum levels of complement components and circulating immune complexes.

Abbreviations: CRA, complex releasing activity (complement-mediated solubilization of immune complexes); CIC, circulating immune complexes; GPB^{++}, phosphate-buffered saline containing gelatin, Ca^{++} and Mg^{++}.

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MATERIALS AND METHODS

Patients. All 8 patients, seen at the University Hospital of Tsukuba, met the preliminary American Rheumatism Association criteria for the diagnosis of SLE (Cohen *et al.*, 1971). Three of these were considered as suffering from active disease according to the clinical criteria (Schur & Sandson, 1968). The remaining five patients were classified as inactive. In two patients, serial studies were made of serum samples collected at both active and inactive stages of the disease.

Sera. Venous blood was allowed to clot in glass tubes for 2 hr at room temperature. After centrifugation, the sera were frozen in small aliquots at −70°C until use. Twenty-three sera from patients with active disease and 40 sera from patients with inactive disease were collected. Sera from 30 healthy hospital workers were pooled and kept frozen at −70°C until use.

Buffers. CRA assays were carried out in phosphate-buffered saline (PBS) containing 0·1% gelatin, $1{\cdot}5 \times 10^{-4}$M Ca^{++} and 5×10^{-4}M Mg^{++}, pH 7·4 (GPB^{++}). PBS containing 0·1% gelatin and 0·01M EDTA pH 7·4 (EDTA–GPB) was also used to terminate the reaction.

Preparation of immune complexes. Ovalbumin (OA) (grade V) was purchased from Sigma Chemical Co., St Louis, Missouri. Rabbit antiserum to OA was prepared by injecting a rabbit with OA incorporated in Freund's complete adjuvant. IgG antibody to OA was purified by passage through a DEAE cellulose column and by affinity chromatography on Sepharose 4B-OA. The IgG was labelled with ^{125}I by the chloramine-T method (McConahey & Dixon, 1966). The specific activity was $5{\cdot}5 \times 10^6$ c.p.m./μg protein.

Immune complexes were prepared with OA and ^{125}I-labelled IgG Ab to OA at equivalence. The mixture was incubated for 30 min at 37°C, then kept overnight at 4°C. The resulting precipitates were washed three times with cold GPB^{++}, and then resuspended to a concentration of 200 μg/ml of Ab in the buffer.

CRA assays. Before use, immune complexes (IC) were centrifuged to remove spontaneously dissociated Ab. Fifty microlitres of serum were mixed with an equal volume of ice-cold GPB^{++} in which were suspended IC containing 2 μg of Ab. The mixture was immediately incubated at 37°C. After 30 min of incubation, a 50 μl sample was withdrawn from the mixture and placed in a plastic tube containing 0·5 ml of 0·05% sheep erythrocytes suspension in ice-cold EDTA–GPB. After centrifugation at 5000 ***g*** for 10 min at 4°C, the tube was decanted, and both pellets (A) and supernatants (B) were assayed for radioactivity in a gamma counter. To estimate the extent of spontaneous solubilization, a tube containing only GPB^{++} and IC was processed in the same manner and pellets (C) and supernatants (D) were counted. Specific CRA was determined by the following formula:

$$\text{per cent solubilization} = 100 \times \left(\frac{B}{A+B} - \frac{D}{C+D} \right)$$

Patient's CRA was then expressed as a percentage of the control pooled serum.

Other complement studies. Serum levels of C1q, C4, C3 and factor B were measured by single radial immunodiffusion using specific antisera prepared in our laboratory. The results were expressed as a percentage of the control pooled serum. CH50 was measured by Mayer's method (Mayer, 1961).

Circulating immune complexes (CIC). Levels of CIC were assayed by the C1q-deviation test (Sobel, Bokisch & Müller-Eberhard, 1975), as follows: serum samples are incubated for 30 min at 56°C to inactivate intrinsic C1q, followed by addition of ^{125}I-C1q to the reaction mixture. Under these conditions, a part of C1q binds to IC in the serum while the rest of C1q remains free. To this reaction mixture sensitized erythrocytes (EA) are added to bind free C1q on EA. By centrifugation, C1q with IC is separated from those with EA. The results of IC assay are expressed as a percentage inhibited by CIC in the test serum.

Statistical analysis. Student's *t*-test and Pearson's correlation coefficients were calculated where appropriate using standard methods.

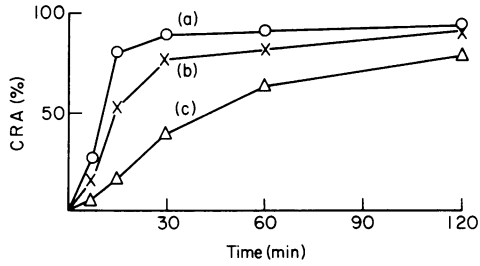

Fig. 1. The effect of incubation time on CRA of sera. Difference is prominent at 30 min. (a) = RA; (b) = normal; (c) = SLE.

RESULTS

Evaluation of the condition for assaying CRA

One volume of GPB^{++} containing IC (40 μg of Ab/ml) was mixed with an equal volume of normal human serum, RA serum or SLE serum. The mixtures were immediately incubated at 37°C. At timed intervals, samples of 20 μl were withdrawn into tubes containing 0·5 ml of 0·05% sheep erythrocytes. After centrifugation, both pellets and supernatants were assayed for radioactivity and then CRA was calculated. As shown in Fig. 1, the results indicated that a reaction time of 30 min best discriminated CRA of the test sera from a normal serum.

CRA in patients with SLE

CRA of sera from SLE patients was $54 \pm 26{\cdot}1\%$ (mean $\pm$ 1 standard deviation) and was decreased as compared to the normal value of $92 \pm 21\%$ ($P < 0{\cdot}001$). CRA of sera collected during active disease was $35 \pm 17{\cdot}8\%$ and that of inactive disease was $64{\cdot}1 \pm 24{\cdot}1\%$; there was a highly significant difference between these two groups ($P < 0{\cdot}001$) (Fig. 2). Interestingly, 20 of 23 sera collected during active disease demonstrated CRA values less than 50% of the control pooled serum. On the other hand, CRA of 29 of 40 sera from inactive disease exceeded the 50% level.

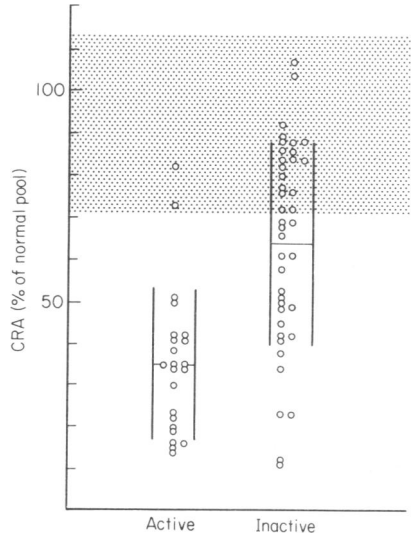

Fig. 2. CRA of sera from active and inactive SLE patients. Shaded area represents the normal range (mean $\pm$ 1 SD). Sera from active stage demonstrated significantly lower CRA than that of sera from inactive stage.

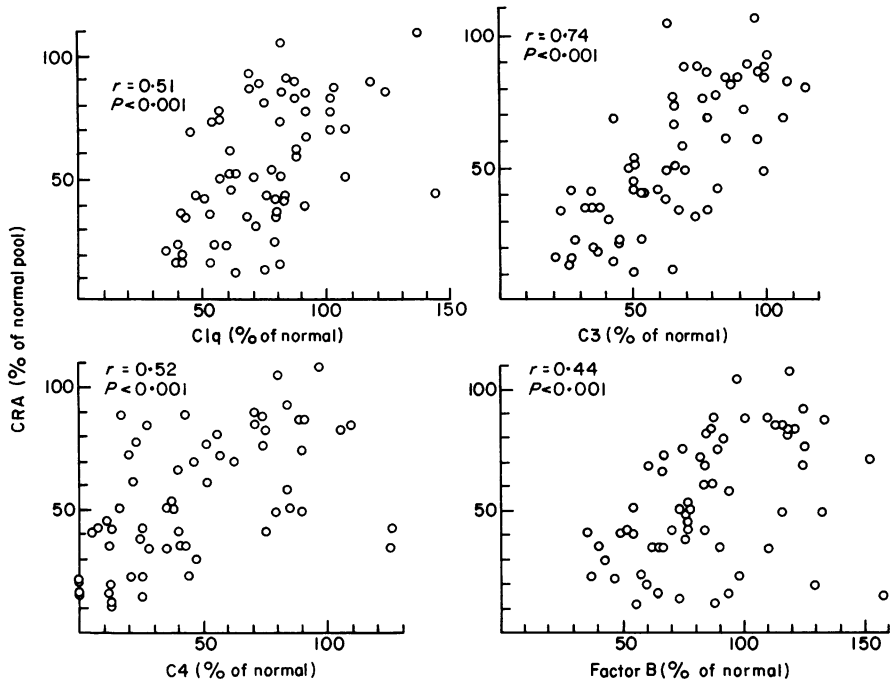

Fig. 3. Correlation between CRA and C1q, C4, C3 and factor B. r = Pearson's correlation coefficient.

Correlation between CRA and CH50, C1q, C4, C3 and factor B

CRA in SLE sera correlated closely with the CH50 ($r = 0{\cdot}72$, $P < 0{\cdot}001$) and with levels of C1q, C4, C3 and factor B (Fig. 3). These correlation coefficients exceeded those between the CH50 and the respective complement components (Table 1).

Table 1. Pearson's correlation coefficient between CRA, CH50 and complement components

	C1q	C4	C3	Factor B
CRA	0·51	0·52	0·74	0·44
CH50	0·40*	0·49	0·71	0·36**

$P < 0{\cdot}001$ *$P < 0{\cdot}002$ **$P < 0{\cdot}003$

Serial determinations of CRA, CIC, C4 and C3 in patients with SLE

Changes of CRA were followed in two patients with SLE during prednisolone therapy (Fig. 4).

In case 1, a diagnosis of SLE was made when she was 20 years old. There were pancytopenia, arthralgia, LE cells, photosensitivity and a high titre of anti-DNA Ab. Following corticosteroid therapy, CRA and levels of C3 and C4 returned to normal in association with the reduction of CIC level. The titre of anti-DNA Ab also became normal. The patient was clinically inactive in 2 months.

In case 2, a diagnosis of SLE was made on the basis of massive proteinuria, arthralgia, LE cells, discoid lupus and high titre of anti-DNA Ab. Following corticosteroid therapy, CRA and levels of C3 and C4 improved and that of CIC decreased.

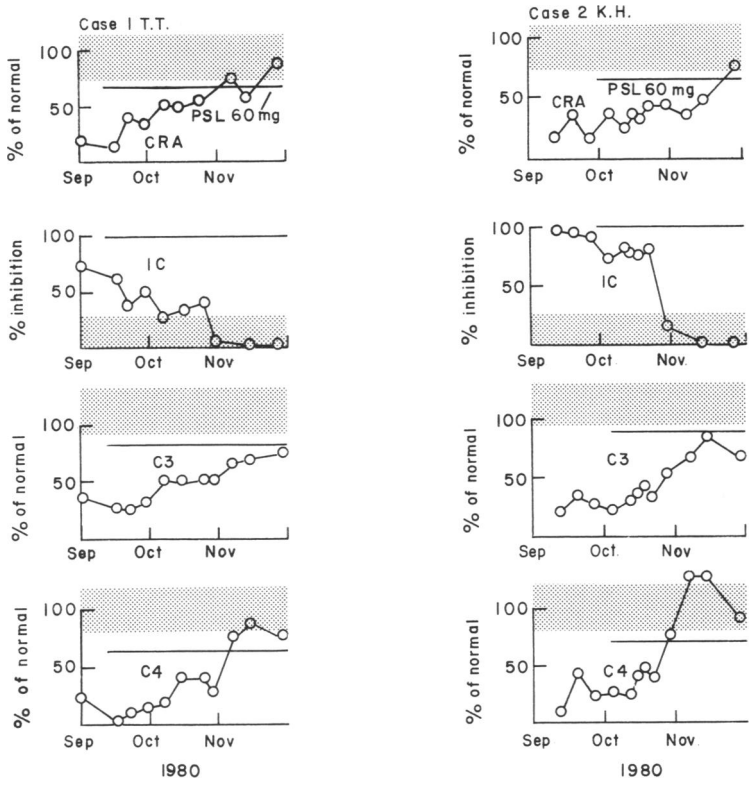

Fig. 4. Serial changes of CRA in SLE patients. Shaded areas represent the normal range (mean ± 1 SD). In both cases, the correlation between CRA and the complement components was positive, while that between CRA and CIC was negative.

DISCUSSION

This is the first detailed report of the determination of CRA in sera of SLE patients. Our studies showed that CRA of active SLE was significantly lower than that of inactive SLE and that CRA changed in association with clinical activity of the disease (Fig. 2). An arbitrarily determined borderline at 50% of the control value was helpful in differentiating CRA of active from inactive SLE. From these facts, we may conclude that CRA is useful for the assessment of disease activity.

It is of interest that the correlation coefficients between CRA and complement components were better than those between CH50 and the respective components. C3 best correlated with CRA, consistent with the previous reports in mice (Czop & Nussenzweig, 1976). In addition, the fact that CRA also correlated with levels of C1q and C4 indicates that the complement components of the classical pathway, in addition to the alternative pathway, are closely involved in CRA *in vivo*. Although factor B is essential for CRA (Takahashi, Tack & Nussenzweig, 1977), its correlation coefficient to CRA was lower than those of C1q and C4. The reason for this may be related to the large amount of factor B in serum.

The presence of CIC in the sera of SLE patients has been well documented. Consistent with the finding that large-sized CIC are formed in complement-deficient states *in vitro* (Schifferli, Bartolotti, & Peters, 1980), large-sized CIC have been confirmed to be present in some cases of active SLE (Tung, DeHoratius & Williams, 1981). In our study, it was clearly shown that the level of CIC decreased as the levels of C3, C4 and CRA increased. These observations lead us to postulate that large-sized CIC in SLE may be subject to solubilization through CRA. Solubilized complexes then become trapped effectively by the reticuloendothelial system, as complexes at an early stage of

solubilization retain binding affinity for the immune adherence receptors (Takahashi, Takahashi & Hirose, 1980). This clearance is accompanied by further improvement of clinical activity.

It remains controversial, however, whether complement plays an important role for the clearance of soluble immune complexes. It has been shown that the liver uptake of soluble complexes is independent of the complement system in animals (Mannik, 1980; Harkiss & Brown, 1981), while complement has been shown to markedly enhance the macrophage uptake of immune complexes (Snick & Masson, 1978; Kijlstra, van Es & Daha, 1979). Our results are compatible with the latter and support the possibility that complement deficient patients may be also deficient in their ability to dispose of CIC with subsequent development of a lupus-like process (Zeitz *et al.*, 1981). Further evidence is needed to substantiate this hypothesis.

The authors gratefully acknowledge the expert technical assistance of Ms Masayo Yamaguchi. This work was supported in part by a grant from the Ministry of Health and Welfare, Research Committee for the Treatment of Connective Tissue Diseases, Japan.

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