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# Neutrophil phagocytosis in sarcoidosis. Reduced C3b receptor-mediated phagocytosis in active and silent sarcoidosis

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## SUMMARY

The phagocytic and complement receptor function of polymorphonuclear neutrophils (PMN) from patients with sarcoidosis was studied using a kinetic assay which allows the distinction to be made between Fc receptor-mediated and C3b receptor-mediated particle uptake. The study included one group (A) of patients with active disease ($n = 20$), and one group (B) with silent or inactive disease who since 10 years had no symptoms or radiological signs of sarcoidosis ($n = 11$). Abnormal C3b receptor function was observed in both groups but the impairment was most pronounced in the A group. The presence of C3b receptor dysfunction in both groups with a quantitative difference between the groups, is compatible with C3b receptor dysfunction being a primary causal factor of sarcoidosis.

**Keywords** C3b receptor neutrophils phagocytosis sarcoidosis

## INTRODUCTION

Defective neutrophil phagocytosis has been noted in a majority of a sarcoidosis population (Hällgren *et al.*, 1982), and we interpreted this to be the consequence of humoral inhibition and defective complement receptor function of the neutrophils. Neutrophil migration is also often abnormal in sarcoidosis (Campbell, 1977; Gange *et al.*, 1977; Maderazo *et al.*, 1976; Schmekel *et al.*, 1984), and one hypothesis for granuloma formation in sarcoidosis is that defective neutrophil function results in abnormal antigen clearance, leading to abnormal stimulation of macrophages and, consequent granuloma formation. The question was raised if the defective neutrophil phagocytosis was a consequence of the disease process or if it had a triggering function for the initiation of granuloma formation and thus would be a causal factor predisposing for the disease development. A large prospective study in healthy individuals with evaluation of neutrophil function might answer this question but was not feasible. Instead we performed a study of PMN phagocytosis in now apparently healthy persons who had passed through the disease years ago (silent group). The results were compared with those in patients with symptoms and a short disease duration (active group). Inflammatory cell markers, chest radiographs and lung function tests were used in the evaluation of disease activity.

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## MATERIALS AND METHODS

*Patients.* Two groups of sarcoidosis patients were studied. One group (A) of 25 sarcoidosis patients had their disease diagnosed during the 3 year period immediately preceding the start of this study. Five of these patients were excluded because of previous erythema nodosum. The mean age of the remaining 20 patients was 39 years (range 26–75 years) and 10 were women. Chest radiographs were normalized in eight cases, bilateral hilar lymphoglandular enlargement was present in eight and in the remaining four cases parenchymal densities were noted. These 20 patients were judged to have active sarcoidosis. Another group (B) of 24 persons had had the disease diagnosed during a time period, occurring 10–12 years before the start of this study. From this group, 13 persons were later excluded because of signs of active sarcoidosis such as chest radiographic abnormality or various subjective symptoms. Persons who previously had erythema nodosum were also excluded. Chest radiograms were normal in the remaining 11 persons and they had no clinical signs of disease. The mean age of these persons was 50 years (range 32–72 years), and 10 were women. These persons were judged to have silent or no disease.

Two persons of group B had had the diagnosis histologically verified in the initial period of disease. Ten patients of group A were examined by mediastinoscopy and were found to have typical histological findings. In the remaining cases the clinical picture was typical of sarcoidosis and follow-ups gave no indication of other diseases.

*Lung function studies.* Total lung capacity, forced expiratory flow in 1 s, vital capacity, phase III slope and closing volume measured by the single breath $N_2$ test, lung compliance and specific compliance were measured as earlier described (Fridriksson *et al.*, 1981). Four patients of group A abstained from the compliance measurement. Lung function variables were related to age and weight, etc., according to earlier published regression equations (Fridriksson *et al.*, 1981).

None of the patients were smokers. The lung function variables were expressed as difference from the predicted normal value in standard deviation units. Values lower than predicted are indexed as minus, and values higher than predicted as plus.

*Blood analyses.* **Phagocytosis** Latex particles coated with IgG (IgG particles) or IgG and complement (C3b particles) from pooled normal serum were phagocytosed by PMN isolated from heparinized venous blood (Håkansson, Hällgren & Venge, 1980). The phagocytic uptake rate was measured as earlier described (Hällgren, Jansson & Venge, 1977), using an electronic thrombocyte counter. PMN isolated from 37 healthy laboratory workers, phagocytosed IgG coated particles at a rate of 0·58/min (s.d. 0·14). C3b particles were phagocytosed at an enhanced rate by normal PMN 0·98/min (s.d. 0·23). C3b receptor-mediated enhancement was defined as the difference in uptake of C3b and IgG particles. The mean normal value was +0·40/min (s.d. 0·24).

**Lysozyme** Lysozyme was measured with a radioimmunoassay as previously described (Venge *et al.*, 1979). The mean serum content was 1,780 μg/l (s.d. 504) in 35 healthy laboratory employees.

## RESULTS

*Phagocytosis*

The phagocytosis of IgG particles by PMN was found to occur at a normal rate in both group A and group B. This result accord with earlier measurements in sarcoidosis patients by Hällgren *et al.* (1982). Our observed value for uptake rate was 0·56/min (s.d. 0·12) in group A, and 0·67/min (s.d. 0·24) in group B, and the difference was not significant.

The uptake rate of C3b particles in group A was found to be 0·45/min (s.d. 0·16) and in group B 0·76/min (s.d. 0·19). Therefore, both group A ($P < 0·001$) and group B ($P < 0·01$) deviated from normal by an apparent defective C3b receptor function (Fig. 1). Thus, the enhancement of uptake rate, normally caused by coating IgG particles with complement was absent in group A (mean −0·11/min, s.d. 0·18), and significantly ($P < 0·001$) less prominent in group B (mean +0·08/min, s.d. 0·18) than normally. The difference in enhancement due to C3b receptor interaction in the A and B groups was significant on the 1% level.

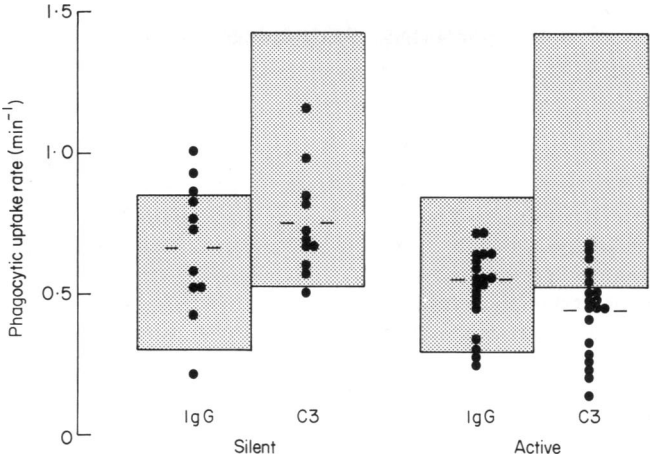


**Fig. 1.** PMN phagocytic uptake rate of IgG or C3 particles in group A (active disease) and group B (silent disease). Normal variation (mean ± 2 s.d.) is indicated by the shaded area (for further information see text).

*Serum analyses*

Serum content of lysozyme in patients (A) with active disease (2,723 μg/l, s.d. 1,544) was significantly higher than normal ($P < 0{\cdot}01$), and higher ($P < 0{\cdot}01$) than in persons (B) with silent sarcoidosis (1,693 μg/l, s.d. 408).

*Lung function tests*

Lung function tests reflecting lung volumes and air flow were normal in both groups. (Data not shown). Decreased compliance was, however, noted in group B (Fig. 2), suggesting increased lung stiffness possibly due to some degree of fibrosis. These tests were normal on an average in group A. The difference between A and B in compliance was significant ($P < 0{\cdot}01$).

*Relationship between* in vivo *and* in vitro *tests*

Individual IgG-mediated or complement-dependent enhancement of phagocytic uptake rates were

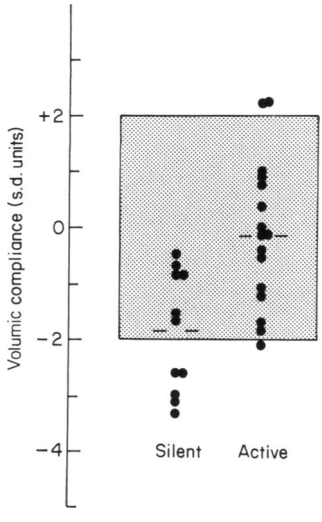


**Fig. 2.** Lung compliance in group A (active disease) and B (silent disease). Deviation from predicted values, expressed as s.d. units, is indicated. Normal variation (mean ± 2 s.d.) is indicated by the shaded area.

not related to the present chest radiographic appearance or to the lung function variables. Nor was there any relationship between individual serum content of lysozyme and Fc receptor-mediated or C3b receptor-mediated phagocytic uptake rates in these groups of patients.

## DISCUSSION

Complement receptor dysfunction of sarcoid neutrophils has previously been observed (Hällgren *et al.*, 1982). Since it has been hypothesized that defective neutrophil phagocytic function could predispose for granuloma formation (Segal & Loewi, 1976), the question was raised if the complement receptor dysfunction was a primary defect of the sarcoid PMN or an abnormality secondary to the disease. Therefore we studied apparently healthy persons (group B) who had had sarcoidosis diagnosed more than 10 years before the start of this study but now free of signs or symptoms. Another group (A) consisted of patients with symptoms who had their diagnosis less than 3 years before the study.

HLA B8 antigen has been shown to be associated with sarcoid arthritis and/or erythema nodosum (Neville *et al.*, 1980). Furthermore, patients with erythema nodosum are suggested to have a favorable prognosis, and possibly sarcoidosis with erythema nodosum involves different pathogenetic mechanisms. Therefore individuals with erythema nodosum were excluded.

Thus, data from persons with clinically silent sarcoidosis were compared with those of patients with ongoing active disease.

Serum lysozyme in persons with silent disease was not significantly different from normal, suggesting minimal amount of granuloma mass in those persons (Selroos & Klockars, 1977). On the other hand, granuloma mass was suggested present in patient with active disease, since lysozyme content in serum from those patients was significantly higher than normal.

Previous investigations on neutrophil phagocytosis have led to the concept that an interaction with the Fc receptor is necessary for the initiation of internalization of e.g. an immune complex. C3b receptors, and possibly C4b receptors, are responsible for the recognition and adhesion (Schribner & Fahrney, 1973) thus augmenting the phagocytic uptake rate. In the present study the IgG particles were phagocytosed at normal rates in both groups of patients, suggesting normal Fc receptor function of the sarcoid PMN. These findings are in accordance with our earlier observations (Hällgren *et al.*, 1982).

The expected complement-mediated enhancement of PMN phagocytic uptake rate was however, below normal in both groups, suggesting abnormal C3b receptor function. More pronounced aberrations were noted in PMN from A patients since the complement-mediated enhancement was not at all seen in this group, B persons had less pronounced aberration of complement receptor function, suggesting that active granuloma formation influences, but is not a pre-requisite condition for the complement receptor dysfunction to occur in sarcoidosis.

Our conclusion therefore is that complement receptor dysfunction of some degree can be found in both active and silent cases of sarcoidosis, which is compatible with the hypothesis that such dysfunction precedes and triggers the onset of the disease. The fact that our silent cases had a lower than normal lung compliance may be explained as disease sequelae and does not prove active disease.

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