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Effect of the R569W Missense Mutation on the Biosynthesis of Myeloperoxidase*

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Human neutrophil microbicidal activity is largely mediated by reactive species generated by the oxygen-dependent myeloperoxidase (MPO) system. Peroxidase-negative neutrophils from many patients with hereditary MPO deficiency possess a 90-kDa MPO-related protein. We recently identified a missense mutation, R569W, in the MPO gene of many subjects with MPO deficiency. In these studies we examined the consequences of R569W on MPO biosynthesis and processing, using stably transfected K562 cells expressing normal MPO or the R569W mutation. K562 cells expressing normal MPO mimicked faithfully many features of MPO biosynthesis in myeloid cells. 1) apopro-MPO was synthesized; 2) a functional heme group was inserted into apopro-MPO, and enzymatically active pro-MPO was thereby generated; 3) pro-MPO underwent proteolytic processing to mature MPO; and 4) hemin augmented the processing of pro-MPO. pREP-R569W cells synthesized apopro-MPO, but heme was not inserted. Neither enzymatically active pro-MPO nor mature MPO was synthesized by transfectants expressing mutated cDNA, confirming our hypothesis that the R569W mutation results in a form of apopro-MPO which does not undergo post-translational processing to enzymatically active MPO species. In addition, these data support previous suggestions that heme insertion into apopro-MPO is necessary for its subsequent proteolytic processing into mature MPO subunits.

The efficient oxygen-dependent microbicidal function of human polymorphonuclear neutrophils (PMNs)[1] depends on the activity of myeloperoxidase (MPO; donor H_2O_2 oxidoreductase, EC 1.11.1.7) (1, 2), a heme-containing lysosomal protein present in neutrophils and monocytes (2–12). In the presence of H_2O_2 generated by the NADPH oxidase of stimulated phagocytes, MPO catalyzes the production of hypochlorous acid and other reactive species that are microbicidal and tumoricidal (13, 14). When MPO function is compromised, either by the addition of inhibitors or by using cells deficient in MPO, the killing of bacteria is retarded and that of *Candida* is absent (2, 15–18). Thus MPO has been assigned a central role in oxygen-dependent, PMN-mediated host defense.

Hereditary deficiency of MPO is relatively common, affecting 1 in every 2,000–4,000 individuals (19). We have previously described a series of unrelated individuals with hereditary MPO deficiency whose PMNs lack spectroscopic and enzymatic evidence of functionally active MPO but possess a 90-kDa protein recognized by a monospecific antibody to MPO (20). Based on these studies, we speculated that this form of MPO deficiency results from synthesis of an aberrant MPO precursor, which is incorrectly processed posttranslationally (20). We have recently reported that a single nucleotide missense mutation in exon 10 of the MPO gene is a common genotype underlying MPO deficiency (21). Based on the amino acid sequence of MPO, one would predict that this mutation results in the substitution of tryptophan for arginine at codon 569 (R569W). The impact of this mutation on MPO biosynthesis is unknown.

In studies using K562 cells transfected with cDNA for normal and for mutated MPO, we describe the effects of the R569W mutation on MPO biosynthesis. These studies demonstrate that the R569W missense mutation results in a maturational arrest in MPO processing at the apopro-form of the enzyme. Furthermore, the data suggest that insertion of heme into the peptide backbone of apopro-MPO may be a prerequisite for proteolytic maturation of pro-MPO.

EXPERIMENTAL PROCEDURES

Reagents—Restriction endonucleases and buffers, specific primers for the polymerase chain reaction, and *Taq*I polymerase were obtained from the DNA Core Facility (University of Iowa); reagents for cell culture were obtained from the Hybridoma Facility (University of Iowa); and radioisotopes were obtained from Amersham Life Sciences Products.

Vectors and cDNA Constructs—Full-length cDNA for human MPO (22) was cloned into the *Bam*HI site of pREP10 (Invitrogen, San Diego, CA) for stable expression in mammalian cells. Site-directed mutagenesis using overlap extension with the polymerase chain reaction (24, 25) was employed to generate the R569W mutation using normal cDNA for MPO in pCMV5 (23) as template. Primers used for mutagenesis were the forward primers c_1 (^{1662}ACGGCCGCACCCTCATCCAACCC^{1684}) and p_{17} (^{1860}ATGAGATC**T**GGGAGCGATTGTTT^{1882}) and the reverse primers p_{18} (^{1875}CGCTCCC**A**GATCTCATCCACTGCA^{1852}) and p_{14} (^{2370}AATGCAGGAAGTGTACTGCAGTT^{2348}). The nucleotide altered to produce the desired mutation is printed in bold type. The 686-nucleotide amplicon was digested with *Kpn*I and the resultant 452-nucleotide fragment cloned into pCMV5-MPO, replacing the fragment of normal sequence (nucleotides 1803–2255) excised with *Kpn*I. The inserted region was directly sequenced on both strands using ^{35}S-dATP and the dideoxynucleotide chain termination method (Sequenase, version 2.0, U. S. Biochemical Corp.). The only change in nucleotide sequence produced was the desired mutation (**C**GG → **T**GG) at codon 569. The wild type or mutated cDNA for MPO was excised from pCMV5 and cloned into the *Bam*HI site of pREP10. Plasmids with the correct orientation were expressed in K562 cells.

Transfections—The human cell line K562 (26), was obtained from the American Type Culture Collection (ATCC CCL 243) and maintained in RPMI 1640 with 10% fetal calf serum, 2 mmol/liter glutamine, and

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[1] The abbreviations used are: PMN, polymorphonuclear leukocyte; MPO, myeloperoxidase; pREP-MPO, pREP expression vector into which cDNA for normal MPO is inserted; pREP-R569W, pREP expression vector into which cDNA with the missense mutation at codon 569 is inserted.

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penicillin-streptomycin at 37 °C in an atmosphere of 5% CO_2. Plasmids with normal or mutated cDNA for MPO were transfected into log phase K562 cells by electroporation (27). Cells were washed, resuspended at 16×10^6 cells/ml in serum-free 1640 RPMI with 20 μg of plasmid DNA and 80 μg of carrier DNA in Hepes/NaCl, and electroporated (300 V, 960 microfarads, time constant 16.9–17.3; Bio-Rad). Stable lines (pREP-MPO or pREP-R569W) were selected using 130 μg/ml hygromycin (Calbiochem, San Diego, CA) 48 h after electroporation. Hygromycin-resistant cells were selected after 48–72 h of culture.

Assessment of MPO Activity and Biosynthesis—Peroxidase activity was determined spectrophotometrically, using *o*-dianisidine as substrate (28). Each cell lysate or culture supernatant was assayed in triplicate. Biosynthetic radiolabeling of proteins with [^{35}S]methionine or [^{14}C]δ-aminolevulinic acid was performed, and radiolabeled proteins were immunoprecipitated with rabbit polyclonal antiserum against MPO as described previously (6, 29). Immunoprecipitated proteins were solubilized in sodium dodecyl sulfate and separated by polyacrylamide gel electrophoresis. Gels were soaked in 1 M sodium salicylate (30), dried, and exposed to x-ray film. In some cases specific radiolabeled proteins were quantitated using PhosphorImager SF (Molecular Dynamics, Sunnyvale, CA).

RESULTS AND DISCUSSION

The primary translation product for MPO is a single 80-kDa polypeptide, which undergoes cotranslational, *N*-linked glycosylation to generate a 92-kDa glycoprotein that is processed by glucosidases to produce a relatively long-lived 90-kDa precursor (3–11). The 90-kDa, enzymatically inactive apopro-MPO is converted to the 90-kDa pro-MPO by the insertion of heme. Although the events associated with the conversion of apopro-MPO to a precursor with peroxidase activity have not been completely defined, there is evidence that incorporation of heme is necessary for the proteolytic processing of pro-MPO into the subunits of mature MPO.

Transfection of K562 Cells with Normal MPO cDNA—We selected K562 cells for expression of MPO cDNA because they are of hematopoietic origin (31, 32) and lack MPO (26). The wild type K562 cells electroporated with salmon sperm DNA did not produce MPO and only cells electroporated with cDNA encoding for MPO synthesized a radiolabeled 90–92-kDa protein immunoprecipitated by the monospecific antiserum for MPO (data not shown). However, the relatively low MPO expression in transfected cells precluded use of the transient expression system for our studies. In order to study in greater detail the processing of normal MPO precursors and the effect of the R569W missense mutation on MPO biosynthesis, we established stable transfectants in K562 cells expressing normal and the R569W cDNA for MPO.

Hygromycin-selected transfectants expressing normal MPO (pREP-MPO) synthesized and secreted an MPO precursor (Fig. 1, *panel a*). As previously shown in cells that naturally express the MPO gene (33), tunicamycin resulted in biosynthesis of a nonglycosylated 80-kDa protein that was not secreted (Fig. 1, *panel b*).

K562 cells stably expressing cDNA with the missense mutation at codon 569 (pREP-R569W) were similarly pulse-labeled and chased for 0, 4, and 20 h. As in myeloid cell lines expressing endogenous MPO cDNA (3–11) and in the pREP-MPO line, a single 90-kDa MPO precursor protein was synthesized (Fig. 2). As in the pREP-MPO line, some of the precursor protein was secreted even after a long chase period.

Peroxidase Activity in pREP-MPO and pREP-R569W Cell Lines—During normal MPO biosynthesis, heme is inserted into apopro-MPO, converting it to the enzymatically active precursor pro-MPO (34). In order to assess directly the ability of pREP-MPO and pREP-R569W to synthesize pro-MPO, cell lines were biosynthetically radiolabeled with [^{14}C]δ-aminolevulinic acid, a precursor in heme synthesis. Both cell lines were radiolabeled with [^{35}S]methionine in parallel and immunoprecipitates demonstrated that each cell line synthesized a 90-kDa

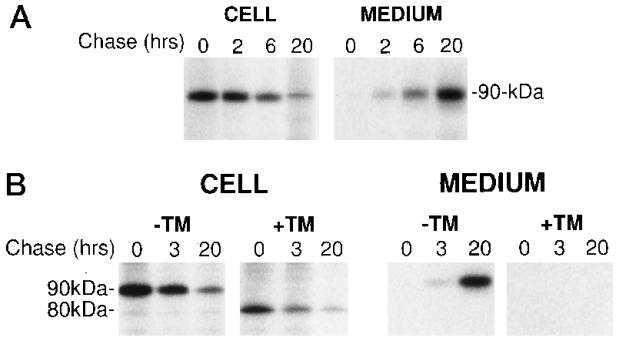

FIG. 1. **Stable expression of MPO-related proteins by pREP-MPO-transfected K562 cells.** Hygromycin-selected K562 cells stably expressing pREP-MPO were pulse-labeled with [^{35}S]methionine and chased for the indicated time periods. At the specific periods of chase, cell lysates and culture media were collected and immunoprecipitated with monospecific antiserum against MPO and the immunoprecipitated proteins separated by SDS-polyacrylamide gel electrophoresis and visualized by autoradiography. *Panel a*, a 90-kDa protein was immunoprecipitated from the cell lysate after the pulse labeling and was gradually secreted into the medium during the chase period. *Panel b*, when pREP-MPO-transfected cells were grown in the presence (*+TM*) of tunicamycin (6.1 μM), the immunoprecipitated protein migrated at 80 kDa, in contrast to the 90-kDa size made in the absence (*−TM*) of tunicamycin. In addition, the nonglycosylated 80-kDa protein was not secreted during the chase period.

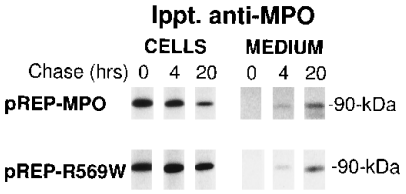

FIG. 2. **Comparison of biosynthesis of MPO-related precursors by stably transfected K562 cells expressing normal (pREP-MPO) and mutated (pREP-R569W) cDNA for MPO.** Transfectants pulse-chase labeled with [^{35}S]methionine were analyzed as described in the legend to Fig. 1. pREP-R569W-transfected cells synthesized a 90-kDa protein identical in size to that made by pREP-MPO cells, cells expressing the normal cDNA for MPO. Similarly, the 90-kDa MPO-related precursor protein was secreted into the culture media of both cell lines over a similar time course.

MPO-related precursor (Fig. 3). However, only pREP-MPO synthesized a 90-kDa MPO-related precursor, which was radiolabeled with [^{14}C]δ-aminolevulinic acid and consistent with pro-MPO (34). In contrast to the immunoprecipitates from pREP-MPO cells, those from pREP-R569W cells lacked protein radiolabeled with [^{14}C]δ-aminolevulinic acid, indicating that these cells did not synthesize pro-MPO. Thus it appears that the R569W mutation results in a maturational arrest in MPO biosynthesis at the apopro-MPO stage.

In order to determine if functional heme was incorporated into pro-MPO synthesized by pREP-MPO, we assayed lysates of wild type K562 cells, pREP-MPO, and pREP-R569W for peroxidase activity (Table I). The parental K562 cells possessed very little peroxidase activity. In contrast, the pREP-MPO cells had significantly more peroxidase activity. Thus it appears that K562 cells transfected with normal cDNA for MPO synthesized enzymatically active MPO-related protein. In contrast, pREP-R569W cells had very little peroxidase activity, similar to that of wild type K562 cells. Based on these data, we conclude that pREP-R569W cells synthesized apopro-MPO but were unable to incorporate heme to create pro-MPO. These findings confirm our hypothesis that patients with the R569W genotype of MPO deficiency lack peroxidase activity in their PMNs because of a posttranslational defect in MPO biosynthesis.

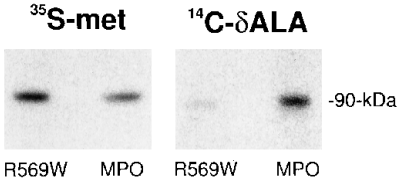

FIG. 3. **Synthesis of pro-MPO by pREP-MPO and by pREP-R569W-transfected K562 cells.** In order to distinguish heme-containing pro-MPO from apopro-MPO, cells expressing pREP-MPO and those expressing pREP-R569W were radiolabeled with [^{14}C]δ-aminolevulinic acid (*^{14}C-δ-ALA*), a precursor of heme, and immunoprecipitated with MPO antiserum. In parallel, cells were radiolabeled with [^{35}S]methionine (*^{35}S-met*) and MPO-related proteins similarly immunoprecipitated. Only pREP-MPO-transfected cells synthesized a heme-containing MPO-related precursor, although both pREP-MPO and pREP-R569W cells synthesized 90-kDa MPO precursor protein.

TABLE I
Peroxidase activities of pREP-MPO and pREP-R569W without and with added hemin (2 μg/ml)

Each sample was assayed for peroxidase activity in triplicate; (*n*) represents the number of samples assayed.

Cell line	No hemin	Hemin added
K562	0.58 ± 0.04 (4)	0.62 ± 0.04 (6)
pREP-MPO	1.57 ± 0.04 (5)[a]	3.54 ± 0.21 (6)[b]
pREP-R569W	0.60 ± 0.02 (4)	0.59 ± 0.01 (6)[c]

[a] *Versus* K562 cells ($p < 0.000001$).
[b] *Versus* K562 cells ($p < 0.000006$); *versus* pREP-MPO cells without hemin ($p = 0.0002$); *versus* pREP-R569W cells with hemin ($p = 0.00003$).
[c] *Versus* K562 cells; not a statistically significant difference ($p = 0.4$).

Proteolytic Processing of MPO Precursors in pREP-MPO and pREP-R569W—Native MPO is a symmetric, heterodimeric protein, each half composed of 59-kDa and 13.5-kDa subunits (33). Studies have suggested that proteolytic processing to the lysosomal form may require incorporation of heme into the precursor (35–37) (*i.e.* when heme incorporation is blocked by succinyl acetone, an inhibitor of heme synthase (38), pro-MPO does not undergo proteolytic processing to the lysosomal form of native MPO).

When pREP-MPO cells were pulse-labeled and chased for 20 h, the 59-kDa heavy subunit of lysosomal MPO was detected within the cells (Fig. 4), demonstrating that K562 cells have the capacity to process pro-MPO into mature protein. On the other hand, pREP-R569W cells pulse-chase-labeled under identical conditions did not generate the subunits of mature MPO. Thus it appeared that K562 cells could synthesize pro-MPO and process it to mature, enzymatically active mature MPO when transfected with cDNA encoding normal MPO. However, the form of apopro-MPO made in pREP-R569W cells could not undergo proteolytic processing to mature MPO.

Proteolytic processing of pro-MPO to MPO in pREP-MPO cells was increased by the inclusion of hemin (2 μg/ml) in the culture medium (Fig. 5). After 20 h of chase in the presence of added hemin, pREP-MPO cells contained 62% of MPO precursor and 257% of the 59-kDa mature subunit in comparison to pREP-MPO cells when exogenous hemin was omitted (Table II). Thus the presence of added hemin resulted in more complete processing of the 90-kDa MPO precursor to mature MPO.

In contrast to pREP-MPO, pREP-R569W cells synthesized a 90-kDa precursor for MPO, which did not incorporate heme (Fig. 3) and did not undergo proteolytic processing into the subunits of mature MPO (Fig. 5). Lysates of pREP-R569W lacked peroxidase activity, suggesting that the precursor synthesized was the apo-form of the enzyme. Neither enzymatic activity (Table I) nor proteolytic processing (Table II) of pREP-R569W was influenced by the addition of hemin to the culture.

In summary, these studies make three important points.

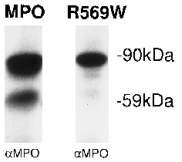

FIG. 4. **Proteolytic processing of the MPO precursor to mature MPO in pREP-MPO- and pREP-R569W-transfected K562 cells.** Stable cell lines expressing pREP-MPO (*MPO*) or pREP-R569W (*R569W*) were pulse-labeled, chased for 20 h, and the cell lysates immunoprecipitated with antisera to MPO (*αMPO*). As in myeloid cells expressing endogenous cDNA for MPO, the pREP-MPO-transfected cells synthesized and processed the 90-kDa MPO precursor into mature, lysosomal MPO, containing a heavy subunit of 59 kDa. In contrast, pREP-R569W-transfected cells failed to process the MPO precursor into the subunits of mature MPO.

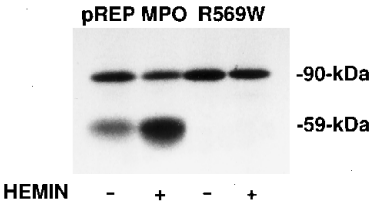

FIG. 5. **Effect of exogenous hemin on proteolytic processing of the MPO precursor to mature MPO.** Cells transfected with pREP-MPO or pREP-R569W were pulse-labeled and chased for 20 h in the absence (−) or presence (+) of added hemin (2 μg/ml). The proteolytic processing of the MPO precursor to mature MPO (represented by the 59-kDa heavy subunit) was augmented 2.57-fold by the addition of hemin. In contrast, the defect in proteolytic processing seen in the cells expressing pREP-R569W persisted even in the presence of added hemin.

TABLE II
Effects of hemin (2 μg/ml) on processing of MPO precursors to mature MPO in pREP-MPO and pREP-R569W stable transfectants

Phosphorimager counts for immunoreactive proteins precipitated from stable transfectants were normalized for the values obtained in the absence of hemin. The mean values of three separate experiments are shown. Faint signals in the 59-kDa region of immunoprecipitates from R569W were not included in the analysis.

	pREP-MPO		pREP-R569W	
	90-kDa	59-kDa	90-kDa	59-kDa
− Hemin	1.00	1.00	1.00	
+ Hemin	0.62	2.57	1.01	

First, studies using the pREP-R569W cell line demonstrate that the R569W missense mutation resulted in a "maturation arrest" in the processing of MPO precursors at the stage of apopro-MPO. Presumably an identical mutant apopro-MPO is the immunoreactive 90-kDa protein previously identified in the neutrophils of subjects with hereditary MPO deficiency and the R569W genotype (20, 21). Thus these findings confirm our hypothesis that the R569W missense mutation results in a defect in the posttranslational processing of MPO (20).

Second, the K562 cells stably transfected with the cDNA for normal MPO demonstrated many of the features seen during the biosynthesis of MPO in myeloid cells. The pREP-MPO cells synthesized apopro-MPO, incorporated heme to make pro-MPO and processed pro-MPO into the mature, lysosomal form of the protein. This is in contrast to previous studies using Chinese hamster ovary cells (39–41), baby hamster kidney cells (42), or baculovirus-infected *Sf9* cells (43)[2] to express MPO cDNA. In none of these systems were all three species, namely apopro-MPO, pro-MPO, and mature active MPO, produced. Thus the K562 cell line provides a mammalian cell line of hematopoietic origin suitable for examination of the biosynthesis of heme-

[2] W. M. Nauseef, unpublished data.

containing myeloid proteins.

Third, in addition to the implications of these findings to understanding the biochemical basis of one of the genotypes underlying hereditary MPO deficiency, our studies using the stable K562 transfectants support and extend previous suggestions that heme insertion is necessary for proteolytic processing of pro-MPO into the subunits of mature MPO (35–37). Exposure of promyelocytic cells to succinyl acetone, an inhibitor of heme synthesis, blocks proteolytic processing of MPO precursors to mature MPO and this inhibition is reversed by inclusion of hemin in the culture medium. Pinnix *et al.* (37) demonstrated that succinyl acetone did not alter mRNA for MPO in treated cells and speculated that heme was essential for the maturation of MPO precursors in the endoplasmic reticulum. In support of that conclusion, pREP-MPO cells possessed more peroxidase activity and synthesized more mature MPO in the presence of hemin. The pREP-R569W cell line, which was unable to incorporate heme and could synthesize only apopro-MPO, was unable to process proteolytically the MPO precursor into the subunits of mature MPO.

Taken together, data from the studies presented provide an experimental framework for characterization of additional features of normal synthesis, processing, and lysosomal targeting of MPO. It is clear that there is molecular heterogeneity underlying MPO deficiency (44), and this system may be useful for identifying specific events in MPO expression which are abnormal in other genotypes of the disorder, including pretranslational (45) as well as posttranslational defects (21, 46). On a larger scale, this expression system may be applicable for the study of biosynthesis of other heme-containing proteins.

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REFERENCES

1. Klebanoff, S. J. (1970) *Science* **169,** 1095–1097
2. Klebanoff, S. J. (1991) in *Peroxidases in Chemistry and Biology* (Everse, J., Everse, K., and Grisham, M., eds) pp. 1–36, CRC Press, Boca Raton, FL
3. Hasilik, A., Pohlmann, R., Olsen, R. L., and Von Figura, K. (1984) *EMBO J.* **3,** 2671–2676
4. Koeffler, H. P., Ranyard, J., and Pertcheck, M. (1985) *Blood* **65,** 484–491
5. Yamada, M., and Kurahashi, K. (1984) *J. Biol. Chem.* **259,** 3021–3025
6. Nauseef, W. M. (1986) *Blood* **67,** 865–872
7. Strömberg, K., Persson, A., and Olsson, I. (1986) *Eur. J. Cell Biol.* **39,** 424–431
8. Nauseef, W. M. (1987) *Blood* **70,** 1143–1150
9. Akin, D. T., and Kinkade, J. M., Jr. (1987) *Arch. Biochem. Biophys.* **255,** 428–436
10. Akin, D. T., and Kinkade, J. M., Jr. (1986) *J. Biol. Chem.* **261,** 8370–8375
11. Olsson, I., Persson, A., and Strömberg, K. (1984) *Biochem. J.* **223,** 911–920
12. Bainton, D. F., Ullyot, J. L., and Farquhar, M. G. (1971) *J. Exp. Med.* **134,** 907–934
13. Albrich, J. M., McCarthy, C. A., and Hurst, J. K. (1981) *Proc. Natl. Acad. Sci. U. S. A.* **78,** 210–214
14. Hurst, J. K. (1991) in *Peroxidases in Chemistry and Biology* (Everse, J., Everse, K. E., and Grisham, M. B., eds) pp. 37–62, CRC Press, Boca Raton, FL
15. Kitahara, M., Eyre, H. J., Simonian, Y., Atkin, C. L., and Hasstedt, S. J. (1981) *Blood* **57,** 888–893
16. Bos, A. J., Weening, R. S., Hamers, M. N., Wever, R., Behrendt, H., and Roos, D. (1982) *J. Lab. Clin. Med.* **99,** 589–600
17. Parry, M. F., Root, R. K., Metcalf, J. A., Delaney, K. K., Kaplow, L. S., and Richar, N. J. (1981) *Ann. Intern. Med.* **95,** 293–301
18. Lehrer, R. I., and Cline, M. J. (1969) *J. Clin. Invest.* **48,** 1478–1488
19. Nauseef, W. M. (1990) *Hematol. Pathol.* **4,** 165–178
20. Nauseef, W. M., Root, R. K., and Malech, H. L. (1983) *J. Clin. Invest.* **71,** 1297–1307
21. Nauseef, W. M., Brigham, S., and Cogley, M. (1994) *J. Biol. Chem.* **269,** 1212–1216
22. Johnson, K. R., Nauseef, W. M., Care, A., Wheelock, M. J., Shane, S., Hudson, S., Koeffler, H. P., Selsted, M., Miller, C., and Rovera, G. (1987) *Nucleic Acids Res.* **15,** 2013–2028
23. Stinski, M. F., and Roehr, T. J. (1985) *J. Virol.* **55,** 431–441
24. Ho, S. N., Hunt, H. D., Horton, R. M., Pullen, J. K., and Pease, L. R. (1989) *Gene (Amst.)* **77,** 51–59
25. Horton, R. M., Hunt, H. D., Ho, S. N., Pullen, J. K., and Pease, L. R. (1989) *Gene (Amst.)* **77,** 61–68
26. Lozzio, B. B., and Lozzio, C. B. (1979) *Leuk. Res.* **3,** 363–370
27. Ulrich, M. J., and Ley, T. J. (1990) *Blood* **75,** 990–999
28. Nauseef, W. M., and Clark, R. A. (1986) *Blood* **68,** 442–449
29. Nauseef, W. M., McCormick, S. J., and Clark, R. A. (1995) *J. Biol. Chem.* **270,** 4741–4747
30. Chamberlain, J. P. (1979) *Anal. Biochem.* **98,** 132–135
31. Marie, J. P., Izaguirre, C. A., Civin, C. I., Mirro, J., and McCulloch, E. A. (1981) *Blood* **58,** 708–711
32. Lubbert, M., Herrmann, F., and Koeffler, H. P. (1991) *Blood* **77,** 909–924
33. Johnson, K. R., and Nauseef, W. M. (1991) in *Peroxidases in Chemistry and Biology* (Everse, J., Everse, K., and Grisham, M., eds) pp. 63–82, CRC Press, Boca Raton, FL
34. Arnljots, K., and Olsson, I. (1987) *J. Biol. Chem.* **262,** 10430–10433
35. Nauseef, W. M., McCormick, S., and Yi, H. (1992) *Blood* **80,** 2622–2633
36. Castañeda, V. L., Parmley, R. T., Pinnix, I. B., Raju, S. G., Guzman, G. S., and Kinkade, J. M., Jr. (1992) *Exp. Hematol.* **20,** 916–924
37. Pinnix, I. B., Guzman, G. S., Bonkovsky, H. L., Zaki, S. R., and Kinkade, J. M., Jr. (1994) *Arch. Biochem. Biophys.* **312,** 447–458
38. Ebert, P. S., Hess, R. A., Frykholm, B. C., and Tschudy, D. P. (1979) *Biochem. Biophys. Res. Commun.* **88,** 1382–1390
39. Moguilevsky, N., Garcia-Quintana, L., Jacquet, A., Tournay, C., Fabry, L., Piérard, L., and Bollen, A. (1991) *Eur. J. Biochem.* **197,** 605–614
40. Jacquet, A., Deleersnyder, V., Garcia-Quintana, L., Bollen, A., and Moguilevsky, N. (1992) *FEBS Lett.* **302,** 189–191
41. Jacquet, A., Deby, C., Mathy, M., Moguilevsky, N., Deby-Dupont, G., Thirion, A., Goormaghtigh, E., Garcia-Quintana, L., Bollen, A., and Pincemail, J. (1991) *Arch. Biochem. Biophys.* **291,** 132–138
42. Cully, J., Harrach, B., Hauser, H., Harth, N., Robenek, H., Nagata, S., and Hasilik, A. (1989) *Exp. Cell Res.* **180,** 440–450
43. Taylor, K. L., Uhlinger, D. J., and Kinkade, J. M., Jr. (1992) *Biochem. Biophys. Res. Commun.* **187,** 1572–1578
44. Selsted, M. E., Miller, C. W., Novotny, M. J., Morris, W. L., and Koeffler, H. P. (1993) *Blood* **82,** 1317–1322
45. Tobler, A., Selsted, M. E., Miller, C. W., Johnson, K. R., Novotny, M. J., Rovera, G., and Koeffler, H. P. (1989) *Blood* **73,** 1980–1986
46. Kizaki, M., Miller, C. W., Selsted, M. E., and Koeffler, H. P. (1994) *Blood* **83,** 1935–1940