Hereditary Myeloperoxidase Deficiency

By Mitsuo Kitahara, Harmon J. Eyre, Yasmen Simonian, Curtis L. Atkin, and Sandra J. Hasstedt

Subjects with neutrophil myeloperoxidase (MPO) deficiency have been rarely reported. In part this may be due to the lack of a simple screening technique that would detect them. With the routine use of a cytochemical leukocyte differential counter that employs MPO stains, over a 40-mo period 8 unrelated probands with partial MPO-deficiency and one with complete deficiency were identified. Family studies have identified 23 additional, partially deficient subjects. The largest pedigrees demonstrate that the carrier state or partial MPO deficiency is inherited in an autosomal dominant pattern. Leukocytes from a subject with complete MPO deficiency and from some subjects with partial deficiency have impaired bactericidal activity against S. aureus. Superoxide generation was increased and chemiluminescence decreased in MPO-deficient leukocytes. Eleven subjects were prospectively followed for 18–30 mo. Only two partially deficient subjects have had serious infections consisting of recurrent streptococcal cellulitis and aseptic meningitis. These data suggest that leukocyte MPO deficiency is a common inherited defect that results in minimal clinical problems, supporting the concept of multiple leukocyte bacterial killing systems.

LEUKOCYTE MYELOPEROXIDASE (MPO)
in the presence of H2O2 and a halide constitutes a potent bactericidal system,1 which is also effective against fungi,2 viruses,3 mycoplasma,4 and mammalian tumor cells.5 However, despite the presence of very large quantities of MPO in normal neutrophils (1%–5% of the dry weight), the relative importance of this killing mechanism is unknown. This is illustrated by the several MPO-deficient subjects previously reported who had no apparent increase in infection rates.5 Only three reported MPO-deficient subjects have had severe infections, all having candidiasis.6–8 Two of these subjects also had diabetes mellitus. Inherited complete MPO deficiency has been described in only 15 cases from 12 families.6–12

Further evaluation of subjects with MPO deficiency may shed some light on the physiologic importance of this enzyme. In the past, such studies were difficult because of the small number of subjects detected. Using an automated cytochemical analyzer for routine hematology work, over a 40-mo time span, 32 subjects with complete or partial MPO deficiency were detected. The results of genetic analysis, neutrophil metabolic and functional studies, and prospective follow-up of these subjects are reported here.

MATERIALS AND METHODS
Automated Cytochemical Screening of Leukocytes

The Hemalog D (Technicon Instruments Corp., Tarrytown, N.Y.), an automated cytochemical analyzer, detects different leukocytes by their size and staining characteristics.13 Neutrophils and eosinophils are stained for MPO activity using 4-chloro-l-naphthol as the electron donor. Monocytes are identified by their esterase activity and basophils by their heparin content. MPO deficiency is indicated when the Hemalog D reports a low neutrophil count and increased numbers of large unstained cells (LUC). Also, the position of the neutrophil cloud on the oscilloscope picture of the MPO channel is shifted toward the low absorption threshold.9 Approximately 18,000 sequential individuals seen at the University Medical Center were screened for MPO deficiency. A total of 120 relatives of 9 deficient probands were also tested.

Preparation of Granulocytes and Zymosan

Leukocytes were isolated at 4°C from heparinized (10–20 U/ml) blood by dextran sedimentation, treatment with 0.15% saponin and hypotonic lysis of red cells, and washing followed by suspension in Hanks’ balanced salt solution (Microbiological Associates, Walkersville, Md.). These preparations contained approximately 85% neutrophils. Zymosan was obtained from Schwartz-Mann, Orangeburg, N.Y. For opsonization, 100 mg of zymosan particles were washed with phosphate-buffered saline, resuspended in 10 ml normal serum, rotated 1 hr at 37°C in a Rotorack (Fisher Scientific, Santa Clara, Calif.), recovered by centrifugation, washed with and then resuspended in 10 ml phosphate-buffered saline.

Measurement of Myeloperoxidase Activity

Two methods were employed to measure MPO activity. The first was by histochemical staining giving a MPO score. Kaplan’s benzidine dihydrochloride stain for MPO was employed on fresh finger stick smears.14 MPO score was defined as the sum of the intensities (0 to 4+) of 100 consecutive neutrophils.3 The second measure of MPO activity was by enzymatic assay. Packaged, freshly isolated leukocytes were suspended and lysed in 0.2% Triton X-100 (Sigma Chemical Co., St. Louis, Mo.) solution. Aliquots of this solution were treated exactly as for horseradish peroxidase solution in a Worthington Manual assay of oxidation of o-dianisidine.15 In this assay, 1 U of enzyme corresponds to that amount of enzyme decomposing 1 μmole of H2O2/min at 25°C. “MPO activity” was expressed as units per 5 x 106 neutrophils, as in Rosen and Klebanoff.16

Electron Microscopy

Isolated leukocytes were fixed in Karnovsky’s fixative at 4°C, pH 7.4, and incubated at 22°C in a saturated solution of 3,3’-diaminobenzidine.17 MPO-positive granules were counted on representative electron microscopy pictures.


**Bacterial Killing Assay**

Bactericidal activity of isolated neutrophils against *Staphylococcus aureus* (502A) was determined by the technique of Quie et al.\(^7\) A suspension of bacteria in water was adjusted to optical density 0.6 at 620 nm, then diluted 50-fold with Hanks' balanced salt solution plus gelatin. Triplicate reaction mixtures each consisted of 0.1 ml diluted *S. aureus* suspension, 0.5 ml 20% normal human serum (for opsonins), and \(5 \times 10^6\) neutrophils in 0.5 ml Hanks' solution. Aliquots (5 μl) of reaction mixture were removed after 0, 30, 60, and 120 min incubation, and added to 10 ml sterile water to lyse granulocytes. After 10 min, 0.1 ml of the water suspension was taken for subculture. With the zero-time incubation taken as 100%, activity was expressed as mean percent viability of triplicate assays.

**Chemiluminescence and Superoxide Generation**

Generation of superoxide radical anion by isolated neutrophils in the presence and absence of opsonized zymosan was assayed by superoxide dismutase-inhibitable reduction of cytochrome-c. This method, run in triplicate, was the same as that of Rosen and Klebanoff.\(^1\) Net production of superoxide was taken as equivalent to net reduction of cytochrome-c at various times, and expressed as nmoles O\(_2^-\) / 10^6 neutrophils. Measurement of the time course of chemiluminescence of isolated neutrophils was performed according to Rosen and Klebanoff.\(^1\) Results were expressed as scintillation rate (cpm) of neutrophils plus opsonized zymosan less the rate from similar suspensions lacking zymosan.

**Clinical Data**

Eleven subjects with MPO deficiency from the families who were willing to be prospectively followed were interviewed and examined at the Clinical Research Center (CRC) of the University of Utah Medical Center. A complete blood count, chemistry survey, urinalysis, and chest radiograph were obtained for baseline values. Whenever any subject developed symptoms suggesting an infection, he or she was examined at the University of Utah Medical Center. The follow-up time from initial evaluation ranged from 18 to 30 mo.

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**Table 1. Measures of Neutrophil Myeloperoxidase**

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<thead>
<tr>
<th>MPO Score</th>
<th>MPO Activity (U/5 x 10^6 Neutrophils)</th>
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<tbody>
<tr>
<td>n</td>
<td>X ± SD</td>
</tr>
<tr>
<td>Normal</td>
<td>20</td>
</tr>
<tr>
<td>Complete deficiency</td>
<td>1</td>
</tr>
<tr>
<td>Partial deficiency</td>
<td>31</td>
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**RESULTS**

**Incidence of MPO Deficiency**

Over a period of 40 mo, automated leukocyte differential counts were done on approximately 18,000 routine blood samples using the Hemalog D. Eight persons with partial MPO deficiency and another with complete deficiency were identified. Subsequently, another 23 partially deficient subjects were identified among 120 tested relatives of the 9 probands. As previously reported for some of these family members,\(^9\) the MPO score and MPO activity showed bimodal distributions in these family members. Table 1 shows the values of these measures for groups of normal and partially MPO-deficient subjects and for the completely deficient individual. There was considerable variability (values of 0 to 3+) in the MPO score of individual neutrophils from some of partially deficient subjects. Numerous electron micrographs of their neutrophils, however, showed that all cells examined had decreased numbers of MPO-positive granules. We believe this evidence rules out the possibility of two cell populations in the partially MPO-deficient subjects. Electron micrographs of the neutrophils from

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**Fig. 1.** Kindred 1. MPO deficiency is present in both sexes and in each generation.
the subject with complete MPO deficiency showed no MPO-positive granules. Normal numbers of MPO granules were seen in eosinophils from all subjects.

Hereditary Pattern

Figures 1–3 show pedigrees of the three largest families with MPO-deficiency. These are descendents of Utah pioneer families who, according to records of the Genealogical Society of Utah, are unrelated over at least 5 generations. The completely MPO-deficient subject is unrelated to the families shown. Kindred 1 has 50 living members within 4 generations, 13 of whom demonstrated partial MPO deficiency. Kindreds 2 and 3 include 11 identified subjects with partial deficiency. The means of the MPO scores of deficient subjects in the three families were not signifi-

Fig. 2. Kindred 2. MPO deficiency showing variable expression.

Fig. 3. Kindred 3. MPO deficiency.
MYELOPEROXIDASE DEFICIENCY

Table 2. Inheritance of Myeloperoxidase—Deficiency

<table>
<thead>
<tr>
<th>MPO Score</th>
<th>Normal</th>
<th>Deficient</th>
<th>LOD Score</th>
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<tr>
<td>Kindred 1</td>
<td>358 ± 29 (27)</td>
<td>240 ± 40 (13)</td>
<td>4.0</td>
</tr>
<tr>
<td>Kindred 2</td>
<td>340 ± 31 (17)</td>
<td>234 ± 74 (7)</td>
<td>3.3</td>
</tr>
<tr>
<td>Kindred 3</td>
<td>357 ± 29 (32)</td>
<td>182 ± 106 (4)</td>
<td>7.4</td>
</tr>
<tr>
<td>All</td>
<td>354 ± 30 (76)</td>
<td>235 ± 71 (24)</td>
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*Logarithm of the odds of a dominant model versus a recessive model.

Significantly different according to Student’s t test (Table 2). Male to male transmission was evident in kindreds 1 and 2. Families of 5 of the 9 probands studied had at least one additional member with partial MPO deficiency. The other 4 probands were studied on more than one occasion and all remained deficient. None had any underlying disease or was taking any medication known to be related to MPO deficiency, and thus, we believe they represent hereditary deficiency but we are not sure this is the case.

Computerized pedigree analysis for dominant versus recessive inheritance of partial MPO deficiency was performed according to Elston, using the unaffected subjects (including some spouses) and affected subjects listed in Table 2 and shown in Figs. 1–3. The logarithms of the odds (LOD scores in Table 2) of a dominant pattern of inheritance were greater than 3 for each of the three families. Thus, the odds for the dominant model of inheritance of partial MPO deficiency in each of these three families were 1000 times greater than for the recessive model of inheritance.

Leukocyte Function Studies

Bactericidal activity of neutrophils from the completely deficient subject and from 3 partially deficient subjects against S. aureus is compared to 15 controls in Fig. 4. Neutrophils from the completely deficient subject and one partially deficient subject showed significantly decreased killing activity (p < 0.05) when compared to normal neutrophils. However, neutrophils from two moderately MPO-deficient subjects (MPO activity >0.45 U/5 x 10⁷ neutrophils) were able to kill organisms as efficiently as control neutrophils.

Superoxide generation by neutrophils from completely and partially MPO-deficient subjects is shown in Fig. 5. As previously shown by others, superoxide generation by completely MPO-deficient neutrophils was augmented. In partially deficient neutrophils, superoxide generation was also increased as compared with control neutrophils.

Chemiluminescence of completely MPO-deficient neutrophils and control neutrophils is illustrated in Fig. 6. The peak chemiluminescence activity of MPO-deficient neutrophils was less than that of controls. These studies confirm that the MPO-deficient neutrophils in this series have the same functional characteristics as those previously reported by other investigators.

Clinical Significance

Eleven representative MPO-deficient subjects were followed prospectively. Only two subjects with partial MPO deficiency have had serious infections. In spite of a MPO level of 0.84 U/5 x 10⁷ neutrophils and
normal streptococcal killing capacity, one subject (proband 6) developed three episodes of streptococcal cellulitis in his lower extremities during the period of observation. All have responded promptly to penicillin therapy. This patient also has long-standing lymphedema secondary to treatment for testicular cancer, which may have predisposed him to these infections. A second subject (kindred 2, IV-11) was hospitalized with aseptic meningitis. He recovered without sequella and is currently well.

DISCUSSION

This report and that of other investigators* demonstrate that neutrophil MPO deficiency is not a rare disorder. When screening techniques were applied to a large hospital population, the frequency of the deficiency among the random sample of individuals screened was approximately 1/2000. Both the Hemalog D and the MPO score can be used for detection of subjects with the enzyme deficiency.\(^6\)\(^9\) The three pedigrees in this report show an autosomal dominant pattern for the inheritance of partial MPO deficiency. This agrees with previous reports\(^6\)\(^7\) of “autosomal recessive” inheritance of complete deficiency. If partial deficiency represents one deficiency allele and complete deficiency two deficiency alleles, then one would observe a dominant inheritance pattern with the ability to detect the heterozygous state. Most of our partially deficient subjects had MPO scores between 200 and 300 (see Table 1). However, 5 individuals among the 3 pedigrees had scores ranging from 83 to 138. They might possibly be homozygous for the deficiency allele or heterozygous for two different deficiency alleles.

The molecular abnormality present in MPO-deficient neutrophils has not been well defined. Biochemical studies of human MPO have shown a variety of isoenzymes with conflicting reports as to their number.\(^20\)\(^21\) Partial MPO deficiency shows variable expression, which may be accounted for by variable inheritance of MPO isoenzymes. Salmon et al.\(^22\) could not detect the presence of MPO in neutrophils of a completely deficient subject by means of gel diffusion or immunofluorescence. However, further studies are required to define whether MPO deficiency represents the absence of normal enzyme or the presence of an altered enzyme with no functional activity. Different families may have different defects.

Lehrer and Cline,\(^7\) Moosmann and Bajanovsky,\(^8\) and Cech et al.\(^9\) reported patients with MPO deficiency and recurrent Candida infections. With two exceptions, our subjects showed no serious infections. Two of our probands were treated with chemotherapy for non-Hodgkin’s lymphoma and Hodgkin’s lymphoma. During treatment, their absolute neutrophil counts fell to 1820/cu mm and 630/cu mm without infectious complications. Furthermore, in spite of decreased in vitro bactericidal activity, patients III-6 in kindred 2 and III-8 in kindred 4 have remained free of infections. That our prospective observations on these MPO-deficient subjects documented only rare infections adds support to the concept of multiple neutrophil killing mechanisms.\(^1\) Cech et al.\(^6\) suggested that lack of fungicidal activity and severe Candida infection seen in MPO-deficient patients with diabetes mellitus are in part due to diabetic metabolic derangement of neutrophils. Lehrer’s original patient with Candida infection also had diabetes mellitus.\(^7\) Numerous publications indicate that neutrophils from diabetic patients demonstrate decreased bactericidal activity.\(^22\)\(^23\) The association of diabetes mellitus with MPO-deficiency might be an important contributing factor to the development of infectious problems. The completely MPO-deficient subject without diabetes mellitus in our series and the proband’s sister in Lehrer’s series (also completely MPO deficient) have not suffered from any significant infectious illness.

Neutrophil metabolic activities of our subjects determined by superoxide generation and chemiluminescence were similar to those reported by Rosen and Klebanoff.\(^14\) It is interesting to note that superoxide generation determined by cytochrome-c reduction in partial deficient neutrophils was less than that of
completely deficient neutrophils, yet both were elevated as compared to normal. Three of the nine probands in this series have had cancer. Two probands had lymphoma and one had testicular cancer. It will be worthwhile to observe other MPO-deficient patients for a long period of time defined.

REFERENCES

Hereditary myeloperoxidase deficiency

M Kitahara, HJ Eyre, Y Simonian, CL Atkin and SJ Hasstedt