

NEUTROPHIL GRANULOCYTE FUNCTION IN PUSTULOSIS PALMOPLANTARIS

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Abstract. The phagocytic bactericidal capacity of circulating neutrophilic granulocytes was estimated in 22 patients with pustulosis palmoplantaris (PPP). The control group comprised 100 healthy individuals. Leukocytes from the patients had a statistically significant reduced capacity to kill *Staph. aureus* in autologous as well as in AB serum. Leukocyte killing of *E. coli* was not reduced. Serum levels of total hemolytic complement, C₃, C₄ and neutrophil myeloperoxidase activity were normal. The findings suggest an intrinsic or cellular defect of circulating neutrophil granulocytes in patients with PPP.

Key words: Neutrophil granulocyte; Phagocytosis; Pustulosis palmoplantaris

Neutrophil granulocytes are abundant in the pustules of pustulosis palmoplantaris (PPP). The pathogenetic role of the neutrophils in this condition is unknown. A reduced phagocytic uptake of yeast particles in the neutrophils isolated from the venous blood of PPP patients has been reported by Molin & Rajka (8) and Molin (7). However, the phagocytic process with subsequent degradation of ingested material and bacterial killing involves several stages beside the engulfment (11) and the present study of patients with PPP was undertaken to gain further information about the phagocytic process of their neutrophilic leukocytes.

MATERIAL

Twenty-two consecutive outpatients with PPP were examined: 20 females and 2 males. Their ages ranged from 26 to 70 years (average 50 years). The mean duration of their skin disease was 8.1 years.

The diagnosis of PPP was based on clinical data (2). Only patients with a duration of PPP for 6 months or more were accepted. Patients with overt psoriasis on the rest of the body were excluded. Fungal infection of the feet was looked for in all patients and specimens were taken for cultivation from all lesions of the feet. No signs were found of nail- or interdigital mycosis and all fungous cultures were negative. At the time of the examination, all patients had actively pustulating lesions: 11 in both the

palms and soles. 14 had periodically had lesions of hands and feet.

Most of the patients had been treated with emollients and topical fluorinated corticosteroids (without occlusion): all treatment was stopped 2 days before blood was drawn for the neutrophil studies.

Two women were on replacement therapy with thyroxine: one for 21 years following a goitre operation and the other for non-operated goitre. Two other females were treated with a beta-blocker for hypertension and one with phenothiazine for a psychiatric disorder.

One hundred healthy male and female blood donors aged 20-55 years served as controls (70 males, 30 females) (10).

METHODS

Blood was taken at the same time at each examination. White blood cell count and a differential count were done by a standard technique. Myeloperoxidase was estimated by a histochemical method according to Jacobs (4) at the hospital's Clinical Chemistry Department. The results are expressed as the percentage of enzyme-positive cells of all peripheral leukocytes in the smear.

Phagocytotic function of leukocytes isolated from the venous blood of patients and normals was analysed in two assays: for phagocytic bactericidal capacity (FBC) and for nitroblue tetrazolium reduction (NBT). The FBC test was done according to Ölling et al. (10). Briefly, washed leukocytes, pooled AB Rh(+) serum, or autologous serum to be tested were mixed with a suspension of either *E. coli* or *Staph. aureus* in a tube and tumbled for 2 hours at 37°C for phagocytic uptake and killing. Water was then added to lyse the leukocytes, followed by addition of concentrated broth. The tube was kept at 4°C overnight and incubated for growth 4-6 hours the following day. By comparing the optical density of three simultaneously prepared tubes which were not incubated for killing, the surviving fraction of bacteria was calculated. These three reference tubes contained 1/1, 1/10 and 1/100 of the original bacterial inoculum. Controls of non-phagocytotic bacterial killing were always included: a tube containing serum but no leukocytes was tumbled, and a tube containing both leukocytes and serum, which was not tumbled. The NBT test was done according to Hultborn & Ölling (3).

Total hemolytic complement, C₃, and C₄ were determined.

Fisher's permutation test was used for statistical evaluations (9).

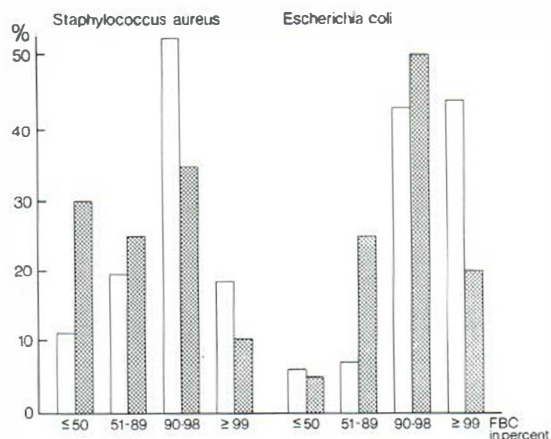


Fig. 1. Allocation of the results in FBC (phagocytic bactericidal capacity) test on leukocytes from 100 blood donors (unfilled) and 20 patients with pustulosis palmoplantaris (filled) against *Staphylococcus aureus* and *Escherichia coli*.

RESULTS

The total number of white blood cells, the neutrophil count, and the concentrations of total serum hemolytic complement, C_3 and C_4 were all within the normal limits of our laboratory. In 19 of the patients, all neutrophils were myeloperoxidase-positive, and in the remaining 3 patients nearly all neutrophils were positive.

Leukocytes from male and female control subjects had a similar bactericidal capacity for *Staph. aureus* and *E. coli*. The results of the FBC test in the patients and in the healthy controls are given in Fig. 1. Using *Staph. aureus* as test organism, leukocytes from the patients had a significantly reduced bactericidal capacity compared with those from the control persons ($p < 0.05$). A similar result was found with autologous serum and the pooled AB Rh(+) serum. With *E. coli* no significant difference between patients and controls was found regarding bactericidal potency.

In all patients the NBT test performed proved normal.

DISCUSSION

Neutrophilic leukocytes migrate profusely and aggregate in the pustules within the upper part of the *stratum malpighii* in patients with PPP. The nature of the triggering chemotactic factor(s) is

unknown. Several reviews on possible mediators of white cell emigration have recently been published (11). The demonstration of the chemotactic ability of the horny layer from psoriasis lesions is interesting in this respect (5, 12).

The epidermal neutrophils are undoubtedly recruited from the circulation. Their chemotactic responsiveness has not been examined but a gross defect of neutrophil mobility seems unlikely. However, a reduced ability of the neutrophils to phagocytose and degrade foreign material, e.g. immune complexes, might be directly involved in the pathogenesis of lesion formation. It is therefore of interest that decreased engulfment by PPP leukocytes has been demonstrated with a yeast particle technique (8). With the same technique, a similar faulty neutrophil function was also observed in patients with erysipelas and discoid lupus erythematosus (8) and in severe forms of atopic dermatitis (6). The significance of these findings for the development of skin lesions in PPP is unclear but in an open uncontrolled therapeutic trial with clofazimine, clinical improvement paralleled an increase in the phagocytic index (7). It cannot be excluded however, that the clinical response in some patients to clofazimine may have been caused by a primary effect on mononuclear cell phagocytosis or a hitherto unknown effect on the immune system (1).

The present method for estimating the phagocytic bactericidal capacity of neutrophilic leukocytes measures the combined efficiency of the leukocytes for ingestion and microbicidal activity. The circulating neutrophils in our patients with PPP exhibited an impaired capacity to kill *Staph. aureus*. As similar results were obtained using autologous and normal serum as opsonin source, an 'intrinsic', or 'cellular' defect may be suggested.

It is not uncommon for patients with PPP to contract a secondary infection of the dermatosis (2) to which the described functional neutrophil defect may contribute.

Our experiments were performed with peripheral blood neutrophils *in vitro* and the implication of their abnormal function for the *in vivo* pathogenesis of cutaneous pustules is unknown. It has recently been suggested, however, that the epidermal pustule in PPP is preceded by a vesicle in which the migration of neutrophils will not take place until the vesicle has reached the stratum corneum (13). Instead, the earliest cellular invasion is by mono-

nuclear cells. This observation motivates continued study of immune mechanisms in PPP.

ACKNOWLEDGEMENTS

We thank Inga Engberg and Helena Kahu for skilful technical assistance; Nils Gunnar Pehrsson, Department of Mathematics, Chalmers Technical High School, for statistical evaluation, and Birgitta Swolin, M.D., Department of Clinical chemistry, Sahlgren's Hospital, for the myeloperoxidase evaluation. This work was made possible by grants from the Medical Faculty, University of Göteborg, Göteborg, Sweden.

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Received March 23, 1978

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