

Circulating immune complexes in sarcoidosis

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ABSTRACT The sera of 50 patients with sarcoidosis were tested for the presence of circulating immune complexes using polyethylene glycol precipitation, followed by single radial immunodiffusion for the amounts of Clq, IgG, IgM, and double diffusion for the presence of IgA. Complexes were detected in 29 (58%) patients. No correlation could be found between the presence of these complexes and the length of history, stage, or activity of disease, nor to steroid therapy. Rheumatoid factor was detected in 14 patients (28%), 13 of whom had circulating immune complexes, and 12 of whom had active disease. Total serum C3, CH50, and Clq were normal, as were immunoglobulin levels. In patients with extrathoracic sarcoidosis, especially skin or joint involvement, complexes were commonly found. The aetiological significance of these complexes remains uncertain.

Circulating immune complexes occur in some patients with sarcoidosis, the proportion varying between 3% and 51% depending on the technique used.¹⁻⁵ The role of immune complexes in sarcoidosis is uncertain. We have investigated sera from an unselected group of patients with sarcoidosis for the presence and composition of immune complexes to see whether these complexes were associated with active or progressive sarcoidosis, as might be expected if these complexes are of pathogenic significance.

Methods

Fifty patients with sarcoidosis were studied (32 male, 18 female). There were the first 50 to be seen at Willesden Chest Clinic or Central Middlesex Hospital during the period of this study. The mean age was 40 years (range 17-63 yr). Twenty-eight were black, 16 white, and six Indian. Length of history ranged from less than one month to over 20 years (mean 4.9 yr). A diagnosis of sarcoidosis was made on clinical features and the result of Kveim testing, lung, mediastinal, lymph node, skin, or liver biopsies in all cases coupled with characteristic radiographic abnormalities. Other causes for non-caseating granulomata were excluded as far as possible. An assessment of whether the disease was active or not was made at the time of the patient's visit

on the basis of clinical, biochemical, or radiological features. At the end of the study the chest radiographs taken on the same day as the blood samples were assessed for the presence of lymphadenopathy, infiltration, or fibrosis without the observer having reference to the patient's name or clinical details. The standard staging of sarcoidosis was made: 0=normal chest film; 1=bilateral hilar adenopathy, alone; 2=bilateral hilar adenopathy plus pulmonary infiltration; 3=pulmonary infiltration or fibrosis alone.

Control sera for serum immunoglobulin and complement levels were taken from 50 healthy individuals. For the polyethylene glycol precipitation, these sera and aliquots from a pool of more than 20 normal people were used (80 estimations in all).

The assay involved the precipitation of soluble immune complexes from serum by the addition of polyethylene glycol, molecular weight 6000 (PEG), a modification of the method described by Nydegger.⁶ The amounts of Clq, IgG, and IgM in the precipitate were measured directly by single radial immunodiffusion (SRID) and the presence of IgA was determined by double diffusion. The values were compared with those obtained in this test from normal sera.

A solution (0.1 ml) of 12% PEG in barbitone buffered saline containing 60 mM EDTA, pH 7.6 was mixed with 0.5 ml of the test serum, used either fresh or after storage at -70°C, and left for 18 hours at +4°C. The precipitated immune complexes were separated from the serum, by

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centrifuging at 1000 g for 20 minutes at +4°C, and washed once in 2 ml of 2% PEG in barbitone-buffered saline containing 10 mM EDTA, pH 7.6. The tubes were drained briefly, the precipitates dissolved in 0.5 ml barbitone buffered saline, mixed well, and left for 30 minutes before assaying for IgG, IgM, IgA, and Clq.

The concentrations of Clq, IgG, and IgM in the PEG precipitate and Clq, IgG, IgM, and IgA in the starting serum were determined by SRID in 1 mm thick gels of 1% agarose in 40 mM EDTA barbitone-buffered saline, pH 8.6. The presence or absence of IgA in the precipitate was shown by double diffusion, against monospecific antiserum.

Each batch of sera tested contained at least two control sera. The values obtained from these sera have provided the confidence limits for the test. Soluble immune complexes were deemed to be present if IgA was present (lower limit of sensitivity 21 µg/ml), or if any of the components assayed were > mean + 2 SD obtained from the control values. These were as follows: Clq > 104 µg/ml (mean 44 µg/ml + 60 µg/ml; IgG > 69 µg/ml (mean 39 µg/ml + 30 µg/ml); and IgM > 114 µg/ml (44 µg/ml + 70 µg/ml). Measurement of supernatant Clq allows detection of complexes which bind all the available Clq. None was found in this series.

CH50 was measured by a standard functional assay, using sensitised sheep red cells, and C3 by SRID. The sera used were raised in rabbits by immunisation with purified components, and standardised by comparison with reference anti-sera.

IgG rheumatoid factor was assayed by a latex globulin reagent (RA-test latex-globulin, Hyland).

Results

IMMUNE COMPLEXES

Circulating immune complexes were found in 29 patients (58%). In 16 sera the complexes contained Clq, in 20 IgG, 14 IgM, and 15 IgA. No correlation was found between presence of circulating immune complexes and the age or sex of the patient.

There was no significant correlation between the length of disease and the presence of immune complexes (fig 1).

Forty patients were considered to have active disease. Twenty-three of these had circulating complexes, thus showing no significant correlation. The composition of immune complexes in relation to activity of disease is shown in fig 2. In addition to this 13 of 15 patients with IgA

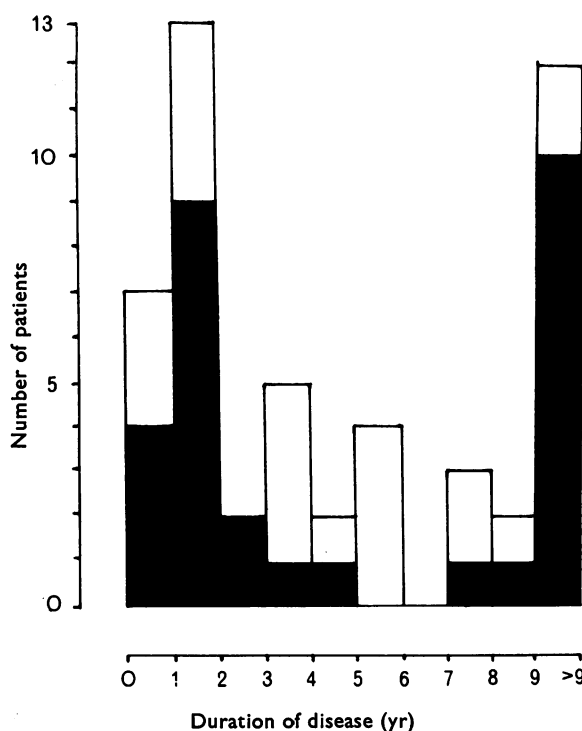


Fig 1 Presence of immune complexes in sera from 50 patients with a varying length of history of sarcoidosis. Dark area represents the number of patients with immune complexes, in each year group.

complexes had active disease.

Steroid therapy had no significant effect upon the presence of immune complexes (fig 2).

No correlation between the presence of immune complexes and stage of disease was found (table 1).

Nine of the 14 patients with extrathoracic sarcoidosis had complexes. All three patients with arthritis and six of the seven patients with skin or nasal involvement had complexes, whereas none of the three patients with eye involvement, and only four of the seven patients with granulomatous hepatitis had evidence of circulating immune complexes.

Table 1 Immune complexes and stage of sarcoidosis

	Number of patients	Number with complexes	Type of complex				Rheumatoid factor
			Clq	IgG	IgM	IgA	
Stage 0	10	6	5	5	4	1	2
Stage 1	13	6	2	4	3	3	3
Stage 2	19	11	6	9	6	7	6
Stage 3	8	6	3	2	1	4	3
	50	29	16	20	14	15	14

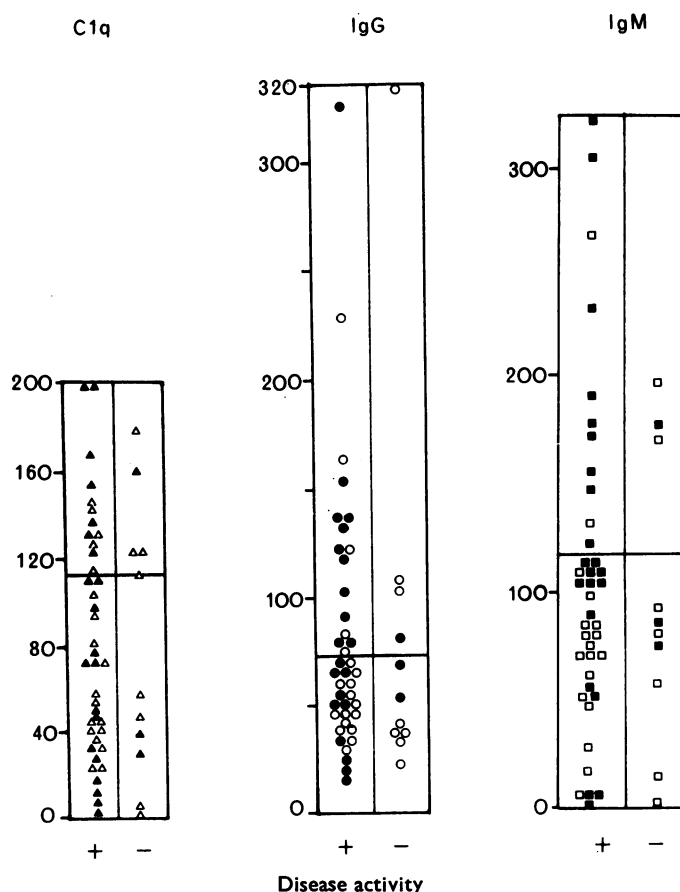


Fig 2 Composition of immune complexes isolated from the sera of patients with active (+), and not active (−) sarcoidosis. The horizontal bar in each column represents the upper limit of normal (mean + 2 SDs for 80 normal control subjects) of 2% PEG precipitable Clq, IgG, and IgM. The closed symbols (▲ ● ■) represent those patients on steroid therapy, and open symbols (△ ○ □) those not on steroid therapy.

Table 2 Immunoglobulin and complement levels in 50 patients with sarcoidosis and 50 normal control subjects

Serum protein	Sarcoidosis patients		Normal control subjects	
	Mean	± SD	Mean	± SD
IgG	106	17	86	15
IgM	92	26	97	21
IgA	146	52	102	41
Clq	110	9	101	8
CH50	99	9	106	7
C3	101	11	102	9

All levels are expressed as a percentage of the normal total amounts. There are no significant differences between patients and control subjects.

RHEUMATOID FACTOR

Rheumatoid factor was present in 14 patients, 13 of whom had circulating immune complexes

(seven Clq, eight IgG, seven IgM, eight IgA). Twelve of the 14 had active disease.

COMPLEMENT AND IMMUNOGLOBULINS

CH50 and the serum concentrations of Clq, C3, IgG, IgM, and IgA did not differ significantly from those of normal control group (table 2).

Discussion

We have found circulating immune complexes using precipitation with 2% PEG in 58% of patients with sarcoidosis. Serum levels of immunoglobulins and complement components were normal. We have found no correlation between the presence of immune complexes and disease activity, length of history, or radiological extent.

of the disease. There was a correlation between skin sarcoidosis and the presence of immune complexes.

Previous studies have described the presence of circulating immune complexes in up to 51% of patients with sarcoidosis.¹⁻⁵ Our results confirm those of Gupta *et al*⁴ who used the Raji cell and monoclonal rheumatoid factor assay who suggested that between 50 and 60% of patients with sarcoidosis have circulating immune complexes. In some studies an association between erythema nodosum and circulating immune complexes was noted.¹⁻³ None of our patients had erythema nodosum at the time of testing.

Gupta *et al*⁴ found circulating immune complexes in 17 of 23 patients with stage 3 disease. We had only eight patients with stage 3 disease, six (75%) of whom had complexes, but with such small numbers it is difficult to draw firm conclusions. All their patients with stage 1 or stage 2 disease with circulating immune complexes also had extrapulmonary features of sarcoidosis. However in our study, of the 17 patients with stage 1 or 2 disease, the nine patients with circulating immune complexes only had intrathoracic disease, as did five of the six whose chest radiograph at the time of testing was normal.

Two previous studies^{1,5} have found an association between acute sarcoidosis (history less than one year) and the presence of immune complexes. We failed to confirm this. Three of our seven patients with this length of history did not have complexes. Similarly, we found complexes in 12 of 21 patients with chronic disease (more than five years), whereas Daniele *et al*⁵ only found complexes in two of 10 such patients. All the patients of Hedfors and Norberg¹ with circulating complexes had a history of one year or less. Like ourselves Daniele *et al*⁵ found no correlation between the presence of immune complexes and the stage of sarcoidosis.

We have confirmed the association between extrathoracic sarcoidosis, especially skin or joint disease, and the presence of circulating immune complexes.^{4,5}

Steroids were given to half our patients, most of whom had immune complexes. It is arguable that the use of steroids may have eliminated the complexes in a further six patients; however this would not have affected our conclusions.

The high prevalence of rheumatoid factor in patients with active disease suggests that the presence of immune complexes acts as an antigenic stimulus for its production. It is not surprising that Gupta *et al*,⁴ using monoclonal rheumatoid factor radioimmunoassay, detected this group of patients.

If immune complexes are of pathogenic significance in sarcoidosis they should be expected to be present in the acute disease and cleared as the disease resolves. The persistence of such complexes should imply active or progressive disease. Our results fail to substantiate this hypothesis and the aetiological significance and constitution of these complexes remains unknown.

References

- 1 Hedfors E, Norberg R. Evidence for circulating immune complexes in sarcoidosis. *Clin Exper Immunol* 1974; **16**:493-6.
- 2 James DG, Neville E, Walker A. Immunology of sarcoidosis. *Am J Med* 1975; **59**:388-94.
- 3 Jones JV, Cumming RH, Asplin CM, Laszlo G, White RJ. Evidence for circulating immune complexes in erythema nodosum and early sarcoidosis. *Ann NY Acad Sc* 1976; **278**:212-9.
- 4 Gupta RC, Kueppers F, DeRemee RA, Huston KA, McDuffie FC. Pulmonary and extrapulmonary sarcoidosis in relation to circulating immune complexes: a quantification of immune complexes by two radio immunoassays. *Am Rev Respir Dis* 1977; **116**:261-6.
- 5 Daniele RP, McMillan LJ, Dauber JH, Rossman MD. Immune complexes in sarcoidosis: a correlation with activity, and duration of disease. *Chest* 1978; **74**:261-4.
- 6 Nydegger UE, Lambert PH, Gerber H, Miescher PA. Circulating immune complexes in the serum in systemic lupus erythematosus and in carriers of hepatitis B antigen. Quantitation by binding to radio labelled Clq. *J Clin Invest* 1974; **54**:297-309.