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Complement-mediated solubilization of immune complexes. Solubilization inhibition and complement factor levels in SLE patients

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SUMMARY

Thirty-two of 36 serum samples from 19 SLE patients showed reduced capacity to mediate complement-dependent solubilization of immune complexes (IC). SLE patients with nephritis exerted the lowest complement-mediated solubilization capacity (CMSC) whereas sera from patients with inactive disease gave the highest CMSC values, with three out of four samples within the normal reference range. Thirty-five of the 36 serum samples showed inhibition of CMSC in a newly developed CMSC inhibition assay. The strongest CMSC inhibition was exerted by sera from newly discovered cases of SLE who received no medical treatment and the lowest inhibition by sera from patients with inactive disease. There was a significant negative correlation between CMSC and CMSC inhibition ($r = -0{\cdot}67$, $P < 0{\cdot}001$). Sera with low concentrations of C1q, C3, factor B or high C3d levels showed markedly reduced CMSC values. Pronounced CMSC inhibition was observed only in samples with normal or high factor H values. No significant correlation was found between CMSC or CMSC inhibition and circulating IC levels, but pronounced CMSC inhibition was registered only in strongly IC positive sera.

Keywords complement solubilization complement factors immune complexes systemic lupus erythematosus

INTRODUCTION

The capacity of serum to exert complement-mediated solubilization (CMS) of pre-formed immune complexes (IC) is dependent upon the alternative activation pathway of complement, and the functional state of the classical pathway influences the initial kinetics of the reaction (Czop & Nussenzweig, 1976; Takahashi *et al.*, 1978). A reduced CMS capacity (CMSC) has been observed in SLE sera and the diminished CMSC has by some investigators been found to correlate with the concentration of certain complement factors, to CH_{50}, and/or to the presence of IC in these sera (Aguado *et al.*, 1981; Sakurai *et al.*, 1982, Schifferli *et al.*, 1981).

The presence of incompletely solubilized IC in some IC positive SLE sera was recently indicated (Baatrup *et al.*, 1983a). Incompletely solubilized IC should be capable of activating the complement system unlike the so called endstage IC. In the present study a CMSC inhibition assay was employed in order to investigate further the presence of such incompletely complement solubilized IC in the

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SLE sera. In addition, the relationship between the concentration of several complement factors and CMSC and CMSC inhibition was studied.

MATERIALS AND METHODS

Reagents and solutions. EDTA (tetrasodium salt) from Sigma, St Louis, Missouri USA. Agarose: Litex HSA from Litex Industri, Glostrup, Denmark and Indubiose A37, Pharmaindustrie, Clichy, France. PBS^{++}, phosphate-buffered saline, (PBS) containing 140 mM NaCl, 10 mM phosphate, 0·15 mM Ca^{++}, 0·5 mM Mg^{++} and 15 mM NaN_3. EDTA-PBS: PBS containing 20 mM EDTA.

Patients. Thirty-six serum samples were obtained from 19 patients (17 females and two males, age 15–60, mean 30 years) with verified SLE (ARA criteria). Three samples were drawn from each of five patients, two samples from seven patients and one sample from seven patients. All were inpatients at Odense University Hospital or Rigshospitalet, Copenhagen. Patients without any of the ARA symptoms at the time of sampling were designated disease inactive. All cases of nephritis (four patients) were verified by laboratory parameters and by biopsy. Five cases of newly discovered SLE received no medical treatment, whereas the remaining patients were given steroids and/or non-steroid anti-inflammatory drugs (NSAID), and/or anti-neoplastic agents (azathioprine or cyclophosphamide).

Normal donor sera. The reference range was based on sera obtained from 24 healthy medical students and 24 blood donors (20 females and 28 males, age 22–65, mean 39 years). No influence of age or sex on CMSC was observed.

Serum handling. Blood samples were collected in ordinary glass tubes and the sera were deep frozen in small aliquots immediately after separation. Samples once thawn were not reused. Unless otherwise indicated, serum dilutions were with PBS^{++}.

CMSC assay. This assay was performed as previously described (Baatrup *et al.* 1983b). In brief: test sera and controls were diluted 1:2 in PBS^{++} and incubated at 37°C for 10 min. ^{125}I-BSA–anti-BSA IC, prepared at maximal precipitation (molar Ab/Ag ratio 3·4) and diluted in PBS with 10% sucrose, were added (2·4 μg/ml of 1:2 diluted serum) and the tubes were incubated at 37°C for 120 min. Two hundred microlitres samples were transferred to 2·8 ml ice cold PBS buffer containing 20 mM EDTA to stop the solubilization reaction. The tubes were centrifuged (3,000**g**, 10 min) and the percentage solubilized (not precipitated) IC was calculated. As controls were used two normal donor sera, three SLE sera and one heat-inactivated normal serum (56°C, 30 min), all with known CMSC values. The CV_T was 0·10–0·17.

CMSC inhibition assay. This assay was performed as previously described (Baatrup *et al.*, 1983a). In brief: 2,100 μl 1:2 diluted NHS was mixed with 200 μl 1:2 diluted test serum or control serum and incubated at 37°C for 20 min. ^{125}I-BSA–anti-BSA IC diluted in PBS (without sucrose) were added (2·4 μg/ml 1:2 diluted serum) and the CMS was calculated after 120 min incubation at 37°C. As controls were used the same sera as in the CMSC assay. When measuring the inhibition of CMS the inhibition (dilution effect) obtained with the heat-inactivated NHS control was subtracted before calculating the specific inhibition.

IC determination. The content of IC in the test sera was determined by the polyethylene glycol complement consumption (PEG-CC) assay (Brandslund *et al.*, 1981a) and the C1q protein A enzyme linked immunosorbent assay (C1q-PA-ELISA) (Bjerrum *et al.*, 1983).

Complement determinations. C1q, C4, C3/C3c, factor B, factor H and factor I were measured by rocket immunoelectrophoresis as earlier described (Laurell, 1972; Weeke, 1973) and C3d by double decker rocket immunoelectrophoresis (DDRIE) (Brandslund *et al.*, 1981b). The antisera against C1q, C4, C3c and C3d were purchased from Dakopatts, Copenhagen, against factor B from Behringwerke Ag, Marburg, and against factors I and H from Seward, London. Sera to be tested were applied in a 1:2 dilution in the electrophoresis buffer, except in the C3d DDRIE assay, where undiluted serum was used. A serum pool from 15 healthy donors was used as reference. In the C3d determinations serum with totally converted C3 (serum incubated 5 days at 37°C with 15 mM NaN_3) was used as standard. CV_T in the complement determinations was 0·04–0·06.

RESULTS

CMSC and CMSC inhibition patterns

The CMSC and CMSC inhibition patterns of the 36 serum samples from 19 SLE patients are shown in Fig. 1. The mean CMSC of all SLE samples was 35%, whereas the mean of the normal donor sera was 76%. Only four SLE samples (11%) were within the reference CMSC range and three of these were obtained from patients showing no clinical disease activity at the time of sample collection. Of 32 samples obtained from patients with active disease only one sample fell within the reference CMSC range (66–85%).

Four patients had kidney involvement as judged by clinical and laboratory parameters and by biopsy. Six serum specimens from these patients exhibited a mean CMSC of 26% and no sample fell within the normal range. Five samples obtained from five patients with newly discovered disease who received no medical treatment, all had CMSC values clearly below the reference range with a mean CMSC of 23%.

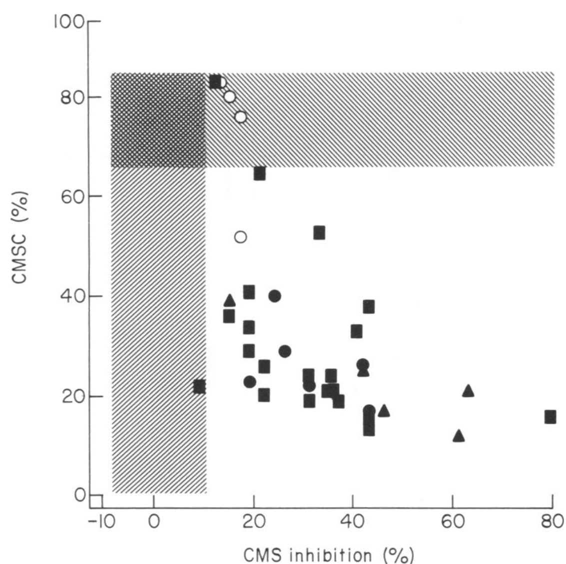

Fig. 1. CMSC and CMSC inhibition of 36 serum samples from 19 SLE patients. The shaded areas indicate the reference range (mean ± 2 s.d.). CMSC and CMSC inhibition were determined in the standard assays as described in Methods. Each value is the mean of duplicate determinations. Samples from patients with active disease, who received medical treatment (steroids and/or NSAID, and/or anti-neoplastic agents) (■), patients without active disease at the time of sample collection, and who received medical treatment (○), patients with nephritis (●) and patient with active disease, receiving no medication (▲). Correlation between CMSC and CMSC inhibition ($r = -0{\cdot}67$, $P < 0{\cdot}001$).

Also shown in Fig. 1 are the CMSC inhibition values. Only one serum sample was within the reference range. The mean CMSC inhibition of all SLE sera was 31%. Mean of the inhibition for the six samples from patients with glomerulonephritis was also 31%, and for the serum specimens from patients with inactive disease 17%. Sera from the five untreated patients with newly discovered disease demonstrated the most potent inhibition (mean 46%). There was a significant negative correlation between CMSC and CMSC inhibition ($r = -0{\cdot}67$, $P < 0{\cdot}001$).

CMSC, CMSC inhibition and complement factor levels

A possible correlation of values obtained by immunochemical determination of each of the individual complement factors Clq, C4, C3/C3c, and factors B, H, and I with CMSC or CMSC inhibition was assessed by Spearmann non-parametrical correlation analysis. No correlation

coefficient exceeding 0·4 was found. However, Fig. 2 a,b & c show markedly reduced CMSC in all serum samples with Clq, C3/C3c or factor B values below 50% of the reference pool value. The same phenomenon, that is, no occurrence of samples with normal or near normal CMSC in combination with reduced C factor levels was observed but to a less extent for factor I. In addition, high C3d values were associated with low CMSC values.

The CMSC inhibition tended to decrease with declining factor H values, but again the correlation was not significant (Fig. 2d). Furthermore, factor B conversion in the SLE sera examined by rocket immunoelectrophoresis did not correlate with either CMSC or CMSC inhibition.

CMSC, CMSC inhibition and IC

The IC contents of the SLE sera were examined by the Clq-PA-ELISA assay. No clear correlation was found with either CMSC or CMSC inhibition, However, all samples with marked CMSC inhibition values exhibited high IC levels in this assay.

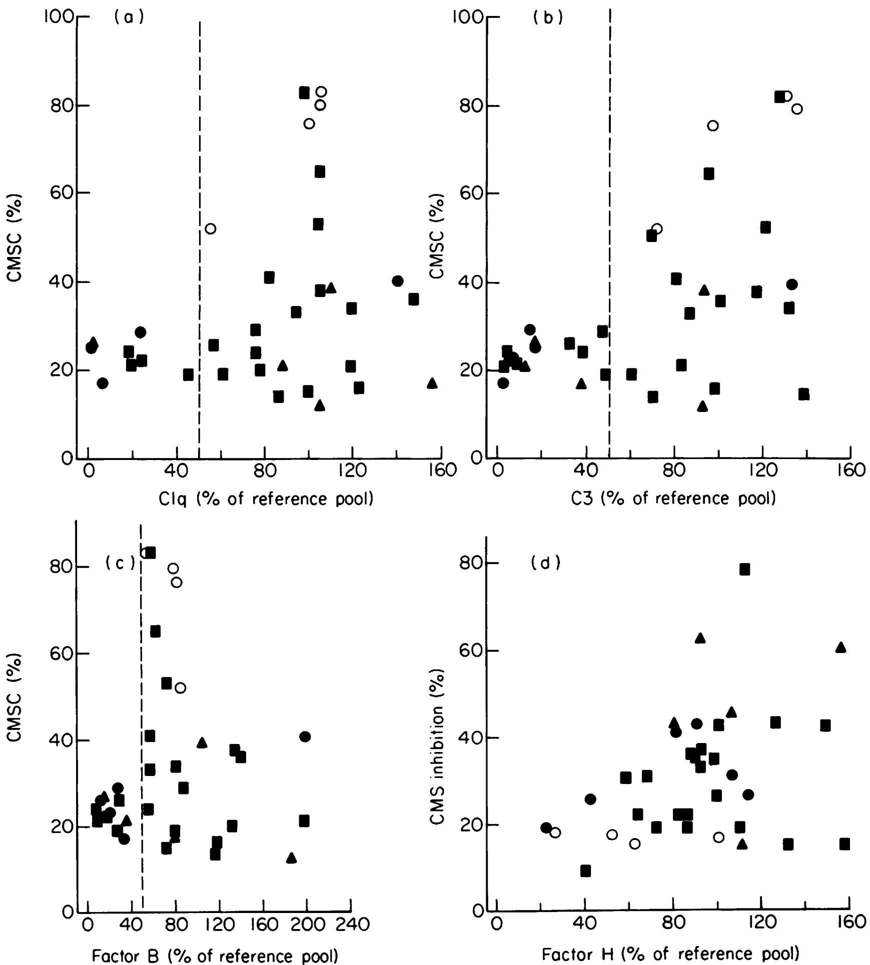

Fig. 2. Relation between complement components and CMSC or CMSC inhibition. The relationship between CMSC and Clq, C3 and factor B, respectively, is shown in Fig. 2 a, b, c. No significant correlation was found but at complement factor levels below 50% of the references (the dashed lines), all samples show strongly reduced CMSC values. In Fig. 2d the relationship between CMSC inhibition and factor H is shown. High inhibition is seen only at factor H levels exceeding 75% of the reference. All values are means of duplicate determinations. Symbols as in Fig. 1.

The CIC levels were also measured by the PEG-CC assay. Strongly positive responses were found with sera showing a high CMSC inhibition, but there was no significant correlation with either CMSC or CMSC inhibition.

CMSC/CMSC inhibition and plasmapheresis

Three patients with active SLE received a total of four plasmapheresis treatments during the period of sample drawing. In one case, 1,000 ml of donor blood was given during the treatment, the other patients received albumin–saline. Samples were drawn just before and after each treatment and they were analysed for CMSC and CMSC inhibition. The treatment resulted in a significant increase in CMSC in one patient and in no demonstrable changes in the other two patients (three treatments). The plasmapheresis effected a significant fall in CMSC inhibition in three of the treatments and no change in the fourth. However, serum from the last patient already had a very low CMSC inhibition before treatment was initiated.

DISCUSSION

The CMSC assay performed according to the previously described method (Baatrup *et al.*, 1983b) has a high power to discriminate between normal donor sera and SLE sera. Thus only four out of 36 SLE serum samples were within the reference range (mean ± 2 s.d.) and three of these sera were from disease inactive patients.

In the CMSC inhibition assay only one of 36 SLE sera was within the normal reference range. When performed on serum, this inhibition assay does not give any indication of the nature of the inhibitory substance(s) in SLE sera. However, we have previously shown (Baatrup *et al.*, 1983a) that a major part of the inhibitory activity could be recovered in PEG precipitated (final concentration 2·75%) material from SLE sera. This would be compatible with the hypothesis that CIC are primarily responsible for the inhibition. Furthermore, BSA–anti-BSA IC incubated with normal donor serum under non-C solubilizing conditions, and then precipitated by 2·75% PEG-6000 similarly inhibited the CMSC. Finally, the addition of human serum as complement source to SLE sera, followed by incubation at 37°C resulted in solubilization of endogeneous IC in the sera and reduced inhibition. These findings point to incompletely solubilized IC as the inhibitory agent, but the possibility that other soluble factors such as nephritic factor may inhibit CMS has not been excluded. The inhibition test as performed in the present study gives an estimation of the total CMS inhibitory activity.

The reduced CMSC and the increased CMSC inhibition in the majority of SLE sera may be the result of an imbalance between the production of IC and the capacity of the complement system to solubilize these IC. This could explain some of the symptoms seen in SLE since the fully solubilized endstage IC are less phlogistic (Takahashi & Takahashi, 1981). Furthermore, the degree of complement-mediated solubilization of endogeneous IC determines the reactivity of such IC to complement receptors on both erythrocytes and leucocytes (Miller, Saluk & Nussenzweig, 1973; Miller & Nussenzweig, 1974) and this in turn is probably of importance for the normal clearance of IC.

Earlier reports (Aguado *et al.*, 1981; Sakurai *et al.*, 1982) have claimed significant correlations between CMSC and C1q, C4, C3 and factor B concentrations. We could not confirm such a clear correlation between CMSC or CMSC inhibition and any single complement factor investigated (C1q, C4, C3/C3c, C3d, B, H and I). However, low levels of C1q, C3/C3c, C4 and B were generally associated with reduced CMSC. Of the 36 SLE sera investigated 24 had values below 50% of the reference pool value for at least one of the factors C1q, C4, C3/C3c and factor B. The CMSC inhibition on the other hand could not be related to reduced complement factor concentrations. High inhibition was seen only when the factor H values exceeded 75% of the reference. Other authors (Takahashi & Takahashi, 1981) have reported an association between depressed CMSC and high factor H concentration. No significant correlation between CMSC or CMSC inhibition and CIC was demonstrated. However, high CMSC inhibition values were seen only in sera with a high IC content, compatible with the hypothesis that endogeneous IC contributed to the inhibition.

The preliminary investigation of the effect of plasmapheresis treatment suggested that CMSC inhibition can be reduced by this treatment.

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