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The value of three immune complex assays in the management of systemic lupus erythematosus: an assessment of immune complex levels, size and immunochemical properties in relation to disease activity and manifestations

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SUMMARY

Sixteen patients with systemic lupus erythematosus, fourteen with renal involvement, have been studied over a 30-month period. Circulating immune complex levels have been assayed by three techniques. The results of tests on 141 sequential sera suggest that the three assays detected overlapping populations of the range of complexes present. Immune complex levels are shown to be good markers of disease activity and certain immunochemical properties of the complexes emerged as better markers than others. Immune complex size is a more important determinant of disease manifestation than are overall immune complex levels. High doses of intravenous methyl prednisolone, which did not reduce overall complex levels, reduced levels of those complexes (molecular weight 600,000–900,000 Daltons) associated with renal involvement.

INTRODUCTION

Neither anti-DNA antibody or serum complement levels have been found to be accurate guides to disease activity in systemic lupus erythematosus (SLE) (Holborow, 1978; Cameron *et al.*, 1976). Levels of circulating immune complexes may be more valuable in the management of these patients since the deposition of such complexes is a major cause of tissue damage in the disease (Cochrane & Koffler, 1973). Each of the currently available methods of immune complex detection is limited by its ability to measure only one of the immunochemical properties complexes may possess and difficulties arise when attempts are made to compare results of one assay with those of another based on a different principle (Lambert *et al.*, 1978).

We have monitored disease activity in a group of patients with SLE; in an attempt to detect a range of complex functions we have used three assay techniques and have estimated the size range of the complexes present in our patients. The results have been correlated with estimates of disease activity, particular disease manifestations and steroid treatment and with other markers of immunochemical injury.

PATIENTS AND METHODS

Patients. Sequential studies were performed in sixteen patients with SLE followed for periods of

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up to 30 months. During this period all but two of the patients fulfilled the American Rheumatism Association Criteria (Cohen *et al.*, 1971) for the diagnosis while in the other two there were good clinical, immunochemical and histological grounds for making the diagnosis. Fourteen of our patients have had clinical, biochemical and histological evidence of renal involvement. All patients had active clinical disease at some time during the study.

Immune complex assays

Blood was kept at 37°C following venesection, separated within 3 hr and aliquots of sera stored at −70°C.

Three immune complex assays were used. Two are dependent on the inhibition of agglutination of latex IgG as described by Levinsky & Soothill (1977).

L_G assay. Anti-human IgG was raised by inoculation of rabbits with pure human IgG. The IgM fraction of rabbit serum was separated by Sephadex G-200 gel filtration and light chain determinants absorbed onto Sigma cell 50 μ (Sigma Chemicals) activated by cyanogen bromide and coated with $F(ab')_2$ prepared by pepsin digestion of human IgG (Hudson & Hay, 1976). The pure rabbit IgM anti-IgG was used to aggregate 1·15–μm diameter latex (Coulter Limited) coated with human IgG. Whole C1 will inhibit aggregation of latex IgG by IgM anti-IgG and C1q will itself aggregate the latex so sera were decomplemented prior to testing. The C1 complex was disrupted by incubation with EDTA and the released C1q adsorbed onto Sepharose 4B gel coated with IgG. Any rheumatoid factors, which would interfere in the assay, were also removed by this step. During decomplementation sera were diluted 1 : 10 reducing monomeric immunoglobulins to levels at which they would not interfere in the assay. Following the decomplementation step the degree to which aggregation of latex IgG by rabbit IgM anti-IgG was competitively inhibited by decomplemented sera was measured using a Coulter Counter ZBI with a 50-μm orifice.

The inhibition of agglutination was calculated from measurements of the monomeric latex particles in an unaggregated control (Co), an aggregated control (C+) and the test reaction (T): $(T-C+)/(Co-C+)\times 100 = \%$ inhibition of agglutination, and is a quantitative measure of IgG-containing complexes in the test serum.

L_C Assay. Pure human Clq was prepared by the method of Yonemasu & Stroud (1971). Clq-binding complexes in serum decomplemented as in the L_G assay were detected by their ability to inhibit competitively the agglutination of latex IgG by C1q, the percentage inhibition being calculated as for the L_G assay.

By adjusting IgM and C1q dilutions in the agglutination reactions the sensitivity of the L_G and L_C assays could be altered and were so set that 90% of a panel of normal sera caused less than 20% inhibition of agglutination.

The third assay used was a solid phase C1q assay (S_C) (Hay, Nineham & Roitt, 1976).

S_C Assay. Polystyrene tubes were coated with human C1q and serum, treated with EDTA, was added. After incubation and washing, radiolabelled anti-human IgG was added to the tubes and, after further incubation and washing, the bound radioactivity counted. The count represents a quantitative estimate of C1q-binding, IgG-containing complexes in the serum. An upper limit of normality for the assay was set using a panel of sera, at 0·4 μg bound radiolabelled anti-IgG per ml of serum.

Immune complex size was estimated by gel filtration on a Sepharose 6B column calibrated with proteins of known molecular weight. Two-millilitre samples of test sera were run on the column and all the fractions between the void volume of the column, which contains proteins of molecular weight 4×10^6 and greater, and the albumin peak were tested for immune complexes using the L_G and L_C assays. CH50, C1q, C4, C3, factor B and anti-DNA antibody were measured by standard techniques (Mayer, 1961; Mancini, Carbonara & Heremans, 1965; Pincus *et al.*, 1969).

Disease activity was assessed in each patient by three scoring systems:

(A) Clinical activity score: a subjective clinical estimate by the physician who looked after the patients: (1) inactive, (2) active and (3) florid.

(B) Disease manifestation score: every chemical, haematological and biochemical abnormality thought to be the result of active lupus and present at any one time in a patient was given a score of 1,

Table 1. Disease manifestations in sixteen patients

Score 1 for each of
Arthralgia/arthritis
Rash
Fever
Hepatosplenomegaly lymphadenopathy
Alopecia
Pleurisy/pericarditis
Raynaud's phenomenon
Oral ulcers
Neurological manifestations
Retinopathy
Neuropathy
Fits
Psychosis
Renal manifestations
Proteinuria > 1 g/day
Sediment
Rising serum creatinine
Hypertension
Haematological manifestations
Coombs +ve haemolysis
Thrombocytopenia
Neutropenia

and the total added up. The maximum score achieved by any of our patients was 6. Table 1 lists the manifestations of lupus present in our patients during this study.

(C) An activity score proposed by Cameron and weighted towards renal disease (Cameron *et al.*, 1976).

Statistics

Linear regression, correlation coefficients, Student's t-test and χ^2 were calculated where appropriate using standard methods.

RESULTS

Immune complex assays

Fig. 1 shows the results from 141 sequential sera assayed for immune complexes. Sixty-four per cent were positive in the L_G assay, 52% in the L_C assay and 55% in the S_C assay. Not all sera were tested in each assay, but of the 141, 77% were positive in at least one assay. This figure rose to 84% in the seventy-three sera to which all three assays were applied.

Correlations occurred between L_G and L_C assay results (Fig. 2; $r=0{\cdot}625$, $P<0{\cdot}001$) and L_G and S_C assay results (Fig. 3; $r=0{\cdot}426$, $P<0{\cdot}001$). There was a population of sera which, positive in the L_G assay, were negative in the S_C, suggesting that IgG-containing non-C1q-binding complexes were present. L_C and S_C assay results did not correlate significantly.

Immune complexes and disease activity

Table 2 summarizes the correlations between our three immune complex assays and the three disease activity scores. The L_G and S_C assays correlated with disease activity assessed by two of the scoring systems but not with that of Cameron. No correlations emerged for the L_C assay.

Fig. 4 shows the correlation between L_G assay results and disease manifestation score and shows that there is a considerable overlap in results, particularly between low levels of activity and inactive disease.

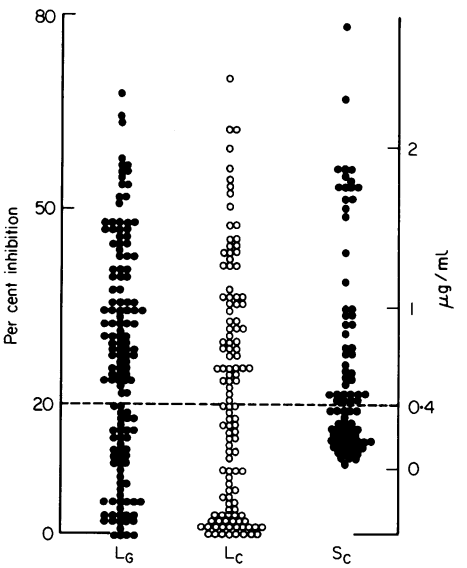

Fig. 1. Immune complex levels by three assays in sequential sera from sixteen SLE patients. Dotted line shows upper limit of normal in each assay. L_G assay (141 sera) 64% positive, L_C assay (119 sera) 52% positive, S_C assay (81 sera) 55% positive.

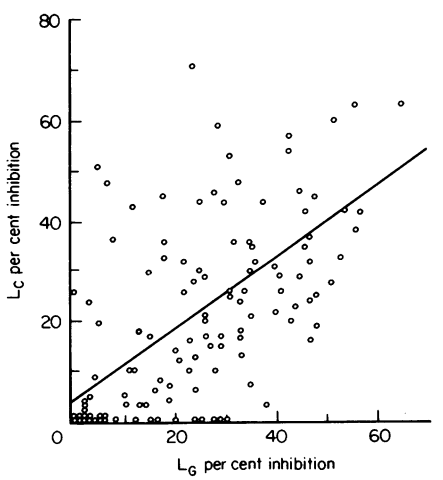

Fig. 2. Correlation between L_G and L_C assay in 118 lupus sera ($r=0{\cdot}625$, $P<0{\cdot}001$).

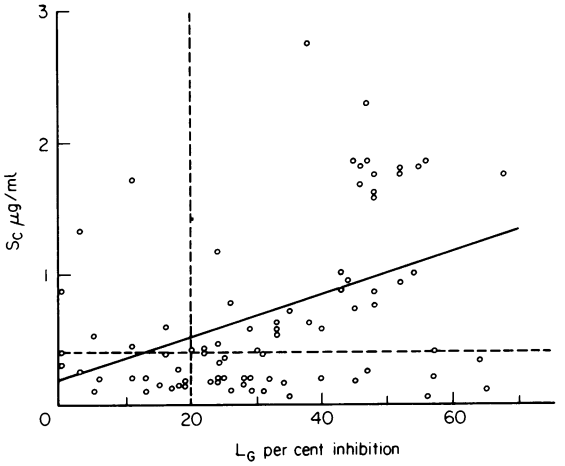

Fig. 3. Correlation between L_G and S_C assay in eighty-one lupus sera ($r=0{\cdot}426$, $P<0{\cdot}001$). Dotted lines show limits of normality in each assay. There is a population of sera positive in the L_G assay which is negative in the S_C, presumably due to non-C1q-binding IgG complexes.

Table 2. Correlation between disease activity and immune complex levels

	L_G	L_C	S_C
Clinical activity score	0·44	n.s.	0·31
Disease manifestation score	0·45	n.s.	0·615
Cameron's activity score	n.s.	n.s.	n.s.

$P<0{\cdot}001$ for each r value shown. n.s.=No significant correlation.

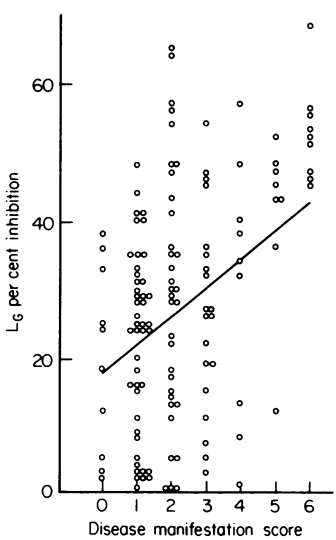

Fig. 4. Correlation between disease manifestation score and L_G complex levels in 134 lupus sera ($r=0{\cdot}45$, $P<0{\cdot}001$).

Complement and disease activity

In individual patients changes in the complement cascade, particularly CH50 levels, were accurate predictors of activity. However, no correlations were apparent between disease activity and levels of individual complement components in the group as a whole. There was some evidence that activation of the complement cascade as indicated by lowered levels of several complement components correlated with disease activity, for significant correlations emerged using a complement component score in which a point was awarded for each low complement factor in a serum (Table 3).

Table 3. Disease activity and complement abnormality score

	Clinical activity score		Disease manifestation score		Cameron activity score	
	1	2 & 3	0–3	4–6	0–3	4–6
Complement abnormality score						
0–2	38	31	61	7	57	10
3–5	7	21	19	10	23	5
	$\chi^2=7{\cdot}265$		$\chi^2=8{\cdot}17$		n.s.	
	$P<0{\cdot}01$		$P<0{\cdot}01$			

Complement and immune complex levels

CH50, C3 and factor B levels showed significant negative correlations with S_C results ($r=-0{\cdot}46$, $P<0{\cdot}001$; $r=-0{\cdot}63$, $P<0{\cdot}001$; $r=-0{\cdot}354$, $P<0{\cdot}05$ respectively) and a correlation exists between the complement abnormality score and S_C complex levels ($r=0{\cdot}55$, $P<0{\cdot}001$). L_G and L_C assay results did not correlate with complement abnormality score or any individual complement component.

Anti-DNA antibody levels

Anti-DNA antibody levels showed no correlation with disease activity in our patients. Neither was there a relationship between anti-DNA antibody and complex or complement levels.

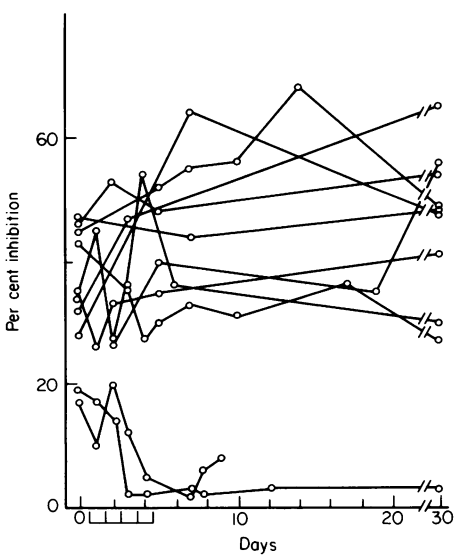

Fig. 5. Serial levels of immune complexes detected by the L_G assay during eleven courses of 'pulse' therapy.

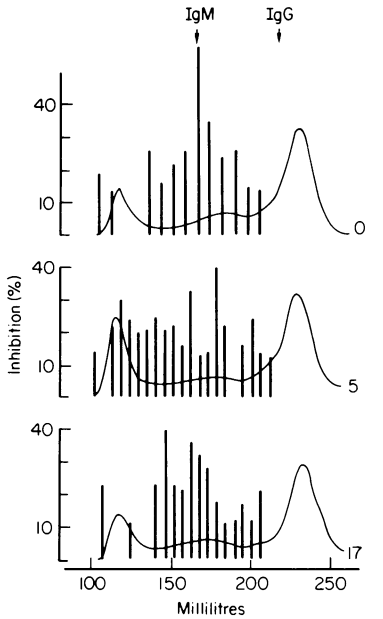

Fig. 6. Effect of 'pulse' therapy on immune complex size (L_G assay) in a patient with active renal disease. Day 0 (top) prior to treatment, day 5 (middle) at the end of course, day 17 (bottom) 12 days after discontinuing treatment. The continuous line shows the elution profile of serum proteins and the volumes at which monomeric IgM and IgG come off the column are marked. The vertical bars express quantitatively the amount of complexes present in each column fraction.

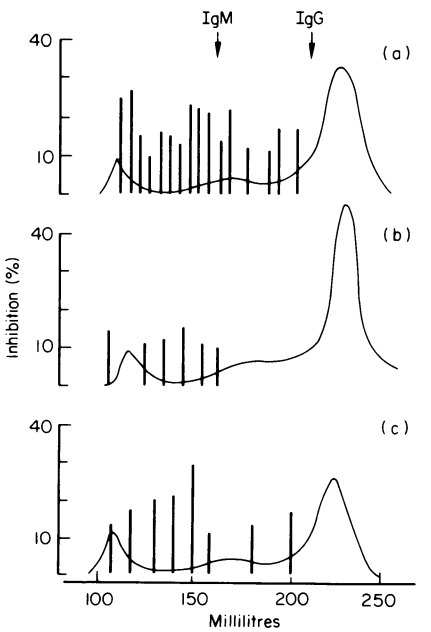

Fig. 7. Immune complex size in three patients with active lupus but no evidence of renal disease (L_G assay). (a) Haemolytic anaemia and pulmonary involvement; (b) peripheral vascular disease; (c) fever, rash, hepatosplenomegaly and lymphadenopathy.

Effect of 'pulse' steroid therapy

Short courses of high doses of intravenous methyl prednisolone, 'pulse' therapy (usually 1 g daily for 5 days) followed by low maintenance doses of oral steroids were used in patients with severe renal and non-renal lupus. Immune complex levels were followed during eleven such 'pulses'. In two of the eleven, L_G complex levels remained normal and in the other nine there was no evidence of a reduction in immune complex levels over periods in excess of 30 days (Fig. 5). The same pattern was apparent in L_C and S_C complex levels.

Immune complex size

Immune complex size has been measured in ten sera from six patients. In two patients with active renal disease the highest levels have been found in the fractions just below those in which monomeric IgM (mol. wt 900,000) comes off the column (Fig. 6), while in non-renal lupus there is no such preponderance of middle range complexes (Fig. 7). Complex size was studied in sequential sera from two patients with active renal lupus treated with 'pulse' therapy and a similar pattern seen in both. A fall in levels of middle range complexes was seen by the end of the 'pulse' and was accompanied by a rise in levels of high molecular weight complexes. These high molecular weight complexes subsequently disappeared and the middle range complexes began to reaccumulate (Fig. 6).

DISCUSSION

Our results demonstrate the limitations of applying only one of the currently available immune complex assays to the study of immune complex disease. Using three assay techniques each of which identifies a separate immune complex function we have been able to detect a wider range of complexes than with any single assay and such multiple assays detect overlapping sections of the spectrum of immune complexes present. The identification of these subpopulations is important since it is clear that certain immunochemical properties of complexes, as detected by different assays, are more closely related to disease activity than others. Two of our three immune complex assays correlate with two of the measures of clinical activity we used. That the distinction between levels in 'inactivity' and 'low grade activity' is not clear-cut may be due as much to the inadequacies of the clinical scoring systems as to the assays.

Any method of assessing activity in lupus has its limitations. Immunological damage may proceed at a level at which clinical or biochemical evidence of injury is absent and many patients without any clinical evidence of renal disease have evidence of immune complex deposition in their kidney biopsies (Cruchaud *et al.*, 1975). Furthermore, any weighting of the score towards phenomena which are felt to be more significant may simply express the bias of the designer and the assumption that those factors given prominence are more directly related to immunological activity may prove invalid. Factors other than activity may be expected to influence complex levels. Steroids, used to treat active disease in all our patients, have an adverse effect on macrophage function (Schreiber, 1977) and so persistence of immune complexes might be predicted in our patients on steroids regardless of their state of disease. Conversely, negative immune complex assay results occurred in some of our patients with active disease. While there is evidence of *in situ* complex formation in animal models of SLE (Izui *et al.*, 1977), even if formed in the circulation, complexes pathogenic by virtue of their propensity to deposit in the tissues might be hard to find in a random serum sample. Recent data (Frank *et al.*, 1979; Lockwood *et al.*, 1979) suggest that immune complex handling is defective in SLE and so levels in individuals may vary widely irrespective of disease activity and the intra-individual variation in levels may therefore reflect more directly changes in activity than the absolute level of complexes measured. Sharp rises in levels may result from the emergence of a specific pathogenic population.

Serum complement and anti-DNA antibody levels have both been proposed as markers of disease activity in SLE. While some authors have claimed very good correlations between anti-DNA antibody levels and activity (Davis, Percy & Russell, 1977) we, like others (Cameron *et al.*, 1976) have not found them helpful, although in our experience the detection of anti-DNA antibody

remains the single best immunochemical guide to diagnosis. Serum complement levels are affected by a variety of influences in SLE. Genetically determined component deficiencies occur while enhanced consumption may account for low levels in active disease (Schur, 1975). A further consideration is that several complement components are acute phase proteins and levels will rise in any inflammatory state. It is not surprising that in such complicated cirumstances clear-cut correlations with disease activity are hard to find. We, like Cameron and co-workers, have not found any single complement component which mirrors disease activity although there are individuals in whom levels accurately predict activity. We have, however, been able to demonstrate a general relationship between complement and activity by measuring multiple complement factors simultaneously. It is then apparent that depressed levels of a number of components occur coincident with active disease. That complement levels showed correlations only with the S_C (IgG, C1q-binding) assay and not the L_C (C1q-binding) assay and that those components which correlated did not include C1q itself, may be a further reflection of the multiple influences on complement activity in SLE.

Short courses of high doses of intravenous methyl prednisolone, 'pulse' therapy, offer advantages in the treatment of severe SLE (Cathcart *et al.*, 1976; Dosa *et al.*, 1978). While some workers have suggested that its efficacy is the result of depression of circulating immune complex levels (Levinsky, Cameron & Soothill, 1977), others have found no effect (Pussell *et al.*, 1978) and we too have found little evidence of a fall in levels. 'Pulse' therapy does not appear to alter the total immune complex load, nor have we detected any resultant immunochemical change in the complexes. Our finding of a preponderance of middle-sized immune complexes in renal SLE agrees with that of Levinsky *et al.* (1977). Like these authors we have not found a similar preponderance in patients without renal involvement. In two renal SLE patients 'pulse' therapy resulted in a rapid decline in levels of these middle range complexes. The appearance of high molecular weight complexes following 'pulsing' may account for the lack of overall decline in complex levels. Since, as already mentioned, steroids impair macrophage handling of immune complexes (Schreiber, 1977) it was to be anticipated that levels would not fall with pulsing, and the high molecular weight complexes which appear are just those, which, by virtue of their size, would normally be well handled by the reticuloendothelial system (Cochrane & Koffler, 1973). Our data suggest, however, that 'pulse' therapy may directly affect immune complex levels, causing a redistribution of their size, although we do not have sufficient information to comment on the relationship of this change to the beneficial effects of therapy.

The maximum information on the state of disease in a patient with SLE will only be obtained by applying multiple immunochemical tests, including anti-DNA antibody, complement and immune complex measurements which between them appear to detect different but overlapping areas of the lupus diathesis. While specific tests such as anti-DNA antibody remain the best laboratory tools for diagnosis in SLE, in our experience sequential measurements of immune complex levels are the best immunochemical method of monitoring disease activity presently available. Despite reservations about the sensitivity of clinical assessment as a guide to treatment in SLE we remain reluctant to treat patients with high levels of immune complexes in the absence of overt disease since it appears that only certain subpopulations of complexes, distinguished by their immunochemical properties and size, may be directly relevant to the activity of the disease and its manifestations. It is particularly interesting that 'pulse' therapy which does not appear to affect greatly the circulating immune complex load or its immunochemical make-up, depresses the levels of those middle range complexes which seem to be particularly associated with renal disease where the benefits of 'pulse' therapy have been demonstrated most clearly (Cathcart *et al.*, 1976; Dosa *et al.*, 1978).

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