Circulating immune complexes in tuberculosis

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ABSTRACT Using a polyethylene glycol precipitation method, immune complexes were detected in 56% of patients with active tuberculosis. After antituberculous treatment, 29% remained positive for immune complexes, a frequency similar to that in a group of racially matched clinic controls (35%). In a study of 17 patients whose immune complex levels were determined soon after diagnosis and again when the therapy had ceased, there was a significant decrease in complexed IgM and C1q. Sequential studies of immune complex levels in tuberculosis merit further investigation; their persistence may indicate the continued presence of antigens, and their disappearance from the circulation may be a guide to successful treatment.

Circulating immune complexes have been shown in the sera of patients with granulomatous diseases such as sarcoidosis¹ and Crohn's disease.² That their presence in these disease correlates with their activity is debatable.^{1,3,4} They have not yet been studied in tuberculosis, which is another granulomatous disease, differing from sarcoidosis and Crohn's disease in that there is an identifiable causative organism and a known curative therapy.

We examined the sera of patients with tuberculosis for the presence of immune complexes to determine whether they were present, whether their presence was related to disease activity, and if the levels diminished with successful therapy.

Methods

PATIENTS

Ninety-six patients (41 male, 55 female), who were either currently affected by tuberculosis or were known to have had a previous infection were studied. These were the first 96 patients to be seen at the Chest Clinic during the period of this study. The mean age was 36 years (range 4-80 years). The racial distribution was Indian 80 (83%), Caucasian 11 (12%), Negro three (3%), and Chinese two (2%).

A diagnosis of tuberculosis was established by using standard clinical, radiological, and bacteriological criteria. Sites of disease were as follows: pulmonary (40); extrathoracic glandular (40); bone (9); abdominal (three); and hilar or mediastinal glands (22); 18 patients had more than one area

affected. The stage of treatment varied, including patients who were studied before its start, and patients who had ceased treatment up to 20 years previously.

In a longitudinal study, 17 patients (eight male, nine female; mean age 38 years, 16 Indian), were initially studied either before therapy had started (five patients) or in the first month of therapy (12 patients), and were restudied after completion of nine months' therapy. The antituberculous chemotherapy used for adults during this study was rifampicin (450 mg or 600 mg once daily) and isoniazid (300 mg once daily for nine months). For the first three months, or until cultures were obtained, daily ethambutol (15 mg/kg) was also given.

CONTROLS

Two control populations were used. A laboratory control group of sera from 80 laboratory and medical personnel was used to define the normal limits for the presence of immune complexes. The upper limit was defined as mean + 2 SD for each precipitated component. For comparison with the 17 patients used in the longitudinal study, we studied a clinic control group of 20 (12 M, eight F) TB contacts or recent immigrants to Brent. (Mean age 34 years (range 18-70); Indian 16 (80%), Caucasian three (15%), and Negro one (5%).)

IMMUNE COMPLEX ASSAY

The method of assay used was that already described,⁵ and in brief involved the precipitation of soluble immune complexes from serum by the addition of polyethylene glycol (MW 6000, Hopkin and Williams Ltd) to a final concentration of 2%, using a

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modification of the method described by Nydegger.⁶ The amounts of C1q, 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 com-

pared initially with those obtained in this test from the laboratory control group. To study the effect of serum immunoglobulin levels on the amount of precipitated immunoglobulin 217 sera from patients with a variety of diseases were studied. We found that the

Fig 1 Amounts of C1q, IgG, and IgM in the 2% PEG precipitations of 96 sera from patients with present or past tuberculosis. All results are expressed in g precipitated from 1 ml of serum. The horizontal bar represents the upper limit of normal (mean + 2 SD) in each column. These values are as follows: C1q 104 µg (44 µg + 60 µg); IgG 69 µg (39 µg + 30 µg); IgM 114 µg (44 µg + 70 µg).

total immunoglobulin levels in serum produce a small variation in the precipitated fraction. Thus for IgG a 50% increase in total serum IgG is associated with a 13% increase in precipitated IgG and for IgM a 50% serum increase produced only a 4% increase in precipitated IgM. Thus the absolute amount of Ig measured in the precipitated fraction is largely independent of changes in serum immunoglobulin levels.

STATISTICAL METHODS

Results had a normal distribution, so comparisons were made between values before and after therapy using Student's paired *t* tests. Unpaired *t* tests were used to compare clinic controls with patients with TB.

Results

INITIAL STUDY OF PATIENTS WITH TUBERCULOSIS

Forty-six of 96 (48%) patients with present or past tuberculosis have circulating immune complexes. The composition of these complexes is shown in figs 1 and 2. Abnormal amounts of C1q were present in 27 of these (59%), IgG in 32 (70%), IgM in 15 (33%), and IgA in 30 (65%). In the sera of 31 patients, more than one component was found.

Fig 2 Diagram showing composition of immune complexes. Three main components are shown—96 tuberculosis patients.

Seventy-eight per cent (7/9) of sera studied before therapy had complexes, while only 45% of sera studied while on or after therapy had them (39/87). Their presence decreased according to the length of

therapy; they were present in 56% of patients in the first and 33% in the last month of therapy, but even when treatment was completed 29% of the patients had them (fig 3).

Fig 3 Number of patients positive for immune complexes (ie, abnormal levels of C1q, IgG, IgM, and IgA), in relation to the state of therapy. "Treated" area represents those patients who have had more than nine months of treatment.

Patients with intra- and extra-thoracic disease both had similar amounts of complexes. Similarly, age, sex, and race had no influence on their presence, though they were present in the sera of 7/20 (35%) of the clinic control group, comprising largely recent immigrants to this country.

LONGITUDINAL STUDY

Because the frequency of complexes in the whole group appeared to decrease with increasing length of therapy (fig 3), we restudied 17 patients after completion of their nine months' therapy. Five were studied first before the start of therapy and 12 in the first month of therapy (figs 3, 4, 5, 6, table). Increased levels of C1q, IgG, and IgM immune complexes were present initially when the disease was active, with a significant fall in the levels of C1q and IgM complexes at the end of therapy. After therapy, levels of C1q, IgG, and IgM complexes were similar to those in both the laboratory and clinic control groups. In eight patients with active disease

Fig 4 Amount of IgG in the 17 patients before and after anti-tuberculous therapy. Laboratory and clinic controls (see text) are included for comparison.

Table Concentration of C1q, IgG, and IgM in PEG precipitates

	Clinic controls	Tuberculosis patients	
		First month therapy	After therapy
Number	20	17	17
C1q	38.6 ± 32.6	†103.0 ± 72.0	‡45.2 ± 28.4
IgG	43.0 ± 26.0	* 67.0 ± 44.0	43.0 ± 18.0
IgM	62.8 ± 66.0	*117.0 ± 62.0	§31.0 ± 35.2

All in µg from 1 ml serum (mean ± SD).

* Significant increase from control subjects $p < 0.05$.

† Significant increase from control subjects $p < 0.001$.

‡ Significantly lower than in the first month of therapy $p < 0.01$.

§ Significantly lower than in the first month of therapy $p < 0.001$.

complexed IgA was detectable. Six of these were unchanged at the end of treatment. The number of components (C1q, IgG, IgM, or IgA) in the complexes was less after completion of therapy (figs 7 and 8).

It is possible that the disappearance of immune complexes might have been related to drug therapy (for example rifampicin) being given at the initial time of testing for immune complexes, and then being finished at the time of the second assay, but the pattern of reduction in immune complexes shown by the five initially untreated patients is identical to that seen in the treated patients (figs 4, 5, and 6).

Fig 5 Amount of C1q in the 17 patients before and after anti-tuberculous therapy. Laboratory and clinic controls (see text) are included for comparison.

Discussion

A high proportion of patients with active tuberculosis have circulating immune complexes, the level of which shows no relationship to the site of disease. In patients with inactive disease the proportion with immune complexes is lower, and a longitudinal study of 17 patients over the course of their treatment showed that the abnormally elevated levels of C1q, IgG, and IgM complexes fell to normal limits at the end of the treatment period. These findings in general suggest that the presence of circulating immune

complexes is related to the activity of the disease—a finding which is similar to that seen in other infections such as sub-acute bacterial endocarditis.⁷ It seems unlikely that any of the initial elevation of immune complex levels is related to therapy with rifampicin.⁸ There was no significant difference in the level of complexes in those studied before or immediately after the start of treatment.

There was a significantly higher frequency of circulating immune complexes in the control group drawn from the Chest Clinic patients. They were matched for race with the patients studied and in so

Fig 6 Amount of IgM in the 17 patients before and after anti-tuberculous therapy. Laboratory and clinic controls (see text) are included for comparison.

far as racial origin was concerned they differed significantly from the subjects who comprised the laboratory controls. The source of this elevation of levels of immune complexes in our "normal" clinic population is not explained, but it does appear to be a characteristic of our patient groups. The levels in the treated and clinically cured patients came down not to the normal level for the laboratory control group but to the normal level for our local population.

It is not clear whether the presence of abnormal amounts of complexes in the serum of patients is of diagnostic value other than as a general indicator of activity of an infectious process.⁹ As a screening test the presence of complexes is not helpful, since at least 40% of the patients with active disease had no circulating immune complexes and they were still present in over 25% of our treated and cured patients. However, it is interesting to speculate that sequential study of levels in individual patients might

Fig 7 Comparison of 17 patients before (untreated) and after (treated) anti-tuberculous therapy. 0 represents absence of immune complexes. 1 represents the presence of one C1q, IgG, IgM, or IgA in the 2% PEG precipitates. >1 represents any combination or all of these.

Fig 8 Tuberculosis—the composition of immune complexes in 17 patients when disease was active and again after therapy—three main components shown.

give some guidance to the activity of the disease and provide useful information about the length of therapy necessary in the individual patient. The value of such a test might be improved if it were possible to identify the specific antigen in the complexes. This has not yet been feasible. Identification of complexes with this specific antigen might improve the accuracy of the test and its value in follow-up of treatment.

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