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Interaction of immune complexes and T suppressor cells in sarcoidosis

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ABSTRACT Circulating immune complexes were present in half of 31 patients with sarcoidosis and raised levels were associated with low numbers of T suppressor cells (Tγ- and Fcγ-receptor-positive lymphocytes). Incubation of T cells with trypsin restored the percentage of Tγ cells to within or above the normal range. Incubation of normal lymphocytes with sarcoid immune complexes significantly reduced the number of detectable Tγ cells. Preincubation of normal lymphocytes with circulating immune complexes significantly reduced their blastogenic response to concanavalin A. These studies suggest an interaction between immune complexes and T suppressor cells in sarcoidosis and emphasise the importance of immune complexes in modulating the immune response.

Sarcoidosis is associated with various alterations in the immune response.[1–4] Cutaneous anergy to tuberculoprotein and other recall antigens is common.[5,6] Patients with sarcoidosis frequently have low numbers of circulating T lymphocytes (E-receptor-positive cells) and an associated decrease of the in vitro T lymphocyte response to mitogens.[7] That the T lymphocyte population contains both T cells having a helper function (Tμ) and ones having a suppressor function (Tγ) is now well established.[8] The possible role of suppressor cells (Tγ) in diseases with aberrant immune responses has been studied by various techniques. We have used the ability of T suppressor cells to bind the Fc portion of immunoglobulin G (IgG) (Tγ or Fcγ-positive cells) to investigate the role of suppressor cells in sarcoidosis. In addition, we have studied the role of immune complexes, as sarcoidosis is commonly associated with increased concentrations of circulating immune complexes.[9–12]

Methods

Subjects Thirty-one patients with sarcoidosis (18 men and 13 women, aged 20–64 years) entered the study. The duration of their disease ranged from one month to 10 years and the sarcoidosis was classified conventionally as stage I (10 patients), stage II (9 patients), and stage III (12 patients). The diagnosis was confirmed by Kveim testing or tissue biopsy. No treatment was being given at the time of study. The control population comprised 21 healthy laboratory personnel matched for age and sex.

Immune complexes Blood was allowed to clot for two hours at room temperature and serum was separated by centrifugation and stored at −20°C. Complexes were measured by two techniques. The Raji cell radioimmunoassay was performed using the technique of Theofilopolous *et al.*[13] These measurements were kindly performed by Dr P Verroust of Paris and are expressed as aggregated human globulin equivalents (AHG) in μg/ml; values of 40 μg/ml and above are pathologically significant. Polyethylene glycol precipitation (PEG) was performed as described by Chia *et al.*[14] Samples from a single batch of pooled AB serum (Flow Laboratories, UK) were used as control sera.

Isolation of T cells (E-rosette positive) Mononuclear cells were isolated from heparinised peripheral blood by Ficoll-Hypaque density-gradient centrifugation. The interface cells were washed three times in RPMI 1640 (Flow Laboratories), which contains 2 g/l sodium bicarbonate and 100 μg/ml penicillin and streptomycin and are resuspended to a final concentration of 5×10^6 cells/ml. A suspension of 1 ml mononuclear cells, 1 ml human AB serum absorbed with sheep red blood cells (SRBC), 1 ml of

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0·5% SRBC, and 2 ml of RPMI 1640 was centrifuged at 200 *g* for five minutes and then incubated for one hour at 4°C. The pellet was gently suspended and the rosetted T cells were separated from the B cells and monocytes by further centrifugation on Ficoll-Hypaque. The SRBC attached to T cells were lysed with buffered ammonium chloride and the cells washed three times in RPMI 1640 containing 20% AB serum. The T cell yield was 97% pure as assessed by EAC rosetting, with 96% viability as determined by Trypan blue exclusion. The samples were free from macrophages as confirmed by non-specific esterase staining.

Anti-ox red blood cell (ORBC) antibodies Antibody to ORBC was obtained from rabbits immunised initially with 1 ml of 50% ORBC intravenously and given three weekly booster injections. Serum was obtained five weeks after the initial injection and IgG prepared by DEAE-cellulose chromatography and adjusted to a final concentration of 10 mg/ml.

ORBC–antibody complexes (OAg) ORBC antibodies were washed in RPMI 1640 three times and a 5% concentration was incubated at 37°C for 45 minutes with a subhaemagglutinating dose of IgG antibody. The sensitised cells were washed in RPMI 1640 three times and used at a 5% concentration.

T cells with receptors for IgG (Tγ—Fcγ-positive cells) Purified T cells (100 μl) (2×10^6 cells/ml) were mixed with 5% OAg (100 μl), centrifuged at 50 *g* for five minutes and incubated at 4°C for one hour. The pellet was resuspended and the number of rosettes were counted and expressed as a percentage of the total T cell population.

Trypsin treatment of T cells T lymphocytes from patients with sarcoidosis were isolated and adjusted to a concentration of 5×10^6 cells/ml. Aliquots of 1 ml were incubated in trypsin (Sigma Chemical Co Ltd) at a concentration of 2 mg/ml for 30 minutes at 37°C. Identical numbers of T cells were incubated in RPMI 1640 under the same conditions. Following incubation the cells were washed three times and readjusted to a concentration of 2×10^6 cells/ml. The percentage of Tγ cells was then measured by rosetting.

Immune complexes and Tγ cells Peripheral T cells from normal individuals were isolated, washed three times in RPMI 1640, and resuspended to a concentration of 2×10^6/ml. Immune complexes obtained by PEG precipitation from sarcoid serum were reconstituted in RPMI 1640 to 50% of the original concentration, to facilitate solubility. One millilitre of cells was incubated for one hour at 37°C with either 1 ml of immune-complex suspension or 1 ml of RPMI 1640. The cells were washed three times and the percentage Tγ cells was measured by rosetting with ORBC antibodies.

Blastogenic response of lymphocytes incubated with immune complexes Lymphocytes were preincubated with immune complexes or RPMI 1640 for 1 hour at 37°C. The cells were washed and adjusted to a concentration of 5×10^6 cells/ml. Cultures were set up in microtitre plates and incubated at 37°C in 5% carbon dioxide. Concanavalin A (Calbiochem, California) at a concentration of 10 μg/ml was added to each well. After 54 hours 1 μCi of tritiated thymidine (specific activity 2 Ci/mmol, Radiochemical Centre, Amersham) was added to each well. The cells were harvested 18 hours later and the radioactivity was measured in a liquid scintillation counter.

Results

Immune complexes Immune complexes were present in 12 out of 23 patients (52%) by the Raji cell assay and 11 out of 19 patients (58%) by polyethylene glycol precipitation. Six patients were tested by both techniques with comparable results.

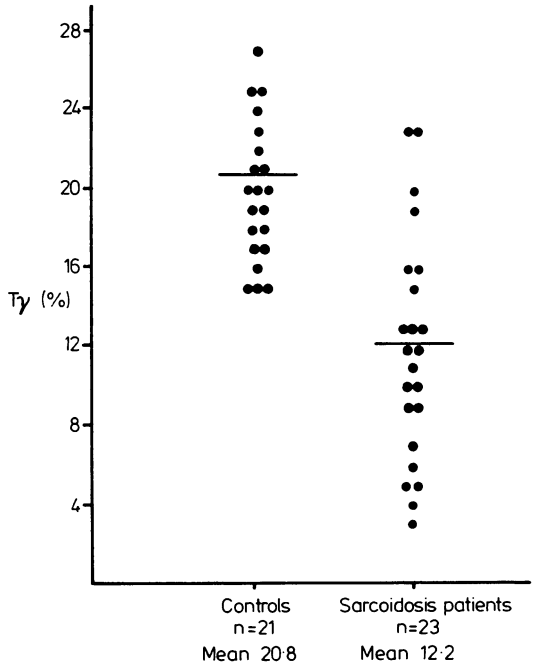

Fig 1 *Concentration of Tγ cells in patients with sarcoidosis and controls.*

Tγ cells The percentage of IgG-receptor-bearing cells was significantly lower in the patients (23 patients) (mean 12·2 ± 5·1) than in the normal controls (20·0 ± 3·9) (Student's *t* test) ($p < 0·001$) (fig 1).

Correlation of Tγ cells and immune complex levels In 23 patients there was a highly significant inverse correlation between raised levels of immune complexes as measured by Raji cell assay and low levels of circulating Tγ *cells* ($r = -0·7$) (fig 2).

Clinical assessment, immune complexes, and Tγ cells There was no correlation between the clinical criteria of duration of disease and radiographic staging and the immune complex and Tγ lymphocyte levels.

Effect of trypsin on sarcoid lymphocytes The percentage of Tγ cells isolated from the peripheral blood of patients with sarcoidosis increased after incubation with trypsin (fig 3). Trypsin, however, had little or no effect on the percentage of T cells isolated from the peripheral blood of normal individuals.

Effect of immune complexes on normal Tγ lymphocytes There was a highly significant reduction in the number of Tγ cells measured in a population of normal lymphocytes after incubation with precipitated immune complexes obtained from the serum of patients with sarcoidosis (paired *t* test: $p < 0·001$) (fig 4).

Lymphocyte blastogenic response The peak blastogenic response of the lymphocytes of six normal controls to concanavalin A was significantly reduced by preincubation with immune complexes from the serum of patients with sarcoidosis (paired *t* test: $p < 0·001$) (fig 5).

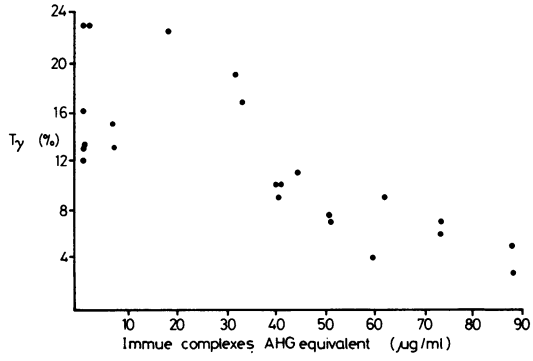

Fig 2 *Relation of Tγ cells and immune complexes in patients with sarcoidosis.*

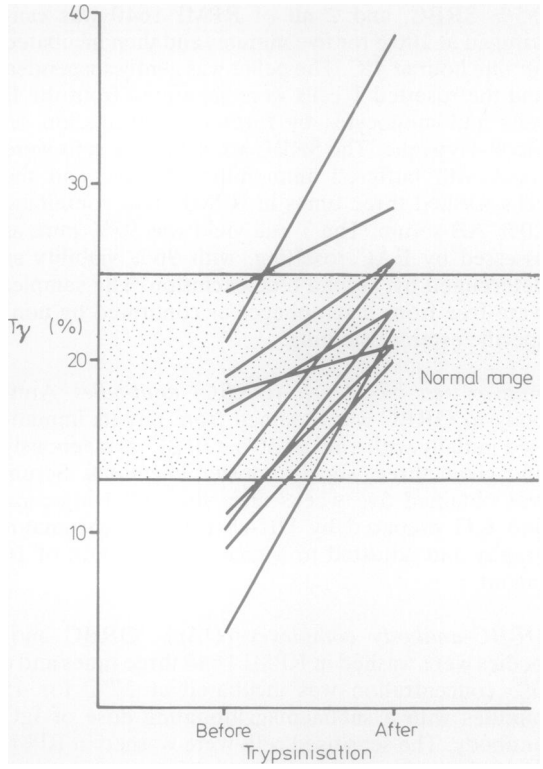

Fig 3 *Effect of trypsin 2 mg/ml on the percentage of circulating Tγ cells in patients with sarcoidosis.*

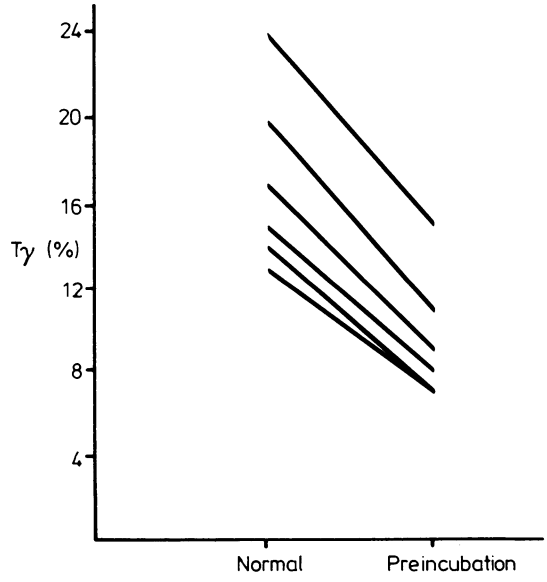

Fig 4 *Effect of preincubation with immune complexes on the percentage of normal Tγ cells.*

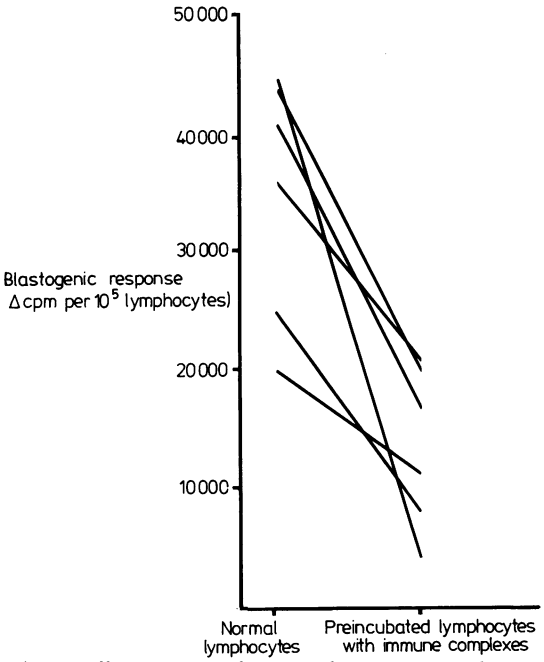

Fig 5 *Effect on preincubation with immune complexes on the blastogenic response of normal lymphocytes to concanavalin A.*

Discussion

The results of this study show a correlation between raised levels of circulating immune complexes and a decreased number of circulating Tγ lymphocytes. About half of our patients had detectable circulating immune complexes, a proportion similar to that found in other reported series.[9-12 15] We have not confirmed an association with the acute form of the disease[9] or with the radiological stage of the disease. The relation of immune complexes to Tγ cells in this study supports Moretta's hypothesis[8] that an interaction with immune complexes is necessary to induce Tγ cell activity. We do not, however, support his view that the surface receptor is lost after activation with immune complexes as trypsinisation of sarcoid Tγ lymphocytes restored the levels to normal values. Similar findings have been reported in Sjögren's syndrome.[16] Further, it has recently been shown that the receptors for naturally occurring immune complexes may be different from those of the Ig-ORBC receptor and loss of the latter receptor may not reflect the stability of the Fc receptor after stimulation with naturally occurring immune complexes.[17] The highly significant reduction in Tγ cells in sarcoid patients suggests that in over half of all patients there are circulating activated suppressor cells and that this phenomenon is directly associated with the presence of immune complexes. Similar studies in primary biliary cirrhosis have also shown a relation between depression of Tγ cells and raised levels of immune complexes.[18] Recently the homogenous nature of the Tγ population has been critically examined by the technique of specific monoclonal antibodies specific for T cell subsets.[19] Cells isolated by E rosetting are thought to contain not only T cells but contaminating "null" cells and monocytes. Those cells previously regarded as T suppressor cells (Tγ) are probably a mixture of true T suppressor cells, null cells, and monocytes. We have excluded the last by non-specific esterase staining but the presence of null cells may influence the results of this study. The cells isolated may be described alternatively as Fcγ-positive E-rosette cells.

Probably activation of suppressor cells in response to immune complex stimulation plays a critical part in the pathogenesis of these disturbances. What determines the persistence of immune complexes in patients with sarcoidosis is unknown but the nature and quality of the antigen and the "affinity" of the antibody may have important roles. Persistence of antigen may stimulate ongoing immune complex formation, resulting in both granuloma formation and activation of the suppressor cell system to reduce the heightened antibody response. Low-affinity antibody may protect the antigen from adequate removal and so perpetuate immunological stimulation. The antibody fraction in our patients did not have cytotoxic ability by microassay and did not contain rheumatoid factor, unlike the serological factors described by Daniele *et al.*[4]

We have also shown functional activity in the isolated immune complexes present in sarcoidosis serum, with the significant inhibition of the lymphocyte response to concanavalin A. The exact mechanism of this effect is not clear; but preliminary studies in our laboratory do not suggest prostaglandin-mediated macrophage activation, as the addition of a prostaglandin synthetase inhibitor (flurbiprofen) failed to prevent suppression.[20]

Comparison of this study with others describing suppressor cell activity in sarcoidosis is difficult.[20-24] High suppressor cell numbers have been shown with an additional incubation period at 37°C, but no previous study has compared circulating immune complexes with the numbers of Tγ cells. This study lends further support to the hypothesis that the immune response in sarcoidosis is influenced both by immune complexes and by the resulting interaction of suppressor and helper lymphocytes.

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References

[1] Buckley CE III, Nagaya H, Seiker HO. Altered immunologic activity in sarcoidosis. *Ann Intern Med* 1966;**64**:508–20.

[2] James DG, Neville E, Walker H. Immunology of sarcoidosis. *Am J Med* 1975;**59**:388–94.

[3] Hedfors E. Immunological aspects of sarcoidosis. *Scand J Respir Dis* 1975;**56**:1–19.

[4] Daniele RP, Dauber JH, Rossman MD. Immunologic abnormalities in sarcoidosis. *Ann Intern Med* 1980;**92**:406–16.

[5] Scadding JG. *Sarcoidosis*. London: Eyre & Spottiswoode, 1967.

[6] Friou GJ. A study of the cutaneous reactions to oidiomycin, trichophytin and mumps skin test antigens in patients with sarcoidosis. *Yale J Biol Med* 191951/52;**24**:533–9.

[7] Hedfors E. Characterisation of peripheral blood lymphocytes in sarcoidosis. *Ann N Y Acad Sci* 1976;**278**:101–7.

[8] Moretta L, Webb SR, Grossi CE, Lydyard PM, Cooper MD. Functional analysis of two human T cell subpopulations: help and suppression of B-cell responses by T cells bearing receptors for IgM or IgG. *J Exp Med* 1977;**146**:184–200.

[9] Hedfors E, Norberg R. Evidence for circulating immune complexes in sarcoidosis. *Clin Exp Immunol* 1974;**16**:493–6.

[10] Verrier Jones J, Cumming RH, Asplin CM, Lazlo G, White RJ. Circulating immune complexes in erythema nodosum and early sarcoidosis. *Lancet* 1976;**i**:153.

[11] Selroos O, Klockars M, Kekomaki R, Penttinen K, Lindstrom P, Wager O. Circulating immune complexes in sarcoidosis. *Clin Lab Immunol* 1980;**3**:129–32.

[12] McI Johnson N, McNicol MW, Burton-Kee JE, Mowbray JF. Circulating immune complexes in sarcoidosis. *Thorax* 1980;**35**:286–9.

[13] Theofilopoulos AN, Wilson CB, Dixon FJ. The Raji cell radio-immunoassay for detecting immune complexes in human sera. *J Clin Invest* 1976;**57**:169–82.

[14] Chia E, Barnett EV, Yamagata J, Knutson D, Restivo C, Furst D. Quantitation and characterisation of soluble immune complexe precipitated from sera by polyethylene glycol (PEG). *Clin Exp Immunol* 1979;**37**:399–407.

[15] Gupta R, Kueppers F, Deremee R, Huston KA, McDuffie FC. Pulmonary and extrapulmonary sarcoidosis in relation to circulating immune complexes. *Am Rev Respir Dis* 1977;**116**:261–6.

[16] Moutsopolous HM, Fauci AS. Immunoregulation in Sjögren's syndrome: influence of serum factors on T-cell subpopulations. *J Clin Invest* 1980;**65**:519–28.

[17] Samarut C, Revillard JP. Active and passive re-expression of Fc gamma receptors on human lymphocytes. *Eur J Immunol* 1900;**10**:352–8.

[18] Sandilands GP, Reid FM, Galbraith I, Mills PR, MacSween RN. Immune complex inhibition of lymphocyte Fcγ receptors in primary biliary cirrhosis—a possible immunomodulatory mechanism. *Lancet* 1980;**ii**:9–13.

[19] Reinherz EL, Moretta L, Roper M, Breard JM, Mingari MC, Cooper MD, Schlossman SS. Human T lymphocyte subpopulation defined by Fc receptors and monoclonal antibodies. *J Exp Med* 1980;**151**:969–74.

[20] Goodwin JS, Dehoratius R, Israel H, Peake GT, Messner RP. Suppressor cell function in sarcoidosis. *Ann Intern Med* 1979;**90**:169–73.

[21] Katz P, Haynes BF, Fauci AS. Alteration of T lymphocyte sub-populations in sarcoidosis. *Clin Immunol Immunopathol* 1978;**10**:350–4.

[22] Hunninghake GW, Fulmer JD, Young RC, Crystal RG. Comparison of lung and peripheral sarcoidosis. In: Williams WJ, Davies BH. *Proceedings of eighth international conference on sarcoidosis and other granulomatous disorders*. Cardiff: Alpha Omega Publishing Ltd, 1980.

[23] Semenzato G, Pezzutto A, Cipriani A, Gasparotto G. Imbalance in Tγ and Tμ lymphocyte sub-populations in patients with sarcoidosis. *J Clin Lab Immunol* 1980;**4**:95–8.

[24] Johnson N, McI Brostoff J, Hudspith BN, Boot JR, McNicol MW. Tγ cells in sarcoidosis: E rosetting monocytes suppress lymphocyte transformation. *Clin Exp Immunol* 1981;**43**:491–6.