

CIRCULATING IMMUNE COMPLEXES IN LUPUS ERYTHEMATOSUS, SCLERODERMA AND DERMATOMYOSITIS

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Abstract. Circulating immune complexes (CIC) were measured by three different methods in serum from 17 patients with systemic lupus erythematosus (SLE), 3 patients with "hydralazine-induced" SLE-like syndromes, 14 patients with discoid lupus (DLE), 8 patients with systemic sclerosis and 5 patients with dermatomyositis. Immune complexes were detected in 13 of the 17 patients with SLE. All patients with lupus nephritis and typical exanthema had circulating immune complexes. The concentration of immune complexes was inversely correlated to serum complements C4 and C3. All 3 patients with "hydralazine-induced" SLE-like syndromes had circulating immune complexes that disappeared after withdrawal of the drug. Immune complexes were detected in 3 of the 14 patients with DLE; all 3 patients with CIC had widespread DLE. In systemic sclerosis, CIC were detected in only 1 of the 8 patients. Four of the 5 patients with dermatomyositis demonstrated CIC in serum. No complement consumption was detected in dermatomyositis and the immune complexes may have been secondary to tissue destruction.

Key words: Immune complexes; Lupus erythematosus; Scleroderma; Dermatomyositis; Collagen disease

The presence of circulating immune complexes (CIC) is well documented in systemic lupus erythematosus (SLE), polyarteritis and rheumatoid arthritis (10, 15). Immune complexes are known to be a pathogenetic factor in the production of glomerulonephritis and vasculitis (9). It is assumed that immune complexes may be of pathogenetic significance in the development of a number of skin diseases (4). In the present study we have investigated the presence of CIC in SLE and related, autoimmune diseases of the skin: "hydralazine-SLE", discoid lupus erythematosus (DLE), systemic sclerosis, and dermatomyositis. In SLE we have investigated the relationship between CIC and the clinical activity of the disease as evidenced by affection of the kidneys and skin. The method used

for detection of CIC is a combination of two radioimmunoassays: the Clq-binding activity (Clq-BA) and measurement of polyethylene glycol precipitable light chain determinants (PP-Lc). A third method used depends on complement consumption (AC-test).

MATERIAL AND METHODS

Forty-seven patients were investigated. Seventeen patients (16 women and 1 man (no. 16)) had SLE according to the criteria of Dubois (3, 6). Twelve patients had a positive LE-cell test and all patients had a positive test for antinuclear antibodies (ANA). Eleven patients were undergoing treatment with prednisone at the time of investigation, and the other patients had previously been treated with prednisone. Ten patients had renal involvement with proteinuria and 4 patients had elevated serum creatinine levels. Renal biopsy was performed in 8 of the patients, revealing histological lesions compatible with SLE and immunofluorescent, granular deposition of IgG, IgM and complement. Seven patients had clinically or histologically characteristic lesions of the skin.

Three patients undergoing treatment with the antihypertensive drug "hydralazine" developed SLE-like syndromes. One patient had a positive LE-cell test. The other 2 patients developed fever, arthralgia, exanthema and positive ANA. All symptoms disappeared after withdrawal of the drug.

Fourteen patients had clinically and histologically verified discoid lupus erythematosus (DLE). Eight patients had widespread discoid lupus (G-DLE) according to O'Leary (16) and 6 had localized discoid lupus (L-DLE). None of the patients had a positive LE-cell test, but 9 patients had a positive test for antinuclear antibodies (ANA). Signs of renal disease were absent. The patients were treated with topical corticosteroids and antimalarials.

Five patients had dermatomyositis (polymyositis). All patients had myositis on histological examination of the skin muscle biopsy, and 4 patients had involvement of the skin. One patient had dermatomyositis and cancer of the breast. At the time of investigation 4 patients were being treated with systemic steroids.

Eight patients had systemic sclerosis according to the criteria of Rook et al. (20). Positive ANA was demon-

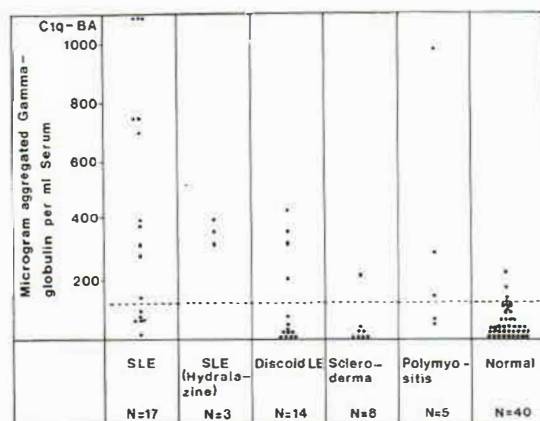


Fig. 1. Measurements of immune complexes by the Clq-BA test, quantitated as equivalent concentrations of heat-aggregated gammaglobulin. Normal values below stippled line.

strated in 3 patients. All patients were undergoing treatment with d-penicillamine or prednisone at the time of investigation.

Detection of immune complexes (CIC). Blood samples from the patients were allowed to coagulate for 2 hours at 20 °C, centrifuged twice at 4 °C and stored at -70 °C until tested. Samples once thawed were not used again.

Three methods were used for detection of CIC. When 2 of the 3 methods were positive, CIC were assumed to be present.

Clq-binding activity (Clq-BA). A modification of the method devised by Nydegger et al. was used (15, 22). In brief: serum was mixed with 125 I-labelled Clq (1 µg/ml). The immune complexes with fixed Clq were precipitated by 2.5% w/v polyethylene glycol (PEG) and centrifuged at 1500 g for 30 minutes. The radioactivity thus precipitated vis-à-vis that precipitated by trichloroacetic acid was measured. The CIC were quantitated as equivalent concentrations of heat-aggregated human gammaglobulin per ml serum (Cohn fraction II, 63 °C, 12 min).

PEG-precipitable light chain determinants (PP-Lc). 0.4 ml patient serum was mixed with 0.1 ml of unlabelled Clq (1 µg/ml) and 2.5 ml 3% w/v PEG in 0.1 M borate, 0.025 M EDTA, pH 8.4. The mixture was incubated 24 hours at 4 °C and centrifuged 30 minutes at 1500 g. The precipitate was washed three times with 2.5% PEG and suspended in 2.5 ml phosphate-buffered saline (0.15 M sodium chloride, 0.041 M sodium phosphate, 1% albumin, pH 7.4). The concentration of light chain determinants was measured by a radioimmunoassay described by us elsewhere (23).

The PP-Lc test was analysed parallel to the Clq-BA test.

Anticomplementarity (complement consumption) AC. Complement consumption was measured as described previously (22). In brief, heat-inactivated serum was mixed with fresh human complement. The sample was incubated with sensitized red blood cells, and the complement consumption was measured by the degree of hemolysis.

Complement. Complements C4 and C3 were measured by radial immunodiffusion (13).

DNase treatment. Sera from 14 patients with SLE were treated with deoxyribonuclease I (DNase) (Worthington Biochemical Corporation, New Jersey) 0.3 mg/ml, 20 °C for 24 hours, and the Clq-BA measured in untreated and in the DNase treated serum.

RESULTS

The results of measuring CIC by three methods: Clq-BA, PP-Lc and AC are shown in Figs. 1 and 2, and in Table 1. It appears that CIC are frequently found in SLE (82% positive by 2 of the 3 methods), "hydralazine-SLE" (100% positive by 2 of the 3 methods) and dermatomyositis (80% positive by 2 of 3 methods). CIC are only rarely detected in systemic sclerosis (13% by 2 of 3 methods) and in DLE (21%). All patients with CIC and DLE had generalized DLE.

The three methods are significantly correlated:

Clq-BA and PP-Lc test: $p < 0.001$ (Spearman's rank correlation test)

Clq-BA and AC test: $p < 0.05$ (χ^2 -test with Yates correction)

PP-Lc and AC test: $p < 0.01$ (χ^2 -test with Yates correction).

The relationship between the clinical features in SLE and the three tests is shown in Table II. All 10 patients with proteinuria had CIC, and all the 7 patients with characteristic clinical and histological skin lesions had CIC. The presence of CIC was not

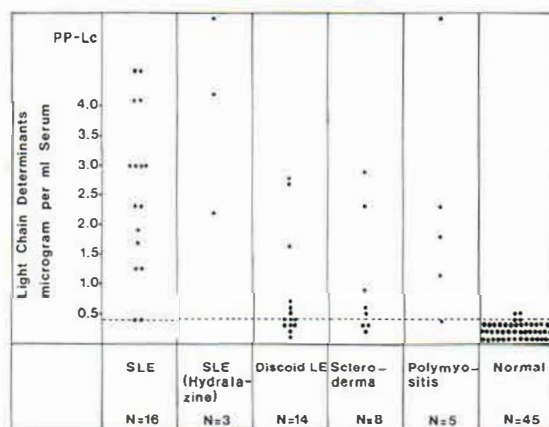


Fig. 2. Measurements of immune complexes by the PP-Lc test quantitated as light chain determinants. Normal values below stippled line.

Table 1. Measurements of immune complexes by the AC test indicated as positive/negative.

The percentage positive is indicated in parentheses

AC-test	No. of patients	Positive	Negative
SLE	17	13* (76)	4 (24)
Hydralazine SLE	3	3 (100)	0 (0)
Discoid LE	14	0 (0)	14 (100)
Scleroderma	8	0 (0)	8 (100)
Dermatomyositis	4	1 (25)	3 (75)
Normal	24	1 (4)	23 (96)

* $p < 0.05$, χ^2 -test.

related to the LE-cell test or to treatment with prednisone.

The results of measurements of complement are shown in Table III. The mean values of C4 and C3 are significantly decreased in patients with SLE ($p < 0.001$, Mann-Whitney rank sum test). In hydralazine SLE the mean values of C4 and C3 are decreased, but due to the small number of patients no statistical calculations were made. All other values are within normal limits. The relationship of C3 complement to Clq-BA for all the patients is shown in Fig. 3. A significant inverse correlation was detected between Clq-BA and C3 and C4 ($p < 0.001$, Spearman's rank correlation test).

Sera from 14 patients with SLE were treated with DNase. A significant decline in Clq-BA was ob-

served during DNase treatment (Fig. 4) ($p < 0.05$, Wilcoxon's signed rank test).

DISCUSSION

Three methods have been used for detection of immune complexes. The Clq-BA and the AC-test depend on the binding of complement to immune complexes. The precipitation of light chain determinants is facilitated by complement (in preparation). Thus all 3 methods are complement dependent, and in this study the 3 methods were found to be significantly correlated. Each method has some potential errors: DNA, heparin and bacterial lipopolysaccharides may produce false-positive Clq-BA; heat inactivation may destroy immune complexes or produce aggregated gammaglobulin in the AC-test. When at least 2 of the 3 methods are positive the risk of obtaining "false-positives" is reduced.

Fourteen of the 17 patients with SLE had CIC detectable by 2 of 3 methods. This is in accordance with the findings of others (5, 10, 15, 25). Ten patients had renal involvement with proteinuria and SLE nephritis was demonstrated by renal biopsy in 8 of the patients. All patients with proteinuria had CIC, thus agreeing with the theory that the nephritis of SLE is caused by immune complexes (1, 19, 21). Seven patients had characteristic clinical and histological involvement of the skin and CIC were de-

Table II. Clinical features in 17 patients with SLE and the measurements of immune complexes by the 3 test systems indicated as positive (+) and negative (-)

HD denotes a patient undergoing regular hemodialysis

Patient no.	LE-cell test	Creatinine (mg %)	Proteinuria	Exanthema	Prednisone	Clq-BA	AC	PP-Lc
1	+	9.1	+	-	-	+	+	+
2	-	7.1	+	-	-	+	+	+
3	+	0.7	+	+	+	+	+	+
4	+	0.5	+	+	+	-	+	+
5	+	0.7	+	+	-	+	-	+
6	+	HD	+	-	-	-	+	+
7	-	0.6	-	+	+	+	+	+
8	+	1.0	-	+	-	+	+	+
9	-	0.9	-	+	+	+	+	+
10	-	0.8	-	+	+	+	+	+
11	+	0.7	-	-	+	-	-	+
12	+	0.7	-	-	+	-	-	-
13	-	1.0	+	-	+	+	+	+
14	+	1.4	+	-	-	-	+	+
15	+	0.7	+	+	+	+	+	+
16	+	1.0	-	-	+	-	-	-
17	+	0.8	+	-	+	+	+	+

Table III. Mean values of measurements of complements C4 and C3

Standard deviation (S.D.) indicated in brackets

	No. of patients	C4 (mg%)	C3 (mg%)
SLE	17	14.6* (7.5)	52.6* (21.1)
Hydralazine SLE	3	18.8 (5.6)	57.0 (6.2)
Discoid LE	14	33.1 (11.4)	76.9 (17.8)
Scleroderma	8	28.1 (14.3)	81.3 (24.9)
Dermatomyositis	5	29.2 (14.2)	88.6 (10.9)
Normal	25	35.6 (11.1)	88.5 (18.6)

* $p < 0.001$, Mann-Whitney rank sum test.

tected in all 7 patients. This indicates that the skin lesions could also be caused by immune complexes. This is supported by the findings of Tappeiner, that Clq-BA are correlated to the deposition of immunoglobulins and complement in uninvolved skin of SLE (25). Thus, CIC are predominantly present in patients with active disease as evidenced by lupus nephritis and typical exanthema. Three patients had neither renal disease nor exanthema, and none of these patients had CIC.

Several antigens may be part of the immune complexes of SLE: DNA, RNA, gammaglobulins, cytoplasmic antigens, clotting factors (1). We have treated sera from 14 patients with DNase and measured Clq-BA before and after treatment. A significant decline was observed, demonstrating that DNA is present in serum either as free DNA or bound in immune complexes. The concentration of circulating immune complexes measured by Clq-BA was inversely correlated to the concentration of C4

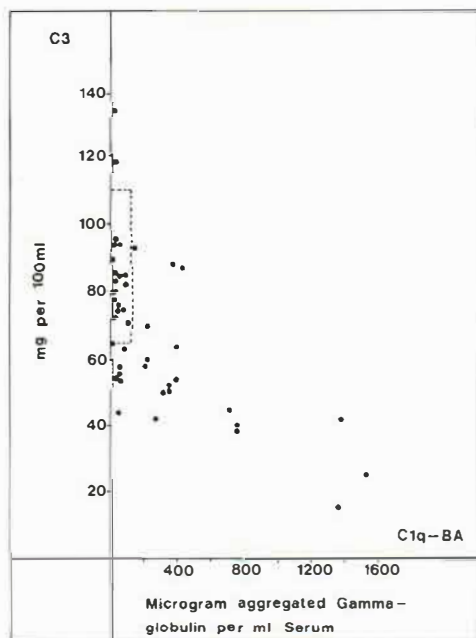


Fig. 3. Relationship between immune complexes measured by the Clq-BA test in all the patients and serum complement C3. An inverse correlation was detected ($p < 0.001$, Spearman's rank correlation test). Stippled area indicates normal values.

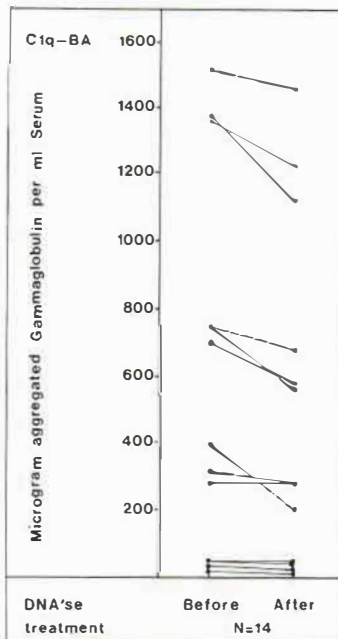


Fig. 4. Measurements of immune complexes by the Clq-BA test before and after treatment with DNase in 14 patients with SLE. A significant decline was detected ($p < 0.05$, Wilcoxon's signed rank test).

and C3 ($p < 0.001$). The mean values of C4 and C3 were decreased in SLE and hydralazine SLE. This complement consumption suggests that immune complexes may be responsible for some of the tissue lesions via the classical pathway of the complement system. Decreased levels of complement have previously been described in association with clinically active disease (2, 5, 7, 19). The same clinical and laboratory abnormalities as found in SLE may be found in patients with "hydralazine-SLE", discoid lupus erythematosus, scleroderma and dermatomyositis, viz. skin eruptions, Raynaud's phenomena, rheumatoid factors and anti-nuclear antibodies. Thus it is possible that immune complexes may also be of significance in these diseases.

Three patients had SLE-like syndromes induced by the antihypertensive drug "hydralazine". Circulating immune complexes were demonstrated during the active phase of the disease in all 3 patients. The immune complexes and all clinical symptoms disappeared after withdrawal of the drug. Thus, circulating immune complexes may be of pathogenetic significance in these patients. Other drugs, such as alpha-methyldopa, gold, procainamide, halothane, have also been thought to produce adverse effects via immune complexes (12, 18, 26, 27).

Fourteen patients had DLE and 3 of the 14 patients had CIC. These 3 patients had generalized discoid lupus (G-DLE). Thus, immune complexes seem related to the widespread form of the disease. By immunofluorescence technique immunoglobulins (particularly IgM) and complement are regularly detected at the dermal-epidermal junction in the affected areas of the skin in DLE, whereas in SLE, immunoglobulins and complement can also be detected in unaffected skin (17). As suggested by Tan (24), a constant discharge of nuclear cytoplasmic antigen from the epidermal cells may react with pre-existing circulating antibodies to produce immune complexes at the dermal-epidermal junction. Immune complexes, however, need not be present in the circulation. This may explain the positive immunofluorescence finding in the affected skin and the rarely positive tests for circulating immune complexes. Those patients with CIC may exemplify the type of patient who will later develop SLE (25).

Eight patients had systemic sclerosis. Immune complexes were detected in only one of these patients. The widespread symptoms of generalized

scleroderma with involvement of the skin and viscera may be the result of changes initiated in the blood vessels (9), and CIC could be the initiating factor. However, we have detected immune complexes in only 1 of 8 patients and no complement consumption was observed. This renders it unlikely that CIC are a major pathogenetic factor in this disease. The patients were being treated with prednisone or penicillamine at the time of investigation. These drugs may decrease the levels of CIC (11, 14), and CIC could be present at an earlier stage of the disease. Treatment, however, did not prevent the finding of immune complexes in the other systemic collagen diseases examined.

Five patients had dermatomyositis (polymyositis). Four of the 5 patients had CIC. No complement consumption was detected, indicating that tissue destruction is not caused via the complement system. The pathogenetic mechanism of dermatomyositis is still not known, but immune complexes may be of significance. Circulating immune complexes may, however, be a secondary phenomena to destruction of tissue and autoimmunization with normally hidden tissue antigens such as nuclear and cytoplasmic antigens from muscle cells. Analogically circulating immune complexes following damage to the heart muscle in acute coronary occlusion has been described (8).

ACKNOWLEDGEMENTS

The study was supported by grants from the Danish Medical Research Council. We thank Anne-Marie Pedersen for technical assistance.

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Received February 19, 1979

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