Hereditary Myeloperoxidase Deficiency

By Petr Cech, Athanassios Papathanassiou, Gaston Boreux, Paul Roth, and Peter A. Miescher

The functional properties of granulocytes in a diabetic patient deficient in myeloperoxidase (MPO) were compared with those of granulocytes in healthy subjects. The granulocytes of this patient had normal phagocytic activity. The microbicidal activity of the granulocytes was partially diminished with regard to Staphylococcus aureus and was almost nil with regard to Candida albicans. Fungicidal activity of normal granulocytes was shown to be impaired during the in vitro artificial hyperglycemic condition. The relationship among diabetes mellitus, MPO deficiency, and serious C. albicans infection was examined. Genetic investigation was carried out in 28 members of the proband's family. In close relatives of the patient, MPO values were found to be diminished to a greater or lesser degree, thus suggesting variable expressivity of the heterozygote state of MPO deficiency.

Hereditary deficiency of myeloperoxidase (MPO) is a very rare condition. So far, about 13 cases from 10 families have been described,1-7 and four of these have been subjected to genetic study. These subjects are generally not abnormally susceptible to serious infection, despite the presence of an intraleukocyte microbicidal defect. We have been able to study another case and to investigate, in particular, the effects of this anomaly on the functioning of granulocytes. Furthermore, the hereditary mode of transmission is analyzed through an examination of 28 members of the family.

MATERIALS AND METHODS

MPO-Deficient Subject

P.M. was a 38-yr-old Portuguese woman who had suffered from diabetes mellitus for the preceding 2 yr. She was admitted to hospital because of diabetic ketoacidosis (serum CO2 content 12.5 mmoles/liter, pH 7.22, serum glucose 341 mg/dl) with fever and chest pain. Diabetes mellitus appeared at the age of 36 yr and was treated with hypoglycemic sulfonamides. The clinical workup revealed a liver abscess due to Candida albicans. She was cured by removal of the abscess and by treatment with amphotericin B. The diabetes mellitus was stabilized by insulin Monotard. Intradermal tests with tuberculin and candidine were positive. 3H-thymidine uptake was normally increased by PHA in lymphocyte culture, as well as by PPD, Candida, and Varidase. The humoral immunity and the chemotactic granulocytic activity, as well as the complement system, were found to be normal. MPO was completely absent from the neutrophils and monocytes.7

Preparation of Granulocytes

Granulocytes were obtained by a slight modification of the technique of Böyum.8 Heparinized blood was placed in an equal volume of Ficoll-Isoaque and centrifuged for 20 min at 700 g (2000 rpm). The layers of Ficoll and plasma containing monocytes and lymphocytes were taken off. The pellet, comprising red blood cells and granulocytes, was suspended in 10 volumes of PBS and centrifuged for 10 min at 700 g. The granulocytes were separated from erythrocytes by dextran sedimentation without
exposure to hypotonic or ammonium chloride solution. Then the cells were washed and suspended at a concentration of $5 \times 10^6$ cells/ml. The erythrocyte contamination of the final preparation was about 5%.

**Biochemical Determination of Granulocytic Enzymes**

MPO was determined by a spectrofluorometric method with kinetic recording using $p$-cresol as substrate. The proband and her close relatives were tested. In summary, the granulocytes were prepared using the Ficoll-dextran technique described previously. The final WBC pellet was washed, counted, homogenized, and frozen (−80°C). Three milliliters of the solution of $p$-cresol (200 μg/ml) in the TRIS-HCl buffer (0.05M, pH 8) and 100 μl of WBC homogenate were pipetted into a test tube that was placed for 3 min in a water bath at 37°C. After addition of 20 μl of H₂O₂ (0.03%), the increase in fluorescence was recorded with a Farrand fluorometer (at 37°C, λex = 320 nm, and λem = 410 nm). The quantity of oxidated $p$-cresol was determined using a standard curve previously established. The enzymatic activity was calculated in international units as micromoles of $p$-cresol oxidized per minute. D-cathepsin activity was determined using tritium-labeled bovine hemoglobin and measuring any radioactivity not precipitable by trichloroacetic acid (TCA). The method of Kato, adapted to our conditions, was used to determine $β$-D-glucuronidase. $p$-Nitrophenyl-$β$-D-glucuronide was used as a substrate, and the $p$-nitrophenol released was measured by spectrophotometry.

**Phagocytic and Microbicidal Assays**

The phagocytic capacity of the patient's granulocytes was determined by measuring the uptake of $^3$H-thymidine-labeled *Escherichia coli* according to the method of Root et al. and Steigbigel et al. The NBT test (nitroblue tetrazolium reduction) was applied as described by Nydegger et al.

Bactericidal activity against *Staphylococcus aureus* (strain S.G. 511) and fungicidal activity against *C. albicans* (IUMM No. 8) were determined by a method developed for the purpose of this study. The strains were used after three successive subcultures. The *S. aureus* subculture was carried out in T.S.A. agar medium and the *C. albicans* in Sabouraud's agar. The time allowed between each subculture was 24 hr for *S. aureus* and 48 hr for *C. albicans*. The *S. aureus* were suspended in a 0.9% NaCl solution and adjusted nephelometrically to an optical density corresponding to a standard suspension of $2 \times 10^7$ staphylococci/ml. The numbers of *C. albicans* were determined spectrophotometrically and adjusted to a concentration of $2 \times 10^7$ fungi/ml. The microbicidal test was carried out in a suspension of $5 \times 10^6$ neutrophils/ml in the presence of 12.5% fresh AB serum with variable amounts of staphylococci or *C. albicans*. The exact numbers of staphylococci and *Candida* in the final test were determined by the method of colony counts in serial dilutions. The final mixture was shaken on a Vortex agitator for 5 sec and then placed in a water bath at a temperature of 37°C. Samples were taken at various intervals up to 120-240 min. After each sampling the cell suspension was shaken for 5 sec on the agitator before being placed once again in the water bath. The samples withdrawn from the test tube were mixed with sterile distilled water in order to lyse the granulocytes. Preliminary experiments showed that a period of 10 min was sufficient for complete lysis of the granulocytes without destroying the *C. albicans* or the *S. aureus*.

After lysis, decimal dilutions were carried out. From the $10^{-1}$, $10^{-4}$, $10^{-7}$, and $10^{-4}$ dilutions, 1 ml was taken and mixed with the corresponding agar. Colony counts were determined after an incubation period at 37°C of 48-72 hr. A control test was carried out with the microbes in AB serum alone.

The fungicidal powers of granulocytes from 8 normal subjects and from the patient (P.M.) were tested against *C. albicans* without the addition of glucose and by adjusting the glucose concentration to 450-500 mg/dl. Otherwise, the test was carried out by the same method as described earlier. The granulocyte/fungi ratio in the test tube was 1:4. The toxicity of the suspending assay medium was measured by osmometer. The paired Student test was used for statistical evaluation of the results of the normal PMN in both conditions.

**Metabolic Study of Granulocytes**

The granulocytes were prepared using the Ficol-dextran technique described previously. The cell suspension had a final concentration of 1.5-3.5 $\times 10^9$ cells/cu mm. Zymosan particles were used in a
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final concentration of 4–6.5 \times 10^7 particles/cu mm to stimulate phagocytosis. Oxygen consumption was measured by the spectrophotometric method of Barzu, with oxyhemoglobin (HbO2) as the oxygen source.\textsuperscript{16}

Some 1 \pm 0.1 \times 10^6 leukocytes were placed in a medium containing 4% dextran (which prevented the suspension from settling), 0.15 mmoles/liter HbO2, and 10% of autologous serum. This medium was covered with a layer of paraffin oil and incubated at a temperature of 37°c. After recording of basal respiration for 5–10 min, stimulation was carried out by injection of zymosan particles, in the numbers determined previously, through this layer with a Hamilton syringe. The granulocyte/zymosan particle ratio was 1:50 + 10.\textsuperscript{16}

Genetic Study

The genetic study was carried out in the Portuguese province of South-East Alentejo (the villages of Povoa, Alqueva, and Santa-Amador). In addition to the patient, 27 relatives were clinically and hematologically examined. Peripheral blood smears were stained immediately after drying by the Kaplow method\textsuperscript{17} with Diff-Quick (Laboratoire Merz-Dade) counterstaining. They were read by two persons independently and in blinded fashion. In order to assess differences in the MPO contents of PMN, these cells were graded as follows: 0 = negative, 1 = weak, 2 = average, 3 = strong. The cumulative score was determined in 100 PMN (maximal score 300). The control smears were obtained simultaneously from 13 normal subjects and were handled under the same conditions as described previously.

RESULTS

Enzymatic Studies

The patient's PMN proved to be completely negative for MPO staining. The quantitative biochemical assay for MPO gave a low value similar to that of the negative control sample (Table I). Thus the cells were considered to be completely devoid of MPO. The following additional enzymes were present in normal amounts in our patient: serum lysozyme, granulocytic D-cathepsin, and granulocytic \(\beta\)-D-glucuronidase.

Phagocytic and Microbicidal Activity

Studies with \textit{E. coli}: Uptake and reduction of NBT by the patient's PMN proved to be no different from uptake by normal PMN: 3% of the patient's PMN and 2% (Table 1). 673.6 ± 355.7

<table>
<thead>
<tr>
<th>Origin of Leukocytes</th>
<th>Count of MPO-Positive Neutrophils (PMN)</th>
<th>MPO Biochemical Determination of Neutrophils</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Weak</td>
</tr>
<tr>
<td>Proband (III/7)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Daughter (IV/8)</td>
<td>11</td>
<td>16</td>
</tr>
<tr>
<td>Son (IV/9)</td>
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<td>3</td>
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<tr>
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<tr>
<td>Husband (III/6)</td>
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673.6 ± 355.7

158–1291

Normal subjects (13)

Mean value

Standard deviation

Extreme values

0 0 6–30 70–94 270–294

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of the control PMN were formazan-positive in the test. On stimulation with *E. coli*, 82% of the patient’s PMN and 84% of the control PMN became formazan-positive. The quantitative phagocytosis test with tritiated *E. coli* showed normal uptake of bacteria by the patient’s PMN (0.76 bacteria/granulocyte/30 min, as compared with 0.80 bacteria/granulocyte/30 min with normal PMN).

Studies with *S. aureus*: The killing rates of *S. aureus* by the patient’s PMN and by PMN of normal subjects are shown in Fig. 1. Initially, no difference was observed. However, after an incubation period of 120 min, a significant decrease in the killing rate was observed with the patient’s PMN.
Studies with *C. albicans*: The killing activity of the patient’s PMN with regard to *C. albicans* appeared greatly diminished at all incubation times investigated (Fig. 2). AB serum alone proved to be devoid of all fungicidal activity.

Studies with *C. albicans* in hyperglycemic condition: Glucose in a concentration of 450–500 mg/dl produced a significant delay in the killing of *C. albicans* by normal PMN. The difference proved significant after a 60-min incubation (Fig. 3). Despite a variable glucose concentration in the test tube, the tonicity of the suspending assay medium remained steady and isotonic.

**Metabolic Study of Granulocytes**

PMN from normal subjects exhibited maximal oxygen consumption 3–6 min after addition of zymosan particles. With the patient’s PMN, maximal oxygen consumption occurred after only 1 min of incubation. Furthermore, maximal oxygen consumption was 60% higher with the patient’s cells than with normal PMN (Table 2).

**Genetic Study**

The family tree is shown in Fig. 4. No consanguinity or serious illness was found in the family. One sister died, paralyzed, at the age of 19 yr, and a brother who suffered from heart disease died at the age of 9 yr. Among the presumed

<table>
<thead>
<tr>
<th>Table 2. Maximal Oxygen Consumption During Phagocytosis</th>
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<tr>
<td>Origin of Granulocytes</td>
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<td>------------------------</td>
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<tr>
<td>Normal subjects (12)</td>
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<td>MPO-deficient patient</td>
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heterozygotes (proband’s parents and children), three (II/4, II/3, IV/9) had moderately diminished total counts of MPO-positive granulations, and two (II/3, II/4) had low neutrophil MPO by biochemical determination (Table 1). The daughter (IV/8) had a very low total count of MPO-positive granulations and a very low MPO activity by biochemical determination (Table 1). Furthermore, four different types of neutrophils with varying degrees of MPO positivity were detected in the daughter’s peripheral blood smears (Fig. 5). Five other members of the family had 1%–4% completely MPO-negative PMN and/or slight diminution of the total count of MPO-positive granulations (Table 1). All other members of the family exhibited normal total counts of MPO-positive granulations. Furthermore, they showed complete absence of MPO-negative PMN.

DISCUSSION

In this diabetic patient with C. albicans liver abscess, hereditary MPO deficiency was discovered. This was only the third report of a patient with hereditary MPO deficiency exhibiting a serious C. albicans infection.3,6

The phagocytic studies showed normal uptake of E. coli with the different methods tested. With regard to NBT uptake and reduction, no impairment of formazan formation was observed, which corroborated the fact that MPO is not implicated in the reduction of NBT. With regard to microbicidal studies, slight impairment of S. aureus killing was observed, and there was very severe impairment of fungicidal activity against C. albicans.

Metabolic studies showed increased consumption of oxygen on zymosan stimulation (Table 2). This finding corroborated the results obtained by Rosen and Klebanoff.5 The difference in oxygen consumption was striking in our case, possibly because of the contributing effect of the diabetic metabolic disorder.18

The microbicidal studies thus confirmed the results obtained by others.3,4,19-21 Of particular importance was the lack of fungicidal activity, which was relevant to the
clinical observation of severe Candida infection in 3 of the 14 subjects with primary MPO deficiency. Furthermore, 2 subjects with this complication were diabetic. It thus might appear that the diabetic metabolic disorder represents a contributory factor for subjects with hereditary MPO deficiency to exhibit severe Candida infection.

The organism disposes essentially two fungicidal mechanisms. The first is T-cell-dependent, and it proved normal in this patient. Its importance has been established in studies on patients suffering from chronic mucocutaneous candidiasis. The second is due to antibody-complement-mediated phagocytosis by circulating and fixed phagocytes. The ultimate fungicidal effect of phagocytic cells is mediated by three types of biochemical events: MPO-H₂O₂-halide, MPO-independent oxidation (hydrogen peroxide, superoxide anions, hydroxyl radicals, singlet molecular oxygen), and the action of lysosomal cationic proteins on the fungal membrane.

The diabetic metabolic disorder is known to interfere with a series of defense mechanisms. Indeed, PMN from poorly controlled diabetic patients exhibit diminished chemotactic, phagocytic, and killing activities. These alterations have been explained by a metabolic intracellular disorder of the glycolytic pathway. In fact, insulin plays an important role in the regulation of phosphofructokinase and pyruvate kinase. Lack of insulin results in diminution of sustained energy production in the form of ATP, a source of energy necessary for effective PMN function. This diminished yield of ATP probably causes a compensatory increase in the rate of oxidative glycolysis via the hexose monophosphate shunt (increased oxygen consumption of diabetic PMN on zymosan stimulation). Furthermore, our
results showed a direct inhibitory effect of hyperglycemia on the fungicidal activity of normal PMN (Fig. 3). In this patient, PMN functions that depend on ATP, i.e., phagocytosis and chemotaxis, were found to be normal. However, the diabetic disorder was well controlled by insulin at the time of this study.

* C. albicans* is a normal inhabitant of the skin, the mucous membranes, and the digestive tract of man. Increased mucocutaneous and urinary tract colonization occurs in diabetics, resulting in mild symptomatic illness. However, to our knowledge, systemic candidiasis has not been described in diabetics.

The local complications seen in diabetic subjects increase the danger of systemic infections. In a diabetic patient under steroid medication a serious candidiasis has been described. In a similar way, a lack of fungicidal activity of PMN in MPO-deficient subjects gains clinical significance. Indeed, the fact that two MPO-deficient subjects encountered serious problems with *Candida* infection once they became diabetic emphasizes the importance of impairment of the fungal defense system due to the diabetic metabolic disorder in addition to MPO deficiency.

It has been thought that MPO deficiency represents an autosomal recessive genetic disorder. Previous genealogic studies have dealt with limited numbers of family members. In these families the incomplete enzyme deficiency in heterozygous family members has been detected by either biochemical or cytochemical methods. We were fortunate in that we were able to include 28 members of the MPO-deficient proband’s family. It is interesting to analyze results in semiquantitative terms (degree of MPO positivity of PMN) and by quantitative biochemical MPO determination (Table 1). According to the results obtained, there was no doubt that our patient was homozygous with regard to this genetic defect. Her daughter was heterozygote, characterized by four different types of PMN with varying degrees of MPO deficiency (Fig. 5). All other suspected heterozygotes in this family appeared only slightly affected. The results of our genetic study (Fig. 4) suggest that this deficiency is indeed an autosomal recessive disorder with varying degrees of gene expressivity in the heterozygous state.

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**REFERENCES**


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