A STUDY OF COMPLEMENT AND IMMUNE COMPLEXES IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS: THEIR VALUE IN MONITORING DISEASE ACTIVITY

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SUMMARY

Patients with Systemic Lupus Erythematosus (SLE) were studied for immune complexes (IC) and complement levels. These tests were carried out sequentially during active and inactive stages of disease over a period of one and a half year. The Clq binding assay (C1qBA) and detection of cryoglobulins were much more sensitive methods of detecting IC (72% & 78% of cases respectively) than the monoclonal Rheumatoid Factor binding assay (mRFBA) (50%) during the disease activity. The fall in CH50 and C4 levels correlated very well with disease activity (83% & 84% of cases respectively). There was an inverse relationship between levels of CH50, C4 and immune complexes in a small sub group of patients. The other sub groups either had a rise in immune complexes levels or a fall in complement levels with disease activity.

INTRODUCTION

Systemic lupus erythematosus is an example of wide spread non-organ specific disorder. Abundant evidence shows that immunologic mechanisms of injury are important in its pathogenesis. A hallmark of the disease is the presence of antibodies to nuclear components, but other immunologic components exist as well. It was not until 1948 when Hargraves, Richmond and Morton discovered LE cell in bone marrow preparations that the wide spread immunologic disturbance of the disease was recognised.[1] In SLE the prototype of immune complex disease, the activation of complement and the deposition of immune complexes play an important pathogenic role. It has been suggested that depressed levels of complement and/or the presence of circulating immune complexes reflect disease activity. Previous studies show a correlation between depressed complement levels and rise of immune complexes with disease activity.[2,15] All these studies were carried out over a duration of 6 months and only during active stages of disease.

In this study we had the opportunity to study 22 patients of classical SLE fulfilling the ARA criteria 1982,[15] over a period of one and a half year. Sixty-seven serum samples were obtained, C4 and CH50 levels and immune complexes were studied by three methods–Cryoprecipitation, ClqBA, mRFBA during active and inactive stages of disease.

The purpose of this study was to determine the role of immune complexes in the pathogenesis of disease, whether a fall in complement levels and a rise of immune complex correlate with overall disease activity. Is there any correlation between fall of complement levels and rise of immune complex levels? Does any specific type of immune complex assay have any correlation with any particular clinical feature? Whether these tests help in

diagnosis, evaluation of disease activity, prognosis and monitoring therapy, either singly or in combination?

MATERIAL AND METHODS

Twenty-two classical SLE female patients whose ages ranged between 15–75 years were studied. They fulfilled the revised ARA criteria 1982[15] i.e. the presence of four or more of the 11 criteria. These criteria are:

1. Malar rash
2. Discoid rash
3. Photosensitivity
4. Oral ulcers
5. Arthritis
6. Serositis
7. Renal disease
8. Neurologic disorder
9. Haematological disorders
10. Immunologic disorder
11. Antinuclear antibody

Immune complexes were detected by three methods:

1. Cryoprecipitation
2. ClqBA
3. mRFBA

Sixty–seven serum samples form SLE patients were studied sequentially during active and inactive stages. Disease activity was based on high DNA binding antibody levels. Blood samples from 30 normal individuals were used as normal control. The controls were laboratory personnel and members of the hospital staff. Both sexes were included, 20 females and 10 males, the ages ranged between 25–60 years. Sera from 5 patients with known high positives were used as positive controls.

Cryoprecipitation

5 ml of blood was drawn by pre-warmed syringes and kept at 37°C for one hour. It was then centrifuged at 1500 G for five minutes in a bucket centrifuge. For detection of cryoglobulins the serum was kept at 4°C for seven days. The method used for the detection of cryoglobulins in the sera of patients is that of E Martin Mola et al.[16]

ClqBA + mRFBA (Fluid Phase)

Immune complexes were detected by fluid phase radio immune assays i.e. by radiolabelled Clq and monoclonol Rheumatoid factor (mRF). Radiodisation of Clq was carried out by the lactoperoxidase technique and mRF by the Chloramine T method. Clq and mRF were purified and donated by the Rheumatology laboratory Hammersmith Hospital. The results are expressed in percent TCA count. Heat aggregated globulins were not used in this assay to express results, as they do not represent true complexes. The normal limit of Clq binding was taken as 10% and mRFBA as 12%.

Estimation of Complement Levels (CH50 + C4)

Blood samples from 10 normal individuals were drawn and allowed to clot at room temperature for 1 hour. They were centrifuged at a speed of 1500 G for 5 minutes. The serum was tested for complement activity by comparing its activity to guinea pig complement (GPC). The serum was stored at 70°C. The pool of normal human serum (NHS) was used as standard for the CH50 and C4 assay. CH50 and C4 in the serum were detected by Functional Gel Diffusion assay. The results of C4 and CH50 were expressed as ± 2SD of controls. The cut off level of C4 and CH50 was 80% in this test.

RESULTS

Immune Complexes and Disease

The levels of immune complexes were measured on 30 controls and 62 serum samples from 22 patients. The Clq binding assay and cryoprecipitation gave positive correlation with disease activity in 72% cases respectively. The correlation with cryoprecipitation is slightly higher (x^2 = 26.33 $p < 0.001$) than Clq binding assay (x^2 = 22.23 $p < 0.001$). The association of both these assays with disease activity is significant. In 12% of the cases the cryoglobulins and Clq binding were positive when the disease was not active. The mRF binding assay showed a smaller degree of correlation with disease activity (x^2 = 6.19 $p < 0.05$). In only 50% there was a positive association with disease activity. In 20% of the cases it was positive when disease was not active (Table–1). However the Clq binding assay showed a positive correlation with mRF binding assay (r = 0.43 $p < 0.001$). The correlation of immune complexes to disease activity varied with the type of immune complex assay used. In some patients the levels of immune complexes remained within the normal range by all three methods. When the disease was active all these patients had renal disease. The disease activity was associated with very low complement levels. The findings agree with previous studies.[5] The mRF binding assay showed higher levels of immune complexes than the Clq binding assay in only three cases. In two cases the patients had mainly cerebral symptoms. The third one had transient ischaemic attacks in the past.

TABLE–1

RAISED LEVELS OF IMMUNE COMPLEXES IN ACTIVE AND INACTIVE STAGES OF DISEASE IN S.L.E.

	Cryoglobulins	ClqBA	mRFBA
Active Disease	78%	72%	50%
Inactive Disease	12%	12%	20%

ClqBA: Clq Binding Assay.

mRFBA: Monoclonal Rheumatoid Factor Binding Assay

Complement and its Relationship with Disease Activity

When the disease was active there was diminution of CH50 in 83% of cases (x^2 = 27.81, $p < 0.001$) and C4 in 84% of cases. In 16% low complement levels were detected while the disease was not active (Table–2).

TABLE–2

DIMINISHED COMPLEMENT LEVEL IN ACTIVE AND INACTIVE STAGES OF DISEASE IN S.L.E.

	CH50	C4
Active Disease	83%	84%
Inactive Disease	16%	16%

Complement and Immune Complexes

An inverse relationship between complement levels and immune complexes was found in 12 out of 22 patients. In some patients there was a considerable fall in the complement levels but the immune complexes remained within normal limits. In these patients the disease activity was predominantly renal. In another group of patients the rise of levels of immune complexes was more than the fall in complement levels.

DISCUSSION

Previous studies carried out to detect circulating immune complexes in patients with SLE have given conflicting results.[12,15] Various methods were used to detect dif-

ferent subpopulations of immune complexes and it may be expected that these assays would have varied correlations with SLE. Cryoglobinaemia provided the first demonstration of immune complexes in SLE.[7] In this study the presence of cryoglobulins was the most sensitive test for detecting immune complexes and correlated well with the ClqBA, indicating detection of a similar group of immune complexes.

The use of ClqBA and mRFBA in parallel allows detection of complement fixing and non-complement fixing complexes. The ClqBA can detect complement fixing immune complexes larger than 196 in size while mRFBA detects immune complexes as small as 8S in size, irrespective of their complement fixing ability; these tests thus detect different but overlapping groups of immune complexes. The ClqBA was shown to correlate with disease activity by some[10,12,14] but others found that this association was not significant.[8] Abrass et al[11] showed an association of disease activity with only solid phase ClqBA but not the fluid phase ClqBA. In this present study 72% of the tests showed a positive correlation between positive ClqBA (fluid phase) and active disease. One patient had very high ClqBA levels while the disease was inactive. This may be due to very large size of these complexes that presumably were unable to deposit in tissues and, therefore, did not produce disease manifestations. A positive correlation existed between ClqBA and mRFBA. However, mRFBA correlated with disease activity in only 50% of the cases. The ClqBA was a more sensitive assay than mRFBA as shown previously by Gabriel et al.[12] The weaker correlation between disease activity and positive mRFBA may be because it detects a different group of immune complexes.

Renal involvement was present in eight patients. In two cases disease was inactive, in three renal diseases was present with other symptoms such as rash, arthritis, vasculitis, leucopenia, mouth ulcers, pleuritis and migraine. In the remaining patients the disease activity was mainly SLE induced renal disease. The immune complexes remained within normal limits by the ClqBA and mRFBA. Cryoglobulins were detected in one case. Previous studies[3,17,18] showed a positive correlation of active renal disease in S.L.E patients and immune complexes. However, others[5,9] showed that such an association was not significant. These studies used the ClqBA and so it was thought that the absence of immune complexes may be due to the inability of the technique to detect non fixing complexes. This hypothesis was supported by Levinsky et al[17] who showed the presence of immune complexes using a method based on the interaction of Rheumatoid factor with immune complexes. It is also proposed that size of the immune complexes is more critical to various disease manifestation than the mere presence of circulating immune complexes. They showed that renal disease was associated with the presence of intermediate size complexes.[3] The failure to detect immune complexes in the above cases may be due to the inability of the technique used to detect intermediate sized complexes.

In three patients the mRFBA was positive while the ClqBA was normal. Two patients had active cerebral lupus. The third patient's disease was clinically inactive at the time although she had a past history of cerebral lupus; it can be explained by the small size of the complexes detected by this method which is easily deposited in the choroid plexus resulting in disturbances of choroidal haemodynamics and integrity of the blood brain barrier. This hypothesis has not been proved and most cases of cerebral lupus are thought to be due to the deposition of immune complexes in the cerebral arterial walls.[4] In

most of the patients with active disease, the levels of immune complexes returned towards normal or decreased with treatment as shown previously.[2,3,17,18]

The levels of C4 and CH50 correlated with disease activity slightly better than immune complex levels in this study. It has been shown on previous studies[5,18] that C3 and Ch50 levels correlated to active disease better than the levels of immune complexes. They also showed that a significant inverse relationship existed between CH50, C3 and C4 levels and immune complexes. In about half the patients with active disease in the present study a fall in complement levels is temporarily associated with a rise in levels of immune complexes. It is presumed that the complement components are being used in immune aggregate dissolution in vivo. This association does not hold in all cases and in a few patients there was a fall in the levels of complements but no rise in circulating immune complexes. It was observed by Harkiss et al[18] that serial studies of immune complexes, DNA binding and complement can be of value in the management of individual patients rather than a single serologic parameter.

This study reveals that these tests are certainly very useful parameters in assessing disease activity and prognosis when done in combination. While singly, they may prove to be of limited value.

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