Evidence for a Pretranslational Defect in Hereditary and Acquired Myeloperoxidase Deficiency

By Andreas Tobler, Michael E. Selsted, Carl W. Miller, Keith R. Johnson, Michael J. Novotny, Giovanni Rovera, and H. Phillip Koeffler

Myeloperoxidase (MPO) is a heme containing enzyme involved in the oxygen-dependent microbicidal activity of human polymorphonuclear leukocytes (PMN). Complete hereditary and acquired MPO deficiencies are defined as lack of peroxidase activity in PMN. Using this criterion, we studied a patient with complete hereditary MPO deficiency, and a MPO deficient variant cell line of HL-60 (HL-60-A7), which we used as a model for acquired MPO deficiency. Western blot analysis showed complete absence of mature and precursor protein of MPO both in PMN from the patient and in HL-60-A7 cells. PMN from both parents had one half of normal levels of these proteins. To study further the molecular basis of this defect, we isolated an intron specific probe for MPO and used it and a cDNA probe. Both normal human bone marrow cells and the promyelocytic HL-60 leukemia cells contained MPO mRNA species of 2.8, 3.3, 4, and 8 kilobase (kb). The transcripts of >8 and 4 kb contained sequences hybridizing to a probe specific for intron 7 of the MPO gene. Bone marrow cells of the MPO deficient patient contained two species of heterogenous nuclear (hn) RNA of >8 and 4 kb, but only trace amounts of the normal sized 3.3 kb MPO mRNA and undetectable 2.8 kb MPO mRNA. HL-60-A7 cells contained both >8 and 4 kb hnRNA, but only small amounts of normal sized 2.8 kb MPO mRNA and undetectable levels of the 3.3 kb mRNA. Southern blot analyses revealed no gross alteration of the MPO gene in both cases. Our results suggest that a pretranslational defect is one mechanism leading to MPO deficiency.

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MATERIALS AND METHODS

Case record. A 31-year-old white woman was found to have MPO deficiency during routine blood examination by automated flow cytochemistry (courtesy of Dr H. Fanselau, Glendale Adventist Medical Center, Glendale, CA). Complete MPO deficiency was confirmed using histochemical staining procedures as described. Differential counts of the peripheral white cells revealed >70% granulocytes. The patient had no history of unusual or recurrent infections and was not taking medication known to interfere with MPO activity, such as sulfonamides, antithyroid agents, phenothiazine, or ascorbic acid. The mother and father of the patient were distant cousins and they had normal leukocyte differential counts.

Cells. The promyelocytic leukemic cell line HL-60,8 the MPO deficient variant HL-60-A7 cells,7,2 and the human lymphotropic virus-1 (HTLV-I) transformed cell line S-LB13 were grown in...
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Fig 1. Myeloperoxidase protein expression in normal and MPO deficient PMN. Western blot. PMN were extracted and subjected to Western blot analyses as described in Materials and Methods. Lane 1, 3 µg purified MPO; lane 2, 5 x 10⁶ cell equivalents of normal PMN; lane 3, 1 x 10⁶ cell equivalent of normal PMN; lane 4, 1 x 10⁶ cell equivalents of normal PMN granules; lane 5, 1 x 10⁶ equivalents of the patient’s PMNs; lane 6, 1 x 10⁶ cell equivalents of the patient’s mother; lane 7, 1 x 10⁶ cell equivalents of the patient’s father; lane 8, 1 x 10⁶ cell equivalents of HL-60 cells; lane 9, 1 x 10⁶ cell equivalents of HL-60-A7 cells; lane 10, 1 x 10⁶ cell equivalents of S-LB1 cells. Molecular weight markers are: 6.2 k, bovine trypsin inhibition; 14.3 k, lysozyme (egg white); 18.4 k, β-lactoglobulin; 25.7 k, α-chymotrypsinogen; 43 k, ovalbumin; 68 k, bovine serum albumin; 97.4 k, phosphorylase B.

alpha medium and 10% fetal bovine serum (FBS) in a humidified atmosphere with 7% CO₂. Bone marrow samples and peripheral venous blood from normal volunteers and the MPO deficient patient were obtained after informed consent. Mononuclear cells were isolated by centrifugation on Ficoll-Hypaque gradients, washed twice in phosphate buffered saline and resuspended in alpha medium containing 10% FBS. The PMN were obtained by subjecting the venous blood to dextran sedimentation (3%) and hypotonic lysis of the RBCs. The preparations contained ≥95% granulocytes, as determined by Giemsa staining.

Preparation of cell free extracts. Cell free extracts were prepared by thawing 1 x 10⁷ PMN in 200 µL of ice cold protease inhibitor solution: 10 mmol/L NaPO₄, pH 7.4; 5 mmol/L iodoacetamide; 1 mmol/L phenylmethylsulfonyl fluoride; 1 mmol/L benzamidine; 1 mmol/L N-ethylmaleimide, 1 mmol/L ethyleneglycolbis-N,N,N,N'-tetraacetic acid, and 5 mmol/L leupeptin. The cell suspension was incubated on ice for 30 minutes after which 800 µL of ice cold 12.5% acetic acid was added. The cell suspension was sonicated (three times, ten seconds each) in a Biosonik IV sonicator fitted with a micro probe. After an additional 30 minutes incubation on ice, the suspension was centrifuged at 13,000 g for ten minutes at 8°C. The clarified supernatant was removed, and extracts (1 x 10⁶ cells/aliquot) were stored at –70°C.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis, Western blotting. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed essentially as described.32 Aliquots (1 x 10⁶ cells) were lyophilized and resuspended in SDS sample buffer containing 10 mmol/L dithiothreitol. After boiling for ten minutes, the samples were electrophoresed for 12 hours at 150 V. Protein was transferred to nitrocellulose with an ABN Polyblot apparatus for 35 minutes, using the buffer system described.33 After transfer, nitrocellulose membranes were processed as described,34 except that both the blocking buffer and washing buffer contained 0.1% Tween-20. Rabbit anti-MPO antiserum (a gift from Dr Kenneth Miyasaki, UCLA) was used at 1:2000 in

Fig 2. MPO RNA accumulation in normal and MPO deficient bone marrow cells. RNA blot. Total RNA of the various cells was isolated and analyzed using a 2.2 kb pMPO2 cDNA probe. Lane 1, normal human bone marrow cells; lane 2, bone marrow cells of the patient; lane 3, HL-60-A7 cells; lane 4, normal PMN; lane 5, HL-60 cells. Lanes 2 and 3 contained 25 µg RNA, while lanes 1, 4, and 5 contained 8 µg RNA in order to enhance detection of MPO mRNA in the experimental samples. (A) shows a two-day exposure of the autoradiogram; (B) is an 8-hour exposure; (C) is an autoradiogram of the same blot after hybridization with the β-actin probe to confirm that the RNAs were intact. Arrow depicts MPO RNA of >8 kb. Analysis was performed as described in Materials and Methods.
RESULTS

Expression of myeloperoxidase. MPO protein levels were examined by Western blot analysis of extracts from \(5 \times 10^6\) (Fig 1, lane 2) or \(1 \times 10^6\) (lanes 3 to 10) cell equivalents. Cell and granule extracts of normal PMN (Fig 1, lanes 2 to 4) contained heavy and light MPO chain units of 55 and 13.5 Kd, respectively, in agreement with previous reports.\(^{\text{17-22}}\) In PMN of the MPO deficient individual, neither the precursor protein of 89 Kd, nor the heavy or light chain subunits of 55 and 13.5 Kd were detectable, even after ten days exposure of the film (lane 5). Cells obtained from both the father and the mother of the MPO deficient patient expressed the heavy and light chain MPO subunits as well as trace levels of precursor protein for MPO (lanes 6 and 7). Cells from both parents had approximately 50% less MPO subunit protein than normal PMN, as determined by densitometry readings. The promyelocytic leukemic HL-60 cells contained both the heavy and light chain units and abundant levels of the precursor protein (lane 8). Mature and precursor MPO proteins were neither detectable in the MPO deficient HL-60-A7 cells (lane 9) nor in HTLV-1 immortalized T lymphocytes (S-LB1, lane 10).

Accumulation of MPO mRNA. Levels and molecular weights of steady-state MPO mRNA were measured by Northern blot analysis of total cellular RNA extracts from

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Fig 3. (A) Sequence of intron 7 of human MPO. Flanking base sequences of exons 7 and 8 and their corresponding amino acids are given. The BamH1 and SstI restriction sites in intron 7 are shown. (B) Southern blot of HL-60 DNA restricted with EcoRI and hybridized with intron 7 (BamHI-SstI), which was labeled with \(^{32}P\). Autoradiogram shows a single 18 kb band.
normal and MPO deficient bone marrow cells, using the 2.2 kb pMPO2 cDNA probe. Normal human bone marrow and HL-60 cells showed MPO RNA species of 2.8, 3.3, -4, and >8 kb (Fig 2A, lanes 1 and 5). The Fig 2B demonstrates a shorter exposure of the same hybridization. RNA of the patient contained hybridizing species of >8 and ~4 kb (lane 2). No 2.8 kb MPO hybrids were detected, and only a very faint band at 3.3 kb was seen after a sixteen-day exposure of the autoradiogram. The HL-60-A7 cells showed MPO RNA species of >8, and ~4 kb (lane 3). No 3.3 kb MPO hybrids were detected, and only a faint band at 2.8 kb was observed (lane 3). MPO RNA was not detectable in normal human granulocytes (lane 4). Identical results were obtained when the same blot was hybridized with the Smal-Smal pMPO2 cDNA probe (data not shown). The faint hybridizing band from bone marrow and HL-60 cells around 1.6 kb is probably caused by displacement of partially degraded myeloperoxidase RNA by 18S ribosomal RNA. The same blot was rehybridized with β-actin DNA probe, which confirmed that the RNA of each sample was intact (Fig 2C).

To characterize further the different MPO mRNA species, we sought to determine whether the ~4 and >8 kb MPO RNA species represented heterogeneous nuclear (hn) MPO RNA, using a probe specific for MPO intron 7. To isolate this intron, we used previously isolated MPO cDNA clones25 that flanked this intron; these were used to probe a λ phage genomic library. Positive clones were sequenced and the entire sequence is shown in Fig 3A. A single 18 kb hybridizing band was seen when a 700 bp intron 7 sequence (BamHI-SstI) was 32P-labeled and used to probe a Southern blot containing human DNA restricted with EcoRI (Fig 3B). This suggested that the 700 kb intron 7 fragment did not contain highly repeated sequences. As further proof, we searched Genbank for similar sequences and found no significant homologies (data not shown).

The MPO intron 7 was used as probe on Northern blots containing RNA from our MPO deficient patient (Fig 4A). We found no 3.3 and 2.8 kb MPO bands of hybridization with RNA from HL-60 cells (lane 1), normal human bone marrow cells (lane 2), bone marrow cells of the MPO deficient patient (lane 3), or HL-60-A7 cells (lane 4). MPO RNA species of approximately >8 and ~4 kb (faint band) were seen from these RNA samples suggesting that these species represent hnRNA. The same blot was rehybridized with a β-actin DNA probe, which confirmed that the RNA of each sample was intact. Using the intron 7 MPO probe, we detected no hybridization to total cellular RNA extracts from MPO nonexpressing cells such as the myeloblastic KG-1 and myelomonocytic U937 cells (data not shown). This demonstrates the specificity of the intron probe.

Southern blot analyses. DNA samples, extracted from HL-60 cells and peripheral blood lymphocytes from the patient, were digested with various restriction enzymes (BglII, PvuII, EcoRI, BamHI) and analyzed by Southern blotting, using the Smal-Smal pMPO2 cDNA probe (Fig 5). No differences in the restriction pattern were observed comparing HL-60 DNA (lanes 1, 3, 5, 7) to those of patient DNA (lanes 2, 4, 6, 8), suggesting that no major alteration of the MPO gene was present in the MPO deficient patient.

DISCUSSION

This study demonstrates a defect in RNA and protein metabolism in myeloperoxidase deficiency. Markedly reduced accumulation of mature MPO RNA occurred concurrently with maintenance of normal levels of the precursor MPO hnRNA in the bone marrow of a patient with hereditary deficiency, and in HL-60-A7 cells, a model for acquired MPO deficiency. Using a cDNA probe and a probe specific for intron 7 of the MPO gene, we have identified two species of MPO mRNA precursors in the nucleus of both normal and abnormal myeloid cells with apparent sizes of >8 and ~4 kb. Because of the inaccuracy of sizing large RNA species on Northern blots, it is not clear whether the >8 kb species
represents a primary transcript of the 11.0 kb MPO gene or a large intermediate. The species of ~4 kb is clearly an intermediate product and not a mature mRNA since it contains the seventh intron of the MPO gene transcript. However, it could represent a partial degradation product of the whole MPO transcript.

Of the two MPO mRNA species detected in the cytoplasm of both normal human bone marrow cells and the promyelocytic HL-60 cells (3.3, 2.8 kb), the 2.8 kb RNA was not detectable in the MPO deficient patient, and only traces of a 3.3 kb species were seen. In MPO-deficient HL-60-A7 cells, the mature MPO mRNA species (2.8 kb) were found in trace amounts, whereas the 3.3 kb species were not detectable. Although low levels of the 2.8 kb and 3.3 mRNA species were present in HL-60-A7 and patient’s cells, respectively, we did not detect MPO protein in either sample. The details underlying this observation remain to be elucidated. The approximate 1.6 kb species observed in HL-60 and normal marrow cells may be a minor RNA by-product of either the processing of MPO mRNA or cross-hybridization to 18S ribosomal RNA since it cannot be easily accounted for by sequence data.25

At the protein level, normal PMN contained the heavy and light chain subunits but lacked precursor protein of MPO, and MPO RNA was not detectable in these cells. The absence of both RNA and precursor protein suggests that MPO is processed to completion in PMN that are terminally differentiated cells. The PMN from the MPO deficient patient possessed neither precursor, nor the heavy and light chain subunits of MPO protein. PMN of both of the parents expressed ca. 50% of the normal amount of the MPO subunits, suggesting that the parents are heterozygous for MPO expression. The promyelocytic HL-60 cells contained abundant levels of both MPO precursor protein and heavy and light chain subunits. The MPO deficient HL-60-A7 cells had neither detectable precursor protein for MPO, nor heavy or light chain subunits.

Our protein study confirms previous work by Nauseef et al that showed complete absence of heavy and light chain MPO protein in MPO deficient PMN.23 Based on the presence of a precursor protein, they concluded that MPO deficiency is due to defective processing of MPO protein. Our findings differ in that we do not detect an 89 Kd precursor protein in either normal or MPO deficient PMN. This difference may reflect the heterogeneity of the underlying defect.

Molecular analyses of thalassemias have shown how a variety of mutations interfere with normal RNA levels and have revealed the genetic heterogeneity of this disease.42,43 Mutations in thalassemia result in abnormal splicing or cleavage of β-globin mRNA, nonsense and frame-shift alterations, and abnormal transcription. Similarly, MPO deficiency may be a heterogenous group of genetic disorders with various mutations resulting in markedly decreased levels of MPO mRNA. These low levels of MPO mRNA could result from a defect in processing or stability of the MPO transcripts, or decreased transcription of the gene. The persistence of MPO hnRNA suggests continued transcription of the gene, yet another alteration at this level cannot be excluded. The molecular bases for the development of MPO deficiency in HL-60-A7 cells also remains unclear. Both HL-60 and HL-60-A7 cells are missing one chromosome 17, which contains the MPO gene.44 Therefore, one possible cause for the MPO deficiency may be a mutation in the one remaining MPO gene.

This study demonstrates that a pretranslational defect is one cause of complete MPO deficiency. Further molecular analysis of this and additional MPO deficient individuals will help to elucidate whether MPO deficiency is a heterogenous
hereditary disorder analogous to the thalassemias. Further investigation of the leukemic HL-60-A7 cells may provide insights into the molecular defect(s) associated with MPO deficiency in myeloproliferative disorders.

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REFERENCES

40. Ponte P, Gunning P, Blau H, Edes L: Human actin genes are single copy for a-skeletal and a-cardiac actin but multicyco for /3-
and γ-cytoskeletal genes. 3' untranslated regions are isotyp specific but are conserved in evolution. Mol Cell Biol 3:2058, 1983


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