

Defective yeast opsonisation of serum in tuberculosis

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ABSTRACT We have studied the opsonising ability of the sera of 92 patients with tuberculosis. Fourteen per cent of these patients showed defective opsonising ability compared with 4% in a clinic control group. This increased frequency in TB was accounted for by the greatly increased proportion with defects found in patients with intrathoracic TB (21%). We may have identified a section of the population with a specific genetically linked abnormality of a host defence mechanism which renders them more susceptible to intrathoracic TB. Further population studies are required.

In recent years the incidence of tuberculosis in some parts of Britain has increased, almost entirely because of the higher incidence in the immigrant population. Various explanations have been proposed. One possibility is that this increase is caused by a defect in one or more of the host defence mechanisms of these patients. We have studied one such mechanism, serum opsonisation, in Willesden—an area of London with both a large immigrant population and a relatively high prevalence of TB.

Opsonisation prepares circulating micro-organisms for phagocytosis. This occurs either through an immune pathway requiring IgG and the classical complement pathway or by a rapid non-immune pathway, which requires a serum factor and the alternative pathway of complement. There is a genetically linked defect of this latter non-immune pathway in approximately 5% of the general population, in that the sera from these patients fail to opsonise yeast for phagocytosis by normal human polymorphonuclear leucocytes. This defect of the effector arm of the host defence mechanism is found excessively frequently both in children with recurrent infections¹ and in adults with sarcoidosis.² We have, therefore, studied patients with tuberculosis for this defect.

Methods

Blood was taken from 92 patients in whom tuberculosis had been diagnosed. These were chosen at random from patients attending Willesden Chest Clinic. A control group of 46 patients with no

Table 1 Patients studied

	Tuberculosis patients	Clinic control subjects
Number	92	46
Sex	39 M 53 F	22 M 42 F
Mean age (yr)	37 (range 4-80)	31 (range 14-74)
Race		
Indian	77 (84%)	28 (61%)
Caucasian	9 (10%)	7 (15%)
Negro	4 (4%)	11 (24%)
Chinese	2 (2%)	—

evidence of TB attending Willesden Chest Clinic for BCG were studied. This group had a racial distribution similar to the patients (table 1). A group of 45 unmatched laboratory staff was also tested.

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.*³ Ten µl of yeast particles and 10 µl of normal human polymorphonuclear leucocytes with a ratio of yeast polymorphs 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 of yeast particles were made up to the same reaction volume and also incubated. Known samples of normal and defective sera were also included in each batch. After incubation the reaction mixture was diluted 1:500 with an automatic diluter (Compupet, Warner Diagnostics). The red cells were lysed by the addition of a drop of Zaponin (Coulter Electronics, Harpenden) to each assay tube and the number of unphago-

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cytosed yeast particles were counted in a Coulter counter. The results were expressed as a percentage uptake of yeast particles by the normal polymorphs.

$$\text{Percentage uptake} = \left(t - \frac{y}{t} \right) \times 100$$

t = total number of yeast particles
y = number unphagocytosed

Immune complexes were precipitated with 2% polyethyleneglycol and the Clq and immunoglobulins determined by radial immunodiffusion.⁴ 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 relevant antisera.

Results

The sera from 13 (14%) of the 92 patients with TB had defective opsonising ability, whereas only 2 (4%) of the 46 clinic control subjects had this defect (figure, table 2). As the frequency of the defect was the same in both the racially matched and unmatched control groups, the results were pooled to obtain a group of similar size to that of the patients with TB. In this pooled control group, five of 89 sera were defective, significantly less than in the TB group. ($p=0.03$ Fisher's exact test).

When the site of disease was considered (table 3), the increased frequency of the defect was almost entirely confined to patients with lung disease (21%) or hilar or mediastinal lymph node enlargement (21%) ($p<0.05$ Fisher's exact test). In patients with peripheral node, pleural, or bone disease the frequency of the defect was normal.

The defect was increased in all racial groups studied (table 4).

Complement values were normal in individual patients and in the groups taken as a whole (table 5).

When an assessment was made as to the severity

and activity of tuberculosis there appeared to be no difference between the patients with this defect and those without, nor did the presence of this defect relate to response to therapy.

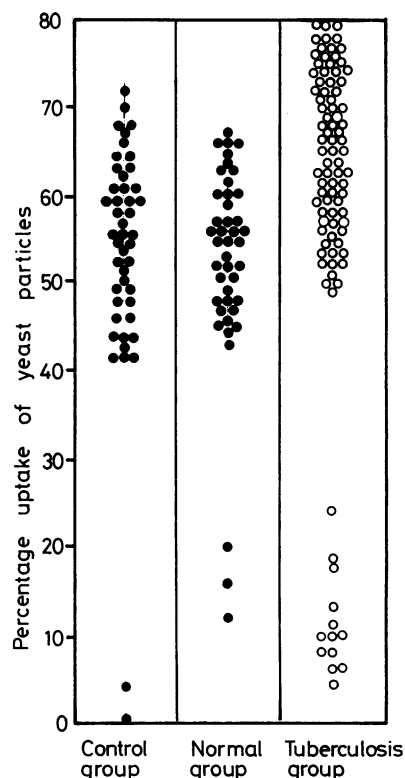


Figure Uptake of yeast particles by normal polymorphs in BCG clinic control subjects, normal laboratory staff, and patients with tuberculosis.

Table 2 Opsonising defect in tuberculosis

	Defect		Total
	Number	%	
All patients with TB	13	14	92
Clinic control subjects	2*	4	46
Laboratory control subjects	3	7	43
Pooled controls	5†	5	89

* Statistical difference from TB patients $p = 0.05$ Fisher's exact test.

† Statistical difference from TB patients $p = 0.03$ Fisher's exact test.

Table 3 Opsonising defect and site affected by tuberculosis

Opsonisation	Control subjects	All TB patients	Site				
			Lung	Hilar or mediastinal glands	Extrathoracic nodes	Pleura	Bone
Total	46	92	34	23	35	6	7
Normal	44	79	27	18	34	6	7
Defective	2	13*	7*	5*	1	0	0

* Significantly different from control subjects $p < 0.05$ (Fisher exact test).

A few patients had disease in more than one site.

Table 4 Racial distribution of defect

Race	Tuberculosis patients	Control subjects
Indian	8/77	1/28
Caucasian	2/9	1/7
Negro	3/4	0/11
Chinese	0/2	—

Table 5 Complement values

	Tuberculosis patients (n = 53)		Control subjects (n = 46)	
	Mean*	± SD	Mean*	± SD
CH50	98	12	106	7
C3	102	13	102	9

* Expressed as percentage of control pool of normal sera. Differences between control subjects and tuberculosis patients not significant.

Discussion

We found an increased frequency of this defect of opsonisation in the serum of patients with tuberculosis, accounted for by the marked increase (21%) in those patients with intrathoracic disease. The frequency of the defect was increased in all races studied, therefore racial selection is unlikely to account for our findings.

It may be argued that defects of the alternative pathway of complement through congenital lack of complement components or consumption of complement might cause defective opsonisation. However, the values of serum complement in individual cases and in groups as a whole were normal.

The presence of this defect in tuberculosis seems to be of little prognostic significance in that the disease responded satisfactorily to therapy in all cases, and patients with this defect did not appear to have the disease more severely than those without. The reason why patients with this defect should have intrathoracic rather than extrathoracic disease is uncertain, but it is interesting that in a previous study the patients with

sarcoidosis who had this defect had a tendency towards pulmonary infiltration.²

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.⁵ The molecular basis for the defect is unknown. Normal opsonisation can be restored by the addition of a small amount of normal plasma.^{1 6} Previous studies have shown this defect to be associated with frequent infection and allergy in childhood and atopy presented with infantile eczema or hay fever.^{1 5} Recent work has also shown there to be an association between this defect and sarcoidosis.²

We may have identified a section of the population with a specific genetic defect which renders them more susceptible to intrathoracic tuberculosis. Further population studies of large numbers of people with this defect are required.

References

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