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PHAGOCYTIC ACTIVITY OF POLYMORPHONUCLEAR LEUKOCYTES IN GENERALIZED PUSTULAR PSORIASIS

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Abstract. The phagocytic activity of polymorphonuclear leukocytes from 10 patients suffering from generalized pustular psoriasis (GPP) has been studied. In all patients intracellular iodination capacity was measured, and 6 were studied regarding hexose monophosphate shunt activity (HMS) and bactericidal capacity. In 2 patients leukocytes from pustules were tested by the iodination reaction. Sera from 7 patients were incubated with normal leukocytes in a bactericidal test to evaluate the opsonizing capacity, which was found to be normal. Leukocytes from patients with GPP after 120 min of incubation with bacteria exhibited significantly impaired lethality compared with the controls, but no significant difference was found after 60 min. Iodination capacity and HMS activity were normal in all patients except in one, who was found to be deficient in myeloperoxidase. The possible significance of the impaired lethality of bacteria in the pathogenesis of GPP is discussed.

Key words: Generalized pustular psoriasis; Phagocytosis; Myeloperoxidase-mediated iodination; Hexose monophosphate shunt activity; Bactericidal test

Generalized pustular psoriasis (GPP) is characterized by acute exacerbations with fever, shivering, leukocytosis and development of subcorneal pustules. Between exacerbations there are few symptoms. The cause of the disease is still unknown, and several aspects of the pathogenesis remain to be elucidated, i.e. why granulocytes accumulate so rapidly as to form subcorneal pustules.

The occurrence of specific chemotactic factors in the skin has been discussed and recently immunoglobulins and C3 have been detected by immunofluorescence in pustules from patients with pustulosis palmo-plantaris (PPP) and GPP (2). In PPP and certain other dermatoses the phagocytosis of yeast cells by granulocytes is diminished (5). The relevance of these observations is still uncertain, but it might be suggested that the development of pustules is linked with the functional state of leukocytes, such as mobilization, random motility, chemotaxis and phagocytic capacity.

Thus, a study of certain aspects of the functional state of the leukocytes causing the pustules, such as phagocytic activity and opsonization, should give insight into the etiology and development of pustular diseases.

One suitable quantitative method for studying the phagocytic activity of leukocytes is to measure the myeloperoxidase (MPO) mediated iodination capacity of phagocytosing cells (6). The iodination reflects ingestion, degranulation and enzymatic activity within the phagocytic vacuole (3). To obtain adequate iodination during phagocytosis, hydrogen peroxide must be available (3). This is generated through the activation of the hexose monophosphate shunt (HMS) (Fig. 1) (10). This activation is assayed by monitoring the CO_2 production from glucose ^{14}C-1 in the phagocytic cells.

The aim of the present investigation was to measure the MPO-mediated iodination, HMS activity, and bactericidal capacity in phagocytosing leukocytes from blood and pustules in patients with generalized pustular psoriasis. Furthermore, the opsonizing capacity of sera from these patients has been evaluated.

MATERIAL AND METHODS

Patients. Ten patients suffering from GPP were examined (Table I). All patients were investigated with respect to iodinating capacity and 6 were studied regarding HMS activity and bactericidal capacity. In 2 patients leukocytes from pustules were tested by the iodination reaction. Sera

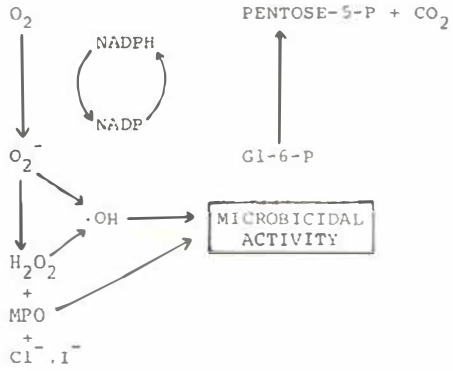

Fig. 1. Proposed antimicrobial mechanisms linked to the metabolism of phagocytosing granulocytes: During phagocytosis, oxygen is reduced to superoxide (O_2-) by an oxidase or directly by NADPH, and further reduced to hydrogen peroxide by superoxide dismutase. H_2O_2 can react with superoxide to form hydroxyl radicals (·OH), or together with myeloperoxidase (MPO) and a halide generate antimicrobial properties.

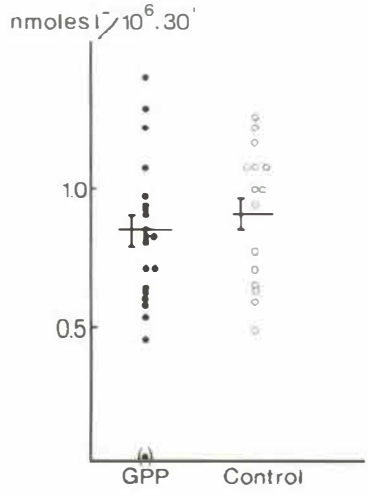

Fig. 2. MPO-mediated iodination in granulocytes from patients with GPP ($n=10$) and controls ($n=9$). The horizontal lines indicate the mean values.

from 7 patients were incubated with normal leukocytes in a bactericidal test to evaluate the opsonizing capacity. In 3 patients and 3 controls the intra- and extracellular iodination was examined.

During the course of the investigation, 7 patients suffered from active disease, whereas 3 patients (Nos. 7, 9, 10) were studied in remission. Four of the patients (Nos. 2, 4, 5, 6) were treated with immunosuppressive drugs (Methotrexate or Hydroxyurea) and 2 of these (Nos. 2, 6) at one sampling time were treated with clofazimine (Lampren®, Ciba-Geigy). The other patients were not exposed to systemic therapy. Apparently healthy volunteers served as controls.

Leukocyte isolation was performed according to Böyum (1) by using Isopaque-dextran sedimentation for the separation of leukocytes from EDTA-blood. After separation, washing and hypotonic lysis of the erythrocytes, the leukocytes were suspended in Krebs-Ringer phosphate buffer containing 10 mM glucose (KRG), pH 7.2, to a concentration of 1×10^7 cells per ml. Serum was collected from healthy blood donors and stored in aliquots at −70°C until use.

Table I. *10 patients with GPP*

Pat. no.	Age	Sex	Duration of disease (y.)	No. of investigations: Iodination	HMS	Bactericidal test
1	67	♂	7	1	–	–
2	60	♂	19	4	2	2
3	22	♀	7	1	–	–
4	71	♂	1	3	2	1
5	67	♀	16	4	2	2
6	45	♂	1.5	6	4	4
7	41	♀	19	2	1	1
8	77	♀	43	2	–	–
9	58	♀	15	1	–	–
10	41	♀	7	2	1	1

Measurement of iodination was carried out as described by Olsson et al. (6). The reaction mixture contained 1×10^6 leukocytes, 10% human serum, 30 nmoles of sodium iodide (1 μCi ^{125}I), 5×10^7 heat-killed yeast cells and KRG to a final volume of 0.5 ml. To determine the extracellular iodination of plasma proteins, 1 ml of buffer was added after the incubation, and the mixtures were centrifuged (1 500 *g*, 10 min) to collect leukocytes and yeast cells in the sediment. The sediment and supernatant fluid were precipitated with 10% trichloro-acetic acid to determine the protein bound (^{125}I) iodine. A differential count was used to determine the iodination per 1×10^6 granulocytes.

Measurement of CO_2 release. To siliconized Erlenmeyer flasks (25 ml) were added: 5×10^6 leukocytes, 0.25 ml human serum, 0.5 ml glucose ^{14}C-1 (0.5 μCi) (New England Nuclear, Boston, USA), 2.5×10^8 heat-killed yeast cells and KRG to a final volume of 3 ml. Immediately following the addition of yeast cells, a rubber stopper was inserted into the flasks. A cellulose filter with 0.3 ml 10% KOH was fastened in the stopper to collect the CO_2. Following one hour of incubation at 37°C with continuous shaking, 0.5 ml 1 N HCl was injected through the stopper to terminate the reaction and release the $^{14}CO_2$ from the medium. After incubation for a further 15 min, the filters were removed, dried, placed in 10 ml scintillation fluid (toluene-PPO-POPOP) and counted in a liquid scintillation counter (Tri-Carb, Packard Instr. Co., Downers Grove, Ill., USA).

Bactericidal capacity of the leukocytes was tested against *Escherichia coli* 0111:K58. The bacteria were grown overnight in nutrient broth (Difco), harvested, washed in cold phosphate-buffered saline and suspended in KRG to a concentration of 1×10^7 per ml. To siliconized test

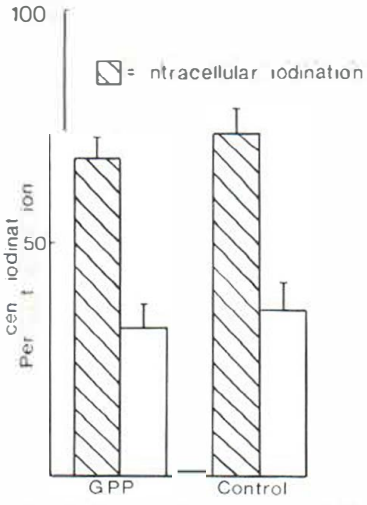

Fig. 3. Intra- and extracellular iodination in patients with GPP ($n=3$) and controls ($n=3$). Vertical bars indicate S.D.

tubes were added 1×10^6 bacteria, 0.05 ml human serum, 2×10^6 leukocytes and KRG to a final volume of 1.0 ml. The control incubation mixture contained 1×10^6 bacteria, 0.05 ml serum, and KRG. The tubes were incubated with continuous shaking for 120 min. At 0, 60 and 120 min, 0.1 ml samples were withdrawn from the tubes and diluted in 2% saponin to lyse the leukocytes. Viable counts were then performed on nutrient broth agar plates. The surviving fraction was calculated as the percentage of bacteria surviving in the presence of leukocytes, compared with the control mixture where the leukocytes had been omitted.

RESULTS

Iodination

There was no significant difference in iodination capacity between leukocytes from patients (mean value 0.84±0.06) and controls (0.91±0.06) (Fig. 2). One patient (No. 6), however, showed impaired iodination, <0.10, due to deficient MPO-activity within his leukocytes (Stendahl & Lindgren, to be publ.). His values are not included among the mean values.

Fig. 3 shows the intra- and extracellular iodination. About 30% of the total protein-bound ^{125}I was precipitated with the extracellular plasma proteins, in the presence of leukocytes from patients as well as from controls.

In 2 patients, leukocytes were isolated from pustules (>90% granulocytes). No difference in iodination capacity was observed, compared with leukocytes isolated simultaneously from peripheral blood (not shown in Fig.).

HMS activity

By measuring the release of $^{14}C\bullet_2$ from phagocytosing leukocytes in the presence of glucose ^{14}C-1, the hexose monophosphate shunt activity was estimated. There was no significant difference in CO_2 release from phagocytosing leukocytes in patients (4 577±620 cpm) and controls (3 683±660 cpm) (Fig. 4). The CO_2 production from glucose ^{14}C-1 increased 7.7 times in the former and 8.3 times in the latter. In resting leukocytes the HMS activity remained at a low level in both groups.

Bactericidal capacity

Fig. 5 shows the bactericidal effect of leukocytes against *E. coli* 0111 : K58. There was no difference in killing rate after 60 min, whereas after 120 min, leukocytes from patients with GPP exhibited significantly impaired lethality compared with the controls ($p<0.05$). The MPO-deficient patient exhibited a still more pronounced impairment. His values are not included among the mean values.

Opsonization

In studying the opsonizing capacity of sera from patients with GPP in the bactericidal test we found no significant difference from control sera (Table 11).

There was no detectable difference in phagocytic activity between patients during exacerbation or

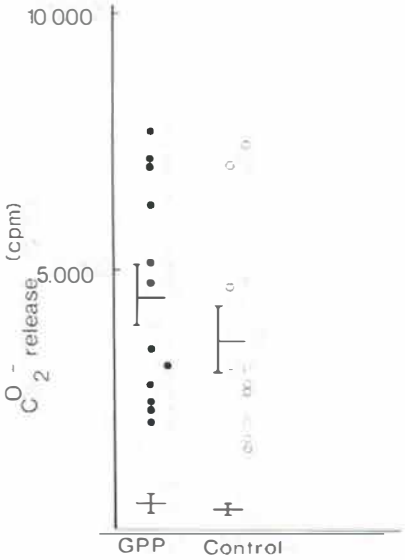

Fig. 4. HMS activity measured as $^{14}CO_2$ release in granulocytes from patients with GPP ($n=6$) and controls ($n=7$). Horizontal lines show the mean values.

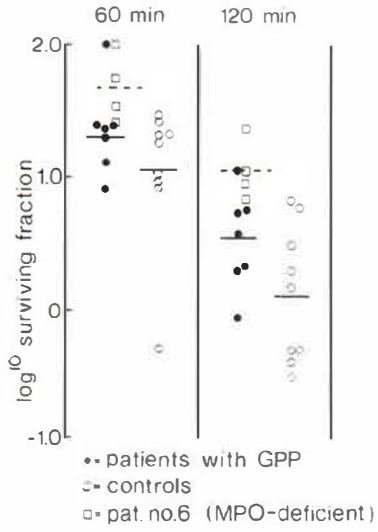

Fig. 5. Bactericidal capacity against *E. coli* 0111 in granulocytes from patients with GPP ($n=6$) and controls ($n=7$). Horizontal lines indicate the mean values. – – – = mean values in MPO-deficient patient.

remission, nor between treated and untreated patients. However, the groups are too small to be evaluated statistically.

DISCUSSION

The bactericidal effect of the phagocytic cell is the result of a complicated series of events. These include the interaction of the microbe with the phagocytic membrane, ingestion with the formation of a phagosome and lysosome–phagosome fusion. Concurrent with these processes are a number of metabolic events, including increased oxygen consumption, stimulation of the hexose monophosphate shunt, production of superoxide (O_2-) and hydrogen peroxide, and MPO-mediated iodination (8). The precise way in which ingested microorganisms are killed, has not yet been clearly elucidated.

In the present investigation we have studied the bactericidal effect, together with certain preceding metabolic events such as HMS activity and MPO-mediated iodination in leukocytes from patients with GPP. Our investigations revealed no difference in iodination or HMS activity between patients with GPP, and controls. These results are not in complete accord with those of Molin & Rajka (5), who found diminished phagocytic uptake of killed yeast cells in leukocytes from patients with PPP. If decreased phagocytic uptake of yeast cells were to occur in GPP, then iodination activity would be diminished, since this reaction depends on the number of ingested cells and the MPO activity within the leukocytes (6). This deduction would indicate that GPP and pustulosis palmo-plantaris may be two different diseases.

The leukocytes from GPP patients showed impaired bactericidal activity against *E. coli*. This impairment was not particularly pronounced and should be regarded as a slower rate of killing, rather than an impairment *per se*. This fact may not have any bearing on the overall propensity to contract bacterial infections, except possibly for certain betahaemolytic streptococci (4). On the other hand it has been stressed that infections, e.g. respiratory diseases, may precipitate an exacerbation of GPP (4).

The major clinical cause of defective ingestion and thus impaired bactericidal capacity of leukocytes is subnormal opsonic activity in the serum (8). Normal opsonization was, however, seen in sera from GPP patients. Since immune complexes are known to inhibit chemotaxis as well as phagocytic uptake (9), and immunoglobulins and C3 are present in the pustules (2), a local influence of pustular constituents on the phagocytic process cannot be excluded. Our experiments show no difference in phagocytic activity between leukocytes isolated from pustules and those isolated from blood, but an opsonizing effect of the pustular contents has not yet been investigated.

The slightly impaired intracellular lethality in GPP leukocytes may be due to exhaustion or immaturity of the cells, as repeated exacerbations may deplete the pool of mature cells. This has been observed during severe infections (7) and recidivating erysipelas (5). In our patients there was no correlation between the duration or the severity of the disease

Table II. *The bactericidal activity of normal leukocytes incubated with sera from patients with GPP and from controls*

A = number of subjects investigated; B = per cent surviving bacteria ±S.E.M.

	A	B	
		60 min	120 min
GPP	7	20.7±2.1	3.8±1.2
Control	10	22.6±2.0	5.6±0.9

and phagocytic activity. Further aspects of the function of the phagocytic cells must be investigated in order to understand the pathogenesis of pustular diseases. At present, random motility, chemotaxis and local factors influencing the phagocytic process are being investigated. Furthermore, chronic infections with aerobic as well as anaerobic microorganisms have to be taken into account.

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