

DECREASED PHAGOCYTIC CAPACITY OF THE NEUTROPHIL LEUCOCYTES IN PATIENTS WITH ATOPIC DERMATITIS

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Abstract. A comparative study has been made of the phagocytic capacity of leucocytes in patients with mild, moderate, and severe atopic dermatitis. For this screening study, the yeast-particle method has been used. Neutrophil leucocytes in patients with severe atopic dermatitis show a pronounced impairment in their phagocytic function, whereas phagocytosis in those with mild dermatitis did not differ from that in healthy controls. The impaired phagocytosis might be associated with the frequent infections in severe atopic dermatitis but might also point to an abnormality of significance for the dermatitis itself.

Defects in the phagocytic function of the neutrophil leucocytes have been demonstrated in several disorders (1, 6, 7). Dermatological disorders found to be associated with defective phagocytosis are discoid lupus erythematosus, palmo-plantar pustulosis and recurrent erysipelas (12). Impaired phagocytosis has also been described in patients with alopecia areata and with accelerated hair loss (15). The mechanism of impaired phagocytosis in these clinically non-related dermatoses is obscure, however.

The main clinical symptoms hitherto known to be connected with impaired phagocytosis are recurring or chronic infections. Since patients with severe types of atopic dermatitis often suffer from frequent staphylococcal and streptococcal skin infections, a study of the phagocytic capacity of the neutrophils in patients with severe atopic dermatitis compared with that of those patients having mild to moderate dermatitis seemed to be of interest.

The ability of the neutrophils to ingest yeast particles is the criterion commonly applied in studies of their phagocytic capacity and this paper presents the results of a screening where this method has been used for evaluation of the phagocytic capacity in atopic dermatitis. In addition,

a preliminary report on the results of a pilot study with a phagocytosis-enhancing drug, clofazimine (Lamprene[®], Ciba-Geigy A.G., Basle, Switzerland), is included (3).

PATIENTS AND CONTROLS

Sixty-five patients, over 15 years of age and having atopic dermatitis, are presented. The severity of their dermatitis was graded on a 1 to 3 scale as described by Öhman (17). Grade 1, mild dermatitis: slightly eczematous changes in the elbow and knee flexures, wrists or ankles. Grade 2, moderate dermatitis: more pronounced eczematous changes in the flexures and also a few patches elsewhere on the limbs, trunk or head. Grade 3, severe dermatitis: often severe excoriations, with pronounced eczematous changes in most flexures on the limbs and also eczematous changes over large areas of the body. The dermatitis of 27 patients was considered to be mild; 20 patients, moderate; and 18, severe.

Controls. Twenty healthy subjects between 15 and 30 years of age served as controls.

The method for study of phagocytic activity is a modification of that described by Brandt (2). Heparinized whole blood was left at room temperature for 30 to 120 minutes, depending upon the sedimentation rate. Leucocyte suspensions were prepared from the buffy coat and the number of mature neutrophils was adjusted to 5 000 per ml by dilution with the donor's cell-free plasma. Admixture of red cells was avoided as far as possible. Before incubation with the particles, the cell suspensions were shaken carefully. Heat-destroyed baker's yeast cells in a concentration of 20 000 per ml in 0.9% saline were used as particles to be phagocytized by the neutrophils. A large batch was prepared at the beginning of the present investigation, stored in tubes containing about 1 ml of the particle suspension and kept frozen at -20°C. In a centrifuge tube, a mixture of leucocytes and particles was made, consisting of 0.4 ml of the leucocytic suspension and 0.4 ml of the particle suspension, and incubated for 30 minutes at 37°C; then 0.1 ml of 0.9% EDTA solution in 0.9% NaCl was added and the mixture centrifuged at 200 rpm for 2 minutes. The supernate was removed, leaving a droplet for suspension of the sediment.

Table I. Phagocytic index of patients with mild, moderate and severe atopic dermatitis

	No. of cases	Mean phagocytic index	Analysis of the difference to the controls	
			t-values	Significance
Controls	20	3.69		
Atopic dermatitis (All patients)	65	3.22	2.61	$0.02 > p > 0.01$
Atopic dermatitis Grade 1	27	3.57	0.54	$0.6 > p > 0.5$
Atopic dermatitis Grade 2	20	3.32	1.48	$0.2 > p > 0.1$
Atopic dermatitis Grade 3	18	2.58	5.05	$p < 0.001$

For evaluation of phagocytosis, droplets of the sediment were spread on slides, air-dried, and then stained with May-Grünwald-Giemsa. Two hundred neutrophils were examined and grouped according to the number of particles ingested. Eight groups were distinguished: the first without any particles and the last with 7 or more particles. A phagocytic index (PI) was calculated as the mean particle number per neutrophil.

In a preliminary trial, 11 patients with severe atopic dermatitis and with a low PI were treated with the phagocytosis-enhancing drug, clofazimine (100 mg three times daily, for at least 3 weeks) to see if it could raise the PI and, if so, if this might affect the dermatitis. The phagocytic index was checked, if possible, once a

week or once every other week and, at the same time, changes in the appearance of the dermatitis were registered according to both the patient's and the doctor's opinion.

RESULTS

The mean of the phagocytic indices found in the various groups is shown in Table I and the distribution of the individual indices is seen in Fig. 1.

The mean PI for the whole group of patients with atopic dermatitis was lower than that found in the controls ($0.02 > p > 0.01$). As seen from Fig. 1, there are large variations between the individual PIs. When the patients are divided into three grades according to the severity of the dermatitis, it is obvious that the mean PI in patients with only mild symptoms (AD Grade 1) does not differ significantly from that of the controls. Nor does the PI for patients with moderate dermatitis differ significantly from that of the control group, although when compared with the control group a tendency to a lowered PI may be seen ($0.2 > p > 0.1$). The patients with a severe dermatitis, however, do have a pronounced lowering of their mean PI when a comparison is made with the controls ($p < 0.001$).

The mean PI of mild atopic dermatitis does not differ significantly from that found in the mod-

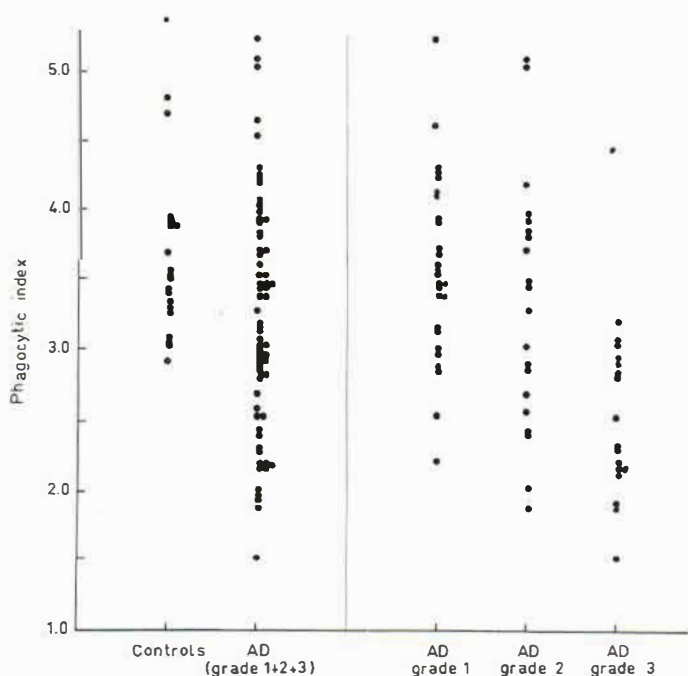


Fig. 1. Individual phagocytic indices in controls and in patients with atopic dermatitis (AD). AD Grade 1 = mild dermatitis, AD Grade 2 = moderate dermatitis, and AD Grade 3 = severe dermatitis.

erate form, whereas the difference between the mild and the severe type is highly significant ($p < 0.001$). The difference between moderate and severe dermatitis is also significant ($p < 0.01$).

The highest serum IgE levels were found in some patients with a severe dermatitis, but there were also some patients in this group who had normal IgE levels. There was no evidence of any correlation between a high serum IgE level and a low PI. Six Grade 3 patients with serum IgE of less than 500 BMW units had a mean PI of 2.56, whereas nine Grade 3 patients with serum IgE 2 000–20 000 BMW units had a mean PI of 2.66.

In 11 patients with severe atopic dermatitis, the phagocytic-enhancing drug, clofazimine, induced an increased PI. The increase was first noted about 1 week after starting the treatment. In 7 of the 11 patients, the treatment was accompanied by a subjective and objective improvement of the dermatitis, beginning about 10 days after the introduction of the clofazimine treatment. The withdrawal of the drug was followed by a recurrence of the dermatitis after about 2 weeks and, at this time, a lowered PI was observed. When the drug was readministered the dermatitis improved and the PI was raised. This procedure has been repeated several times in some of the patients with the same results. In 4 patients, however, there was no effect on the dermatitis although an increased PI was induced. A more detailed study on results of clofazimine treatment will be published separately.

DISCUSSION

The increased interest in the phagocytic function of neutrophil leucocytes seems to have been stimulated by the description in 1957 of the clinical entity termed chronic granulomatous disease (CGD) of childhood (4). This inborn disorder is characterized by recurrent, suppurative, bacterial infections with organisms of low-grade pathogenicity. The patients frequently develop eczema, lymphadenopathy and hepatosplenomegaly. Although the neutrophils ingest the bacteria normally, the neutrophils from these patients have, *in vitro*, an impaired ability to kill various pathogenic as well as non-pathogenic bacteria (14). This defect seems to be associated with impaired H_2O_2 production by the neutrophils. In another syndrome characterized by

severe eczematoid dermatitis and repeated local and systemic infections which was described by Miller *et al.* (10) and Miller & Nilsson (11), a deficiency of serum-enhancement of *in vitro* phagocytosis has been demonstrated. This deficiency was shown to involve a malfunctioning of the fifth component of complement. Ward & Schlegel investigated a child with frequent, severe and prolonged respiratory and cutaneous infections (16). In this child the neutrophil leucotaxis was found to be impaired and attributable, at least in part, to the presence of an inhibitor of the leucotactic function of neutrophils in the patient's serum. It is obvious that there are several mechanisms behind the dysfunction of phagocytes. In most of the hitherto described syndromes associated with an impaired phagocytic function, the tendency to a lowered resistance to infections may dominate the clinical picture; however, in some of the syndromes the infections are not the primary symptoms but are preceded by other changes, among them eczematous dermatitis.

An association between leucocyte dysfunction and these primary changes has not been much discussed. Neutrophil leucocytes take part not only in the defence against infections but are also involved in most inflammatory reactions as producers of a number of mediators, such as various proteases. It might be speculated that there is a possible connection not only between the increased susceptibility to infections and the impaired leucocyte function in these syndromes, but also between the primary changes and the leucocyte function.

The lowered phagocytic index in patients with a pronounced atopic dermatitis has not previously been described. This finding might, to some extent, explain the tendency to frequent bacterial skin infections in these patients. A defect in the ability of the phagocytes to ingest yeast particles does not, however, necessarily mean a defective capacity to ingest and to kill bacteria. Studies on the bactericidal effectivity of the phagocytes are under way in order to obtain more detailed information on the mechanism and relevance of impaired phagocytosis for the infections, as well as for the dermatitis itself. Experiments are also in progress with various combinations of the patient's leucocytes and plasma replaced by those from healthy subjects.

No studies have yet been published on the

phagocytic capacity in the other atopic syndromes but there is some evidence that phagocytosis might be impaired in these syndromes as well. Thus, in preliminary studies in patients with allergic rhinitis, Nilzén found a lowered PI which was increased during hyposensitization (13).

Studies of atopic dermatitis during the last few years have been focused mainly on the levels of IgE and especially on the significance of increased IgE levels for the intensity of the dermatitis (8, 9, 17). High serum IgE levels are more frequently present in patients with a severe atopic dermatitis than in those with a moderate form but severe atopic dermatitis may occur with normal IgE levels. A connection between resistance to bacterial infections and serum IgE levels has been discussed but not proved. Extreme hyper IgE and undue susceptibility to infections in two boys were described by Buckley et al. (5). In these patients, however, phagocytic studies with nitroblue tetrazolium reduction and on bactericidal capacity were normal. In our patients with atopic dermatitis no correlation was revealed between a low PI and the levels of serum IgE.

The positive results from the preliminary trial of the phagocytosis-enhancing drug, clofazimine, are encouraging. The mode of action of this drug, hitherto used as an antileprosy drug, is not yet known. A more detailed double-blind study on its clinical effects is needed, where special attention is paid to its influence on infections as well as on the dermatitis itself.

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