

Defective yeast opsonisation of serum in sarcoidosis

N McI JOHNSON, M W McNICOL, A KAPOOR, J E BURTON-KEE AND
J F MOWBRAY

From the Willesden Chest Clinic, and the Department of Experimental Pathology, St Mary's Hospital Medical School, London

ABSTRACT We have studied the opsonising ability of the sera of 49 patients with sarcoidosis. The serum of 11 (22%) patients was defective in this ability, whereas only two (4%) of 46 clinic control subjects and three (7%) of 43 laboratory control subjects showed this defect. The difference between the prevalence of the defect in sarcoidosis and the control groups was statistically significant ($p < 0.01$). Although the patients with sarcoidosis who had this defect tended to have pulmonary infiltration, this relationship was not statistically significant. Similarly, there was no correlation between the activity of sarcoidosis and this defect, although there was a significant ($p < 0.05$) relationship between the opsonising defect and the persistence of circulating immune complexes. Serum opsonisation is a genetically linked host defence mechanism. Our findings suggest that the presence of this serum defect may render a person more susceptible to sarcoidosis.

Approximately 5% of sera from a normal population fail to opsonise yeast for phagocytosis by normal human polymorphonuclear leucocytes. This defect is common and is thought to underlie much frequent infection and allergy in childhood.¹ The process of opsonisation is an important part of the effector arm of host defence mechanisms. We have examined the sera of patients with sarcoidosis for the presence of this defect, because in this granulomatous disease it is presumed that the persistence of antigen or immune complexes may lead to chronic disease, and opsonisation is an important host defence mechanism that might prevent it.

Patients

Forty-nine patients with sarcoidosis were studied (31 men, 18 women). These were the first 49 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 years), racial distribution was West Indian 28 (57%), white 16 (33%), and Indian five (10%). Length of history ranged from less than one month to over 20 years (mean 4.9 years). A

diagnosis of sarcoidosis was made on clinical features, chest radiography, and histology. 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 patients' visit on the basis of clinical, biochemical, or radiological features. At this visit a blood sample was taken for the measurement of opsonising activity, immune complexes, complement, and rheumatoid factor. At the end of the study the chest radiographs taken on the day the blood samples were taken were assessed for the presence of lymphadenopathy, infiltration, or fibrosis without the observer having reference to the patients' names or clinical details. The standard staging of sarcoidosis was made: 0 = normal chest radiograph; 1 = bilateral hilar lymphadenopathy only; 2 = bilateral hilar lymphadenopathy with pulmonary infiltration; 3 = pulmonary infiltration or fibrosis alone.

Twenty-two male and 24 female control subjects were tested, the mean age was 31 years (range 14-74 years). The racial distribution was West Indian 11 (24%), white seven (15%), and Indian 28 (61%). These were healthy adults asked to attend Willesden Chest Clinic as recent immigrants to Brent or as contacts of patients with tuberculosis. Forty-three normal laboratory and medical staff were also tested.

Laboratory methods

Sera were separated within four hours of collection and stored at -70°C until analysed.

The opsonisation test used was a slight modification of that described by Levinsky *et al.*² Ten μl of yeast particles and 10 μl of normal human polymorphonuclear leucocytes with a ratio of yeast to polymorphs of 5:1 were mixed together with 5 μl of test serum in a Luckham plastic tube and the reaction mixture was made into a total volume of 200 μl , with barbitone buffer (pH 7.6) with calcium (10 mM), and magnesium (10 mM). The mixture was incubated at 37°C for 30 minutes on a rotating mixer. Control samples of 10 μl yeast particles were made up to the same reaction volume and also incubated. Known samples of normal and defective serum samples were also included in each bath. After incubation the reaction mixture was diluted 1:500 with an automatic dilutor (Compupet, Warner Diagnostic). The red cells were lysed by the addition of a drop of Zaponin (Coulter Electronics, Harpenden) to each assay tube and the number of unphagocytosed yeast particles were counted in a Coulter counter. The results were expressed as percentage uptake of yeast particles by the normal polymorphs.

$$\% \text{ uptake} = \frac{t-y}{t} \times 100$$

t = total number of yeast particles

y = number unphagocytosed

Immune complexes were precipitated with 2% polyethylene glycol and the Clq and immunoglobulin determined as described by Nydegger *et al.*³ CH50 was measured by a standard functional assay, using sensitised sheep red cells, and C3 by single radial immunodiffusion. The antisera were raised in rabbits by immunisation with purified components, and standardised by comparison with reference antisera.

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

Results

The sera from 11 (22%) of the 49 patients with sarcoidosis had defective opsonisation ability, whereas only two (4%) sera out of 46 from healthy subjects showed this defect ($p < 0.01$ Fisher's exact test).

The racial distribution of the defect in sarcoidosis was white five (31%), West Indian

five (18%), and Indian one (20%). Of control subjects with the defect one was white and the other Indian. Three (7%) of the 43 normal laboratory staff showed the defect.

Results showing the prevalence of immune complexes and rheumatoid factors in these patients will be presented elsewhere. There was a significant relationship ($p < 0.05$ Fisher's exact test) between the presence of circulating immune complexes and the opsonising defect (table 1). Nine (82%) of the 11 patients with sarcoidosis with the defect had evidence of circulating immune complexes, whereas only 19 (50%) of the 38 patients without the defect had complexes (figure). Rheumatoid factor was present in 14 (29%) of the 49 patients. Only four patients with

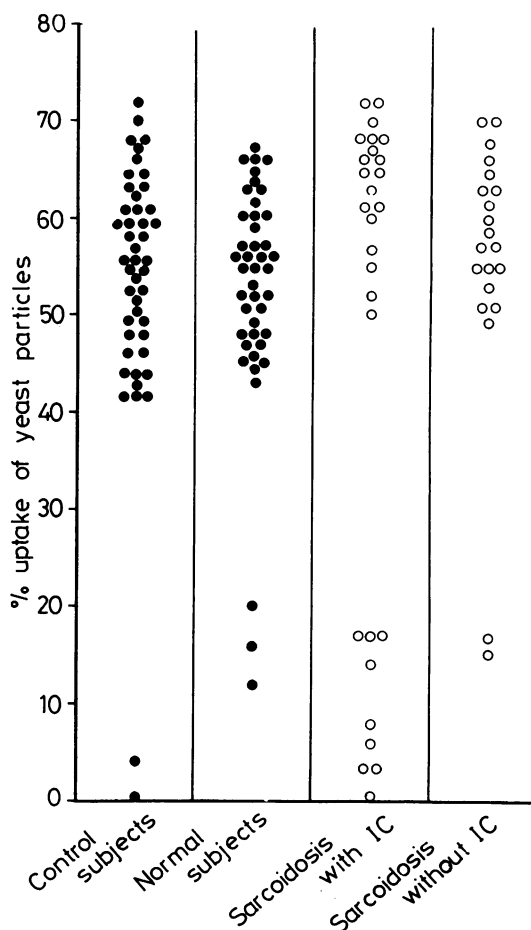


Figure Percentage opsonisation of yeast particles in control subjects, normal subjects, and patients with sarcoidosis with and without immune complexes (IC).

Table 1 *Opsonising defect and circulating immune complexes in sarcoidosis*

Opsonisation	Immune complexes		
	+	-	Total
Defective	9*	2	11
Normal	19	19	38
Total	28	21	49

*Significant difference $p < 0.05$ (Fisher's exact test)

the defect had rheumatoid factor, thus showing no correlation between the defect and its presence.

There was no correlation between the presence of the opsonising defect and the activity of sarcoidosis. The disease was judged to be active in nine (82%) patients with the defect and 30 (61%) without the defect.

Table 2 *Stage of sarcoidosis and opsonisation in 49 patients*

Stage	Defect	Normal
0	1	9
1	2	11
2	6	12
3	2	6

When stage of disease was considered (table 2) there was no definite pattern; however, of the patients with pulmonary infiltration or fibrosis (stages 2 and 3) eight out of 26 (31%) had the defect, whereas only three out of 23 (13%) of those without lung infiltration had the defect. This difference was not statistically significant ($p < 0.09$). Complement levels were normal (table 3).

Table 3 *Complement levels*

Complement	Sarcoidosis patients ($n = 49$)		Control subjects ($n = 46$)	
	Mean*	$\pm SD$	Mean*	$\pm SD$
Clq	110	9	101	8
CH50	99	9	106	7
C3	101	11	102	9

*Expressed as percentage of control pool of normal sera. Differences between normal and disease groups not significant.

Discussion

We have shown that 22% of sera from the patients with sarcoidosis and only 5% of the control sera had a defective opsonising ability, indicating a significant association between this defect and sarcoidosis. The prevalence of this defect in our control subjects is in agreement

with that found in previous studies.^{1,2} We found it impossible to obtain exact racial matching in our control group; however, the increased frequency of this defect was seen in each race tested and the increase was greatest in the whites. Thus it is unlikely that lack of exact racial matching between our groups could account for the differences observed.

If the defect was caused by the disease, one might expect to find complement consumption but the normal levels of complement do not support this. It is known that the opsonising defect is genetically controlled and may be associated with gene products of the HLA region of the sixth chromosome.⁴ The molecular basis for the defect is unknown but can be restored by the addition of small amounts of normal plasma.^{1,5} Previous studies have shown this defect to be associated with frequent infection and allergy in childhood, and atopy presenting with infantile eczema or hay fever.^{1,4}

The patients with the defect tended to have pulmonary infiltration, although this difference was not statistically significant. The significance of our findings is uncertain. The increased frequency and the association of the defect with the persistence of circulating immune complexes suggests that it might have aetiological significance by impairing antigen and immune complex elimination, thereby leading to occurrence and persistence of the disease.

Further studies are necessary not only to find whether there is an increase in the incidence of sarcoidosis in patients with defective opsonisation but also to see whether patients with sarcoidosis and the defect develop progressive disease. At present the association we have found between sarcoidosis and the opsonising defect does not appear to be of prognostic significance.

References

- 1 Soothill JF, Harvey BAM. Defective opsonisation. A common immunity deficiency *Arch Dis Child* 1976; **51**:91-9.
- 2 Levinsky RJ, Harvey BAM, Paleja S. A rapid objective method for measuring the yeast opsonisation activity of serum. *J Immunol Methods* 1978; **24**:251-6.
- 3 Nydegger UE, Lambert PE, 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.
- 4 Turner MW, Mowbray JF, Harvey BAM

Brostoff J, Wells RS, Soothill JF. Defective yeast opsonisation and C2 deficiency in atopic patients. *Clin Exper Immunol* 1978; **34**:253-9.

5 Miller ME, Seals J, Haye R, Levitsky LC. A familial plasma-associated defect of phagocytosis. *Lancet* 1968; **2**:60-3.