Myeloperoxidase Modulates the Phagocytic Activity of Polymorphonuclear Neutrophil Leukocytes. Studies with Cells from a Myeloperoxidase-deficient Patient

O. Stendahl, B.-I. Coble, C. Dahlgren, J. Hed, and L. Molin

Departments of Medical Microbiology and Dermatology, Linköping University Medical School, 581 85 Linköping, Sweden

Abstract. Patients lacking the primary granulae enzyme, myeloperoxidase (MPO), do not usually show any increased susceptibility to infection or altered inflammatory response, in contrast to several other biochemical defects in polymorphonuclear neutrophils. We have now evaluated the role of MPO on phagocyte function in a patient with complete MPO deficiency suffering from generalized pustular psoriasis. We found that the MPO-deficient neutrophils showed enhanced phagocytosis (>200% of normal) of IgG- and C3b-opsonized yeast particles and prolonged *N*-formylmethionyl-leucyl-phenylaline-mediated stimulation of superoxide production.

When purified human MPO was added to normal neutrophils during cell adhesion, their Fc- and C3b-mediated phagocytosis was reduced without affecting cell viability. 1 μg/ml of MPO reduced the Fc and C3b phagocytosis to 47 and 65%, respectively, whereas 10 μg/ml reduced the activity to 20 and 54%. Both attachment and ingestion were reduced to a similar extent, indicating that MPO affected the receptor function per se. When MPO was added to the hyperactive MPO-deficient cells, phagocytosis was reduced more rapidly. Catalase, azide, and methionine eliminated the inhibitory effect, and catalase and methionine, in fact, enhanced the phagocytic activity of adherent neutrophils.

These data indicate that, apart from being a potent antimicrobial system, the oxidizing activity of the MPO-H_2O_2-halide system may modulate the inflammatory response by impairing certain receptor-mediated recognition mechanisms of phagocytic cells, which otherwise could elicit inflammatory reactions and tissue injury.

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Introduction

The role of polymorphonuclear leukocytes (PMNL) in host defense is to destroy invading microorganisms, and several antimicrobial systems have been described in normal human PMNL[1] (1). One powerful antimicrobial system is composed of myeloperoxidase (MPO), hydrogen peroxide (H_2O_2), and a halide cofactor (1). The release of the granule enzyme MPO and the generation of highly reactive oxygen products (O_2^-, H_2O_2) is not only initiated in PMNL by phagocytosis, but also as a result of cell adhesion, aggregation, and as a response to chemotactic substances (2, 3). Apart from showing a potent microbicidal activity, the MPO-H_2O_2-halide system is also toxic to several types of mammalian cells (4). Recently, the MPO-H_2O_2-halide system was also shown to stimulate platelet (5) and mast cell degranulation (6, 7). Furthermore, the MPO-H_2O_2-halide system is capable of inactivating certain leuko-attractants, including complement (C)-derived C5a and the synthetic peptide, *N*-formylmethionyl-leucyl-phenylalanine (f-Met-Leu-Phe) (8, 9). It is thus evident that during inflammation, when the PMNL undergo metabolic activation and degranulation, these events may lead to an accumulation of reactive oxygen metabolites and granule enzymes, including MPO, in the extracellular environment (3), where these may serve as modulators of the inflammatory response.

In the present investigation, we show that Fc- and C3-mediated phagocytosis and metabolic activation are enhanced in PMNL from a patient with MPO-deficiency, and that the hyperactive phagocytosis in MPO-deficient PMNL can be reduced after addition of purified MPO.

Methods

PMNL preparation. Blood was obtained from apparently healthy adult volunteers and from a previously described MPO-deficient patient suf-

1. *Abbreviations used in this paper:* C, complement; cyt c, cytochrome c; FITC, fluorescein-isothiocyanate; f-Met-Leu-Phe, *N*-formylmethionyl-leucyl-phenylalanine; f-norLeu-Phe, *N*-formyl-norleucyl-phenylalanine; H_2O_2, hydrogen peroxidase; HSA, human serum albumin; KRG, Krebs-Ringers phosphate buffer supplemented with 10 mM glucose; MPO, myeloperoxidase; PMA, phorbol myristate acetate; PMNL, polymorphonuclear leukocytes.

fering from generalized pustular psoriasis (10) by using EDTA-vacutainer tubes (Becton-Dickinson & Co., Orangeburg, NY). After dextran sedimentation, the PMNL were separated according to the method of Bøyum (11). The remaining erythrocytes were removed by hypotonic lysis, and the PMNL were washed twice in Krebs-Ringers phosphate buffer supplemented with 10 mM glucose, pH 7.2 (KRG), and finally suspended to 1×10^7 cells/ml in the same buffer supplemented with human serum albumin (HSA) (1 g/100 ml). Viability was tested by using trypan blue exclusion.

Reagents. f-Met-Leu-Phe, *N*-formyl-norleucyl-leucyl-phenylalanine, (f-norLeu-Leu-Phe), phorbol myristate acetate (PMA), cytochrome (cyt c), superoxide dismutase, sodium azide, catalase, L-methionine, and luminol (5-amino-2,3-dihydro-1,4-phtalazine-dione) were obtained from Sigma Chemical Co. (St. Louis, MO). HSA was obtained from Kabi (Stockholm, Sweden). MPO isolated from human leukocytes was a generous gift from Dr. I. Olsson, Lund, Sweden. 1 μg protein contained 0.80 μg MPO with a specific activity (Worthingtons Enzyme Manual, 1977, Worthington Biochemical Corp., Freehold, NJ) of 66.4 units/mg.

Chemiluminescence measurements. Measurements of chemiluminescence were done in a luminometer 1250 (LKB-Wallac, Stockholm, Sweden) kept at 22°C (12). Samples for chemiluminescence were obtained by adding 0.4 ml KRG, 0.01 ml luminol (1 mg/ml), and 0.1 ml PMNL suspension to disposable 4-ml polypropylene tubes. The tubes were placed in the luminometer and allowed to stand until a stable background of chemiluminescence was obtained (<2 min). To activate the systems, 0.1 ml of a stimulus (f-Met-Leu-Phe or PMA) diluted to appropriate concentration in KRG was added, the tubes stirred, and the light emission recorded.

Measurement of O_2^- production. A continuous assay for estimating the O_2^- production was utilized essentially as described by Cohen and Chovaniec (13), by using a Beckman DB-G double beam spectrophotometer (Beckman Instruments, Inc., Fullerton, CA) with a temperature regulator (37°C). In a standard assay, both sample and reference cuvette contained 2.5×10^6 PMNL and 300 nmol cyt c in 2 ml KRG. The reference cuvette, furthermore, contained 200 μm/ml of superoxide dismutase. At zero time, 0.1 ml PMA (5×10^{-6} M) or 0.25 ml f-Met-Leu-Phe or f-norLeu-Leu-Phe (5×10^{-6} M) was added to both cuvettes and the absorbance change accompanying cyt c reduction was monitored at 550 nm.

Motility measurements. Cell migration was measured by using an application (14) of the fluorescence quenching method described by Hed (15). The granulocytes (2×10^4 cells) were allowed to adhere to glass slides, to which fluorescein-isothiocyanate (FITC)-labeled yeast particles (1.5×10^4 particles/cm^2) were prefixed. After nonadhering PMNL had been removed, 100 μl normal human serum (25%) or KRG was added to the slides. After incubation in a moist chamber for 30 min, the slides were rinsed, and the migratory activity was quantitated by counting the percentage of PMNL-associated yeast particles. The chemotactic response was expressed as the difference between the values in the presence and absence of serum. To test the effect of MPO, the enzyme was added to the PMNL during the adherence phase. The agarose technique as described by Stendahl et al. (16) was also used in some experiments to study the effect of f-Met-Leu-Phe.

Phagocytosis assay. The fluorescence quenching assay described by Hed (15) was used as follows: to multispot glass slides (Dynatech Lab, Inc., Alexandria, VA), 0.1 ml PMNL suspension (2×10^5/ml) was added. The slides were then incubated in a moist chamber for 20 min to allow the PMNL to adhere to the slides. Nonadherent cells were then removed with warm (37°C) KRG-HSA. To the PMNL slides, 0.1 ml FITC-labeled yeast particles (*Saccharomyces cerevisiae*) (2×10^6/ml) opsonized with either rabbit antiyeast IgG (20 μg/ml) or normal human serum (50%) were added (17). The particles were designated yeast-IgG and yeast-C3b, respectively. The slides were incubated in a moist chamber at 37°C for 30 min, then rinsed in cold (4°C) KRG, and kept in cuvettes on ice until read in the fluorescence microscope. Immediately before microscopic examination, a few drops of Trypan blue solution (2 mg/ml in carbonate buffer, pH 4.4) were added to each spot. The dye extinguished the fluorescence of the extracellularly located FITC-labeled yeast particles, leaving the ingested ones fluorescent. 100 PMNL were examined and the number of attached and ingested particles were calculated. Phagocytosis in suspension was carried out as follows: 0.2 ml PMNL (2×10^6/ml) and 0.2 ml IgG-yeast particles (6×10^6/ml) in KRG with or without MPO were incubated for 10, 20, and 30 min at 37°C in siliconized glass tubes. After incubation, one drop of the reaction suspension was mixed with one drop of Trypan blue and read in the fluorescent microscope as described above. It is known that MPO, H_2O_2, and chlorides may chlorinate the FITC, and thereby, quench the fluorescence. Lowering the pH can also reduce the fluorescence (18). We measured the fluorescence of FITC-labeled yeast particles preexposed to MPO (2 μg/ml), H_2O_2, and chloride in a microfluorometer (E. Leitz, Inc., Rockleigh, NJ). The fluorescence of the FITC-labeled particles was reduced by 26% after exposure to the MPO system. This reduction was not enough to give artifactually low phagocytic uptake in normal, MPO-containing PMNL, since they still fluoresced brightly when inspected visually.

To selectively study the attachment of IgG- and C3b-opsonized yeast particles, glass slides with adherent PMNL prepared as described above were kept at 4°C and incubated with 0.1 ml of IgG- or C3b-yeast (10^7/ml) at 4°C for 30 min. The slides were then rinsed and examined as described above.

Measurement of PMNL iodination. Iodination was carried out essentially as described earlier (16). The reaction mixture contained 1×10^6 PMNL, 10% pooled human serum, 30 nmol of sodium iodide (0.5 μCi of ^{125}I), 5×10^7 yeast particles or PMA (10^{-7} M), and KRG to a final volume of 1.0 ml. The tubes were incubated at 37°C and the reaction was terminated after 30 min with 0.1 ml of a 0.1 M sodium thiosulfate solution. 5 ml of cold 10% trichloroacetic acid was then added. After centrifugation, the precipitates were washed three times with 5 ml of trichloroacetic acid. The iodination was expressed as nanomoles of ^{125}I precipitated per 1×10^6 PMNL per 30 min.

Results

Oxidative metabolism. We have earlier described a patient with a complete MPO deficiency in his PMNL, who suffered from generalized pustular psoriasis (10). As a consequence of the MPO-deficiency, the candidicidal and bactericidal activity of the PMNL were reduced. No other abnormalities were revealed at that time. Reevaluation of the PMNL showed that the MPO-mediated iodination was virtually absent (8 and 3%, respectively, of control values) after challenge with yeast particles or PMA. Iodination and chemiluminescence were reconstituted after addition of 1 μg/ml of purified human MPO, indicating a normal production of oxidative metabolites (O_2^-, H_2O_2). This was further tested by measuring superoxide production after stimulation with PMA or f-Met-Leu-Phe. The response to PMA was similar in normal and MPO-deficient cells both with respect to lag phase, rate, and maximum response after 30 min. We did not

observe any significantly enhanced O_2^- production in the MPO-deficient patient after 60–90-min incubation with PMA in contrast to the findings of others (19). The response to the chemotactic peptide f-Met-Leu-Phe showed another time course. The response was more rapid (shorter lag phase and increased rate), but terminated earlier (within 5 min). This response was altered in the MPO-deficient cells in that the rate of superoxide production was reduced by 30–40% but continued for a longer period of time; whereas superoxide production in normal PMNL terminated after 5 min, MPO-deficient cells continued to generate superoxide for 10–15 min, reaching a higher maximum value than control cells (Fig. 1).

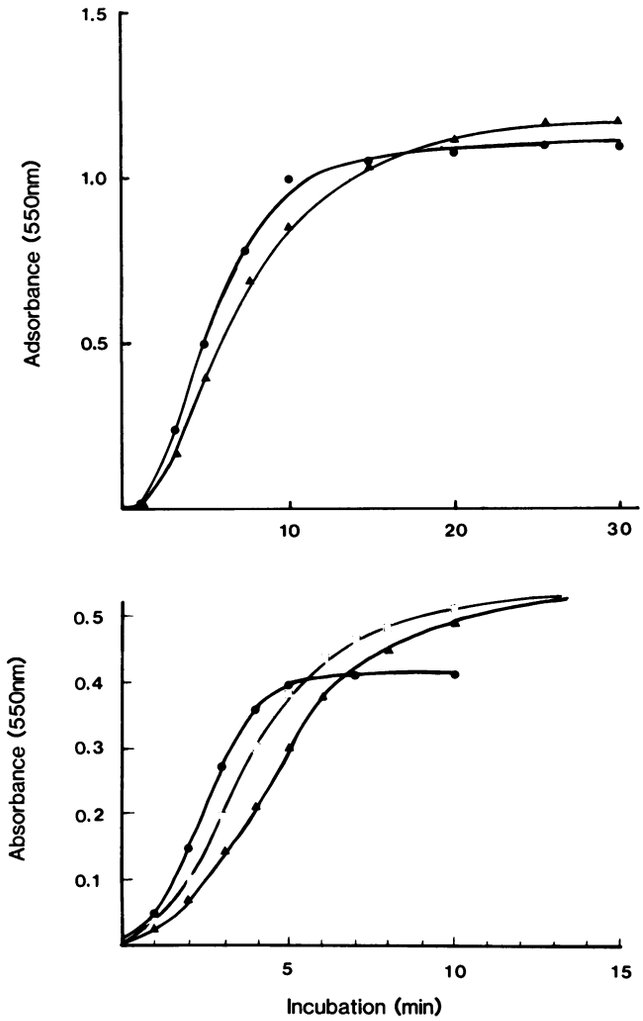

Figure 1. Superoxide production in normal (●) and MPO-deficient (▲) PMNL after stimulation with PMA (10^{-7} M) (*top*) and f-Met-Leu-Phe (10^{-7} M) (*bottom*). (□) Superoxide production in normal PMNL after stimulation with f-norLeu-Leu-Phe (10^{-7} M). Mean of two to three experiments.

The peptide, f-norLeu-Leu-Phe, which is more resistant than f-Met-Leu-Phe to oxidative inactivation, induced a more prolonged response also in normal PMNL (Fig. 1).

Chemotaxis. When studying cell locomotion, the MPO-deficient cells showed normal random migration and normal chemotactic response (not shown by figure), when using activated human serum or f-Met-Leu-Phe as stimulus.

Inactivation of f-Met-Leu-Phe. As shown by Clark et al. (8, 9) the MPO-H_2O_2-halide system can inactivate chemotactic factors by oxidation of methionine. This inactivation can be carried out also by activated normal PMNL, but not by MPO-deficient PMNL (9). To evaluate if f-Met-Leu-Phe exposed to PMNL lost its ability to elicit an oxidative response, we preincubated f-Met-Leu-Phe with normal and MPO-deficient PMNL. Table I shows that f-Met-Leu-Phe exposed to different concentrations of normal PMNL is unable to elicit a chemiluminescence response in normal PMNL. In contrast, f-Met-Leu-Phe exposed to MPO-deficient cells retained its ability to trigger a chemiluminescence response in normal PMNL.

Phagocytosis activity in normal and MPO-deficient PMNL. When IgG and C'-dependent phagocytosis was tested by using yeast particles opsonized with anti-yeast IgG or C3b, we observed a pronounced enhancement of IgG as well as of C3b-mediated phagocytosis in the MPO-deficient cells; the association and ingestion of yeast particles were doubled compared with normal PMNL (Table II) and the percentage of ingested yeast-C3b was enhanced from 69 to 87%. The binding of IgG- and C3b-opsonized yeast particles to PMNL was also assayed at 4°C to inhibit ingestion. The binding of both particles to the MPO-deficient PMNL was significantly increased over the control cells (Table III). No difference in phagocytosis was found between normal and MPO-deficient PMNL in suspension (Table IV). PMNL from five other patients suffering from pustular psoriasis

Table I. MPO-mediated Inactivation of f-Met-Leu-Phe by Normal and Deficient PMNL

f-Met-Leu-Phe (10^{-6} M) preincubated with:	Chemiluminescence
	mV
KRG*	49.9
PMNL (10^3)	31.5
PMNL (10^4)	2.0
PMNL (10^5)	1.1
MPO-deficient PMNL (10^5)	45.0
MPO-deficient PMNL (10^6)	36.0

* The reaction mixtures contained 0.1 ml f-Met-Leu-Phe (10^{-6} M), 0.5 ml KRG or 0.4 ml KRG, and 0.1 ml PMNL or MPO-deficient PMNL. The total number of cells present in the reaction mixture is indicated within parenthesis. After incubation for 30 min at 37°C, the reaction mixture was centrifuged (200 g, 10 min). 0.1 ml of the supernatant was added to 0.45 ml KRG, 0.05 ml PMNL (10^7/ml), and 0.1 ml luminol (1 mg/ml) and chemiluminescence was measured. The indicated values are peak values after ~9 min.

Table II. Phagocytosis Activity in PMNL from an MPO-deficient Patient, Patients with Pustular Psoriasis, and Normal Controls

	Yeast particles/100 PMNL*			
	IgG-yeast		C3b-yeast	
	Association	Ingestion	Association	Ingestion
MPO-deficient PMNL	160±15	133±10 (83)	416±32	361±21 (87)
PP PMNL‡	61±7	53±6 (87)	153±20	129±18 (84)
Control PMNL§	73±12	62±10 (83)	196±15	136±14 (69)

* Number of associated and ingested FITC-labeled yeast particles, opsonized with IgG and C, respectively. Mean of four experiments±SEM. Values within parenthesis express the percentage of the associated yeast particles that are ingested.
‡ Cells isolated from five patients suffering from pustular psoriasis.
§ Cells isolated from four healthy controls.
PP, pustular psoriasis.

showed no enhanced IgG- or C3b-mediated interaction (Table II).

Effect of MPO on PMNL function. It is evident that the MPO-deficient cells were "hyperactive" with respect to phagocytosis. Therefore, we focused on the question whether MPO may influence the function of the PMNL. MPO (1–5 μg/ml) that was added to PMNL during oxidative stimulation (O_2^-) and chemotaxis did not alter the response to PMA or f-Met-Leu-Phe. When MPO was added to normal PMNL before phagocytosis, a clear dose-dependent reduction of cell-associated and ingested yeast particles was seen (Fig. 2). The inhibition of IgG-mediated phagocytosis was more pronounced than that of C3b, and maximal inhibition (~80%) was reached when 5–10 μg/ml was added. C3b-mediated interaction was not inhibited by more than 50% with 10 μg/ml of MPO. Trypan blue exclusion revealed no decrease in viability after exposure to MPO. The presence of MPO during the initial cell adherence phase at 37°C also inhibited the subsequent binding at 4°C of IgG- and C3b-particles (Table III).

Since MPO has a tendency to bind to the glass surface and may be present on the glass surface also after cell adherence, it

Table III. Attachment of IgG- and C3b-opsonized Yeast Particles to Normal and MPO-deficient PMNL at 4°C

	Attached yeast particles/100 PMNL	
Cells	C3b-yeast	IgG-yeast
Control PMNL	99±14*	144±26
Control PMNL + MPO (1 μg/ml)	46±20	85±15
MPO-deficient	182±20	205±16

* Mean±SEM of four experiments.

Table IV. Phagocytosis Activity in Normal and MPO-deficient PMNL, Assayed in Suspension

	Yeast particles/100 PMNL*			
	IgG-yeast		C3b-yeast	
Cells	Association	Ingestion	Association	Ingestion
MPO-deficient PMNL	92‡	87	188	169
Control PMNL	101	95	195	175

* Incubation time, 30 min. No difference between the two cells was revealed when yeast/PMNL interaction was assayed after 10 or 20 min.
‡ Mean of two experiments.

was important to evaluate if the MPO-H_2O_2 system affects the opsonized particles directly. When opsonized particles were preexposed to MPO (1 μg/ml) and H_2O_2 (generated by glucose-glucose oxidase), the phagocytic uptake of yeast-C3b by normal

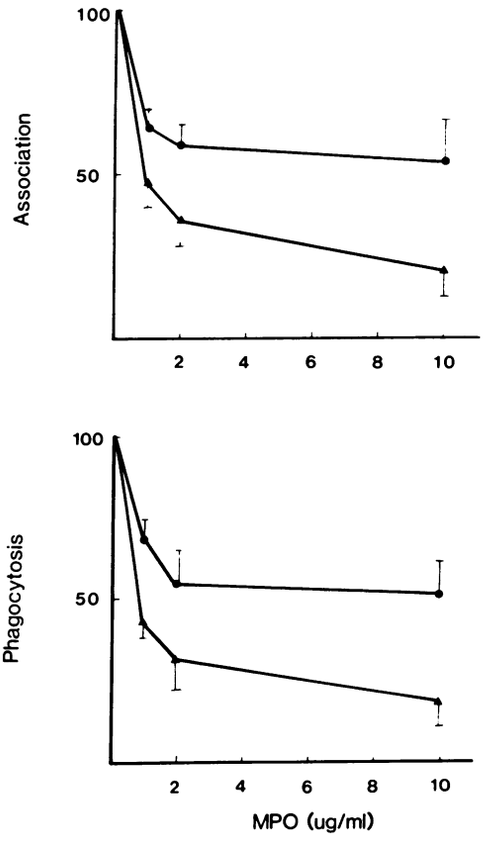

Figure 2. The effect of different concentrations of purified human MPO on association (*top*) and phagocytosis (*bottom*) of IgG-yeast (△) and C3b-yeast (●), expressed as percentage of controls in the absence of MPO. The vertical bars depict SEM of three to six experiments.

and MPO-deficient PMNL was reduced by 50 and 35%, respectively. The uptake of MPO-treated yeast-IgG was reduced by 20% for both normal and MPO-deficient cells. With lower concentrations of MPO (<0.1 μg/ml), this effect was less clear. This shows that the MPO system may affect the opsonized particles directly, particularly the yeast-C3b.

To evaluate the specific requirements for MPO and endogenously produced H_2O_2, sodium azide and catalase were added (Fig. 3) to normal PMNL. Azide eliminated the inhibiting effect as did catalase. Catalase, in fact, enhanced the phagocytic activity by 50–100%, both in the presence and absence of MPO. It thus appears as if MPO and H_2O_2, which are released during adherence, and phagocytosis may reduce the activity of normal granulocytes. Table V shows that if MPO is present only during the adherence of PMNL to the glass slides before any yeast is added, the inhibitory effect is similar to cells exposed to MPO during the whole process. In contrast, when PMNL were kept in suspension in the presence of MPO, little effect on phagocytosis was observed (Table VI). It has been shown that MPO-mediated methionine oxidation causes inactivation of chemotactic peptides (9). To evaluate the role of MPO-mediated oxidation on Fc and C3b receptor activity, different concentrations (0.1–1 mM) of the reducing agent methionine were added to the PMNL during adherence. The phagocytic activity of adherent PMNL was significantly enhanced (Table VII). Methionine also blocked the effect of added MPO (5 μg/ml).

Effect of MPO on MPO-deficient cells. To "reconstitute" the MPO-deficient PMNL, the cells were exposed to MPO. This drastically reduced their phagocytic activity (Fig. 4). 1 μg/ml of MPO reduced IgG-yeast phagocytosis by 50% with no effect on normal PMNL, and 0.5 μg reduced C3b-mediated phagocytosis by 50%, whereas 1–2 μg were needed to affect normal PMNL. Catalase enhanced the phagocytosis response, also, of the MPO-deficient cells. When comparing the effect of 1 μg/ml of MPO on the phagocytosis activity in normal and MPO-deficient PMNL, one can observe that the phagocytosis activity of the hyperactive MPO-deficient cells approaches that of the normal cells.

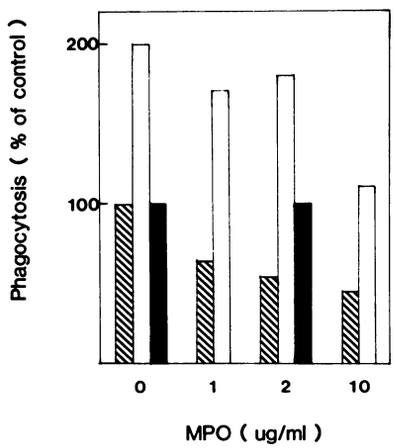

Figure 3. The effect of different concentrations of MPO (▨) on normal PMNL phagocytosis of IgG-yeast, in the presence of catalase (4,000 μm/ml) (□) and azide (1 mM) (■).

Table V. Effect of Myeloperoxidase on Normal PMNL during the Adherence and Phagocytosis Process

	Phagocytosis*	
Supplements	F_c	C3
PMNL	100	100
PMNL + MPO during adherence‡	67±4‖	77±7
PMNL + MPO during adherence and phagocytosis§	43±5	67±5

* Expressed as percentage of control (no MPO added) of IgG- and C3b-mediated phagocytosis of opsonized yeast particles.
‡ MPO (1 μg/ml) added to the PMNL suspension before and during the adherence of the cells to the glass slides. The slides were washed in KRG-HSA before adding the yeast particles.
§ MPO (1 μg/ml) added to the PMNL suspension before and during the adherence and phagocytosis process.
‖ Mean of five experiments±SEM.

Discussion

It is evident from most reports that patients lacking the primary granulae enzyme, MPO, do not show any increased susceptibility to infections (19, 20). In fact, many MPO-deficient individuals are discovered during routine testing (20), and only few patients have shown signs of infections (21, 22) and other inflammatory reactions (10).

When reevaluating the activity of the PMNL from an MPO-deficient patient suffering from generalized pustular psoriasis (10), we found that the PMNL showed enhanced phagocytic activity and prolonged f-Met-Leu-Phe-mediated stimulation of

Table VI. Effect of Myeloperoxidase on the Phagocytosis Activity of Normal PMNL in Suspension

	PMNL-IgG yeast interaction*	
Supplements	Association	Phagocytosis
PMNL	100	100
PMNL + MPO (2 μg/ml)	94±1	98±1
PMNL + MPO (5 μg/ml)	90±8	91±8
PMNL + MPO (10 μg/ml)	82±12	85±13

* Expressed as percentage of control (PMNL without addition of MPO) of PMNL-IgG-opsonzied yeast interaction. Mean of four experiments±SEM.

Table VII. Phagocytosis Activity of PMNL Exposed to MPO and Methionine

Supplements	IgG yeast particles-PMNL interaction	
	Association	Ingestion
PMNL	100*	100
PMNL + methionine (0.1 *mM*)	161	150
PMNL + methionine (1 *mM*)	164	163
PMNL + MPO (5 *μg/ml*)	39	29
PMNL + MPO (5 *μg/ml*) + methionine (0.1 *mM*)	100	102
PMNL + MPO (5 *μg/ml*) + methionine (1.0 *mM*)	98	98

* Expressed as percentage of control. IgG-yeast interacting with PMNL in the absence of MPO or methionine. Mean of two experiments.

the oxidative metabolism. We did not observe this pronounced increase in other patients suffering from pustular psoriasis. MPO may be released and present extracellularly also in nonactivated PMNL (23). Apart from showing a potent antimicrobial activity, the MPO-H_2O_2-halide system can be cytotoxic to certain tumor cells (4) and can inactivate chemotactic factors (8, 9), lysosomal enzymes (24), and protease inhibitors (25). The question, thus, arises whether the presence of MPO can explain the difference in phagocytic activity observed in MPO-deficient and normal PMNL.

The present experiment shows that MPO-mediated iodination is restored after addition of 1 μg/ml of MPO. This indicates that release of H_2O_2 in the MPO-deficient cells is sufficient to mediate the H_2O_2-dependent reaction, and that MPO present in the extracellular environment can function adequately.

When normal PMNL are stimulated with f-Met-Leu-Phe, a rapid increase in chemiluminescence and superoxide production occurs which subsides within 5 min. In contrast, the MPO-deficient cells respond with a slower rate of O_2^- production that, on the other hand, continues for a longer period of time. The rapid termination in normal PMNL could be due to inactivation of the chemotactic peptide by the MPO system. Lack of inactivation in the absence of MPO allows the peptide to stimulate the cell for a longer period of time. This was supported by the observation that the more resistant peptide, f-norLeu-Leu-Phe, induced a prolonged response in normal PMNL cells. We could, however, not conclusively show that addition of MPO to the cells changed the f-Met-Leu-Phe-induced superoxide response to that of normal cells (not shown by figure). The reason for this could be that inactivation takes place intracellularly, or that cyt c acts as a scavenger. It has been suggested that MPO may mediate termination of respiratory burst by inactivating the oxidase system (26). The lack of any MPO effect on chemotaxis suggests that the chemotactic receptors are either resistent to the MPO system or are not exposed to the extracellular MPO.

The most pronounced difference between normal and MPO-deficient cells was found in IgG- and C3b-mediated phagocytosis. Kay et al. (27) have shown that chemotactic factors or other inflammatory mediators may enhance the C3b but not the Fc receptor on PMNL. Sinc both receptors are equally enhanced in the MPO-deficient patient, some other mechanism is operative. Addition of MPO to either normal or MPO-deficient cells caused a pronounced decrease in both C3b- and IgG-mediated phagocytosis. In contrast to Håkanson and Venge (28), who showed that only C3b-mediated phagocytosis of latex particles were impaired by MPO, our results show that the Fc-mediated

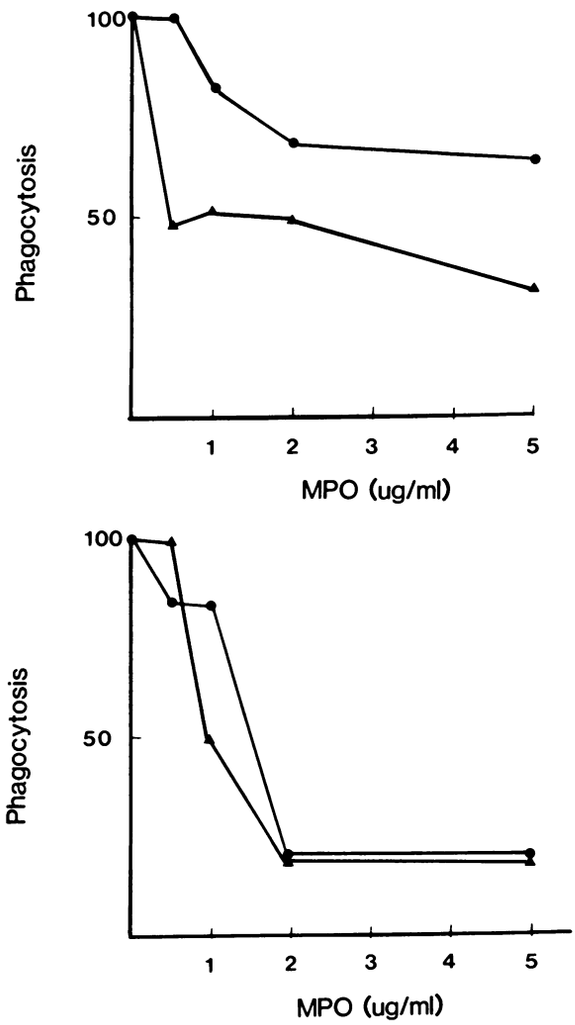

Figure 4. The effect of added MPO on C3b-yeast (*top*) and IgG-yeast (*bottom*) phagocytosis in normal (●) and MPO-deficient PMNL (▲), expressed as percentage of controls in the absence of MPO. Mean of two to three experiments.

interaction is more sensitive to the MPO effect than the C3b receptor. Furthermore, the fact that the MPO effect and the difference between MPO-deficient and normal PMNL is not observed in suspension but only after adhering the cells shows that the generation of oxidative metabolites during phagocytosis of yeast particles in suspension is apparently not sufficient to affect the receptor function. The reason could be that (*a*) there are relatively few yeast particles per PMNL, (*b*) that they activate the cell only when they already have bound to their receptor and (*c*) that cells in suspension release less oxidative metabolites than adherent cells. Furthermore, these experiments show that the MPO-deficient and normal PMNL may have a similar number of Fc and C3b receptors when isolated from peripheral blood. However, when migrating or adhering, the receptor may be altered by MPO-mediated events.

The enhancing effect of catalase also in the absence of MPO indicated that H_2O_2 by itself, as shown before (29, 30), alters the function of PMNL. When MPO was added, the Fc- and C3b-mediated phagocytosis was further inhibited. The inhibitory effect of low concentration of MPO (0.5–1 μg/ml) was evident only in the MPO-deficient cells. This may be due either to enhanced release of H_2O_2 or to the fact that the MPO-deficient cells were more sensitive in their "hyperactive" state.

It is not clear how the MPO-H_2O_2-halide system affects the receptor activity of the PMNL. Both the receptors on the cell and the ligands on the particle can be affected. The attachment experiments at 4°C show that the Fc- and C3b-receptor activity is reduced by MPO, but, at 37°C when H_2O_2 is generated, the surface-bound MPO may also inactivate the IgG and C3b ligands on the particles. The molecular basis for the MPO effect is unclear. Several highly reactive oxidants are formed by the MPO-system (halogens, chloramines, aldehydes, hypochloric acid, and singlet oxygens) (2), of which some may affect the function of PMNL (31). It has been demonstrated that particularly the thioeter linkage of methionine is sensitive to oxidation (9, 32). Whether these oxidative reactions mediate alterations in the receptor activity directly or via general membrane changes awaits further investigation. It is however worthwhile pointing out that mature human PMNL are remarkably resistant to the cytocidal effect of the MPO-H_2O_2-halide system in contrast to more immature myeloid cells (Stendahl et al., unpublished observations). The MPO-H_2O_2-halide system is also reported to inactivate several humoral inflammatory mediators: slow-reacting substance of anaphylaxis (33), prostaglandins (34), chemotactic factors (8, 9), lysosomal enzymes (24), and α_1-antitrypsin (25). It is thus a distinct possibility that lack of these different modifying effects of MPO on the biological properties of the inflammatory response may in connection with certain unknown predisposing and precipitating factors cause disease.

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