Molecular Cloning of the Human Eosinophil Peroxidase
Evidence for the Existence of a Peroxidase Multigene Family

By R. M. Ten, L. R. Pease, D. J. McKean, M. P. Bell, and G. J. Gleich

From the Department of Immunology, Mayo Clinic, and Mayo Foundation, Rochester, Minnesota 55905

The cytoplasmic granules of eosinophils are composed of a crystalloid core surrounded by a matrix and contain the proteins responsible for the effect of these cells in helminth infection and hypersensitivity reactions (1). Eosinophil peroxidase (EPO), an abundant protein in the matrix of the eosinophil granule (1), when combined with H₂O₂ and halide, is a potent toxin for parasites (2) and mammalian cells (3), as well as a mediator of several hypersensitivity mechanisms (4–6). EPO also kills helminths and cells in the absence of H₂O₂, indicating that the molecule itself is a toxin (6, 7). EPO is a heme-containing protein (8), composed of 14,000- and 58,000-dalton subunits (9) that presumably are translated from the same mRNA into a larger precursor that is subsequently cleaved (10). Although its biologic activities are well established, the structure, amino acid composition, and nucleotide sequence of EPO are unknown.

Here, we report the purification and partial amino acid sequence of EPO subunits. This information was used to isolate and determine the nucleotide sequence of a cDNA clone representing human EPO mRNA from a cDNA library of eosinophils from induced human umbilical cord mononuclear cells (MNC). The comparison of the nucleotide and the predicted amino acid sequences of EPO to those of other reported peroxidases suggests the existence of a peroxidase multigene family.

Materials and Methods

EPO Purification and Molecular Weight Determination of the Subunits. Eosinophils were obtained by cytopheresis of the peripheral blood of patients with hypereosinophilic syndrome. EPO was purified from eosinophil granules on Sephadex G-50 and CM-Sepharose columns (Pharmacia Fine Chemicals, Piscataway, NJ), as previously described (11), and the 415/280 nm ratio of the peak fraction from the CM-Sepharose column was 0.9. Peroxidase activity of the purified EPO was determined by measuring A₄₈₅ nm of the reaction of EPO with H₂O₂ and D-phenylenediamine (data not shown). To purify EPO subunits, it was dissolved in 6 M guanidine hydrochloride (Whittaker M. A. Bioproducts, Walkersville, MD) at a final concentration of 2 M guanidine hydrochloride.
centration of 1%, reduced with 0.01 M dithiothreitol, carboxymethylated with 0.02 M iodoacetic acid, and applied to a Sepharose CL 6B column (Pharmacia Fine Chemicals) that had been equilibrated with 6 M guanidine hydrochloride, as described previously (12). Fractions corresponding to molecular weights of the heavy or the light subunits were desalted on a G-15 column (Pharmacia Fine Chemicals) by elution with 0.5 M acetic acid. The fractions corresponding to each EPO subunit peak were pooled, concentrated in a YM-10 filter (Amicon Corp., Danvers, MA), and stored at −20°C.

**Partial Amino Acid Sequence Analysis.** The amino acid sequence of the NH2 terminus of each chain was determined by subjecting the peptides to Edman degradation using a protein sequenator (890D; Beckman Instruments Inc., Palo Alto, CA), and the resulting derivatives were identified by reverse-phase HPLC (13).

**Construction of EPO Probes.** Mixtures of 17-base oligonucleotides of 64–192 degeneracies were synthesized from the determined amino acid sequence in a DNA synthesizer (380A; Applied Biosystems, Inc., Foster City, CA), by using the phosphoramidite method (14). Oligonucleotides were purified on G-50 Sepharose columns and stored at −20°C. When ready for use, the oligomers were 5’ labeled with T4 polynucleotide kinase and γ-[32P]ATP (E. I. du Pont de Nemours & Co., Inc., Boston, MA).

**Cell Cultures.** Human umbilical cord blood (up to 100 ml) was collected in heparinized flasks immediately after delivery. The MNC (10^7–2 × 10^8 cells/donor) were separated by centrifugation on Histopaque 1077 (Sigma Chemical Co., St. Louis, MO) and cultured in RPMI 1640 media (Gibco Laboratories, Grand Island, NY), containing 10% calf serum (HyClone Laboratories, Logan, UT), 50 μM 2-ME (Sigma Chemical Co.), 2 mM L-glutamine (Gibco Laboratories), 100 U/ml penicillin, 0.1 mg/ml streptomycin (Sigma Chemical Co.), and 10% T cell supernatant (Electronucleonics, Silver Spring, MD), from cells stimulated with PHA as described before (15). Cells were cultured at a density of 2 × 10^6/ml at 37°C, in humidified incubators with 5% CO₂, for 4 wk and half of the media was replaced weekly. The protocol for obtaining umbilical cord blood was approved by the Mayo Foundation Institutional Review Board.

**EPO Staining.** Cord blood cell cultures were monitored for the expression of cyanide-resistant peroxidase by a modification of a previously described method (16). Briefly, cytospin preparations were fixed in formalin-acetone for 30 s and stained for 10 min in phosphate buffer containing 75 mg 3,3′diaminobenzidine tetrahydrochloride (Sigma Chemical Co.), 0.3 ml 3% H₂O₂, and 39.2 mg NaCN. Slides were counterstained in hematoxylin (Sigma Chemical Co.) and mounted with permount (Fisher Scientific Co., Pittsburgh, PA).

**Construction of the cDNA Library from Cord Blood Cells.** Cells (10^6) were lysed on day 7–8 of culture, and the total cellular RNA was isolated by the guanidine-isothiocyanate/CsCl₂ method (International Biotechnologies Inc., New Haven, CT and Boehringer Mannheim Biochemicals, Indianapolis, IN) (17). Poly(A)^+ RNA was purified by oligo(dT)-cellulose (Collaborative Research, Lexington, MA) column chromatography (18). The integrity of the mRNA was tested by in vitro translation in rabbit erythrocyte lysates (19). A custom cDNA library was constructed in the λ-zap vector (Stratagene, La Jolla, CA) as described (20).

**Screening of the cDNA Library and DNA Sequence Analysis.** Independent recombinant clones (8 × 10^3) in the cord blood cell library were screened by plaque hybridization using the ^32P-labeled oligonucleotides as probes (21). One clone hybridizing to the EPO probes was isolated from the cord blood cell library (22). This clone was digested with Eco RI and Sau 3A (International Biotechnologies Inc.), subjected to electrophoresis in a 1% agarose gel, and blotted by standard procedures (23) on a gene screen membrane. The membrane was hybridized with the three ^32P-labeled mixed EPO probes and with a mixed probe made from the amino acid sequence of the eosinophil cationic protein (24), and autoradiographed.

Restriction fragments of the clone were subcloned into single-stranded M13mp10 (25). Both strands were sequenced by the dideoxynucleotide chain termination method either from the subcloned fragments or directly from the double-stranded plasmid excised from the λ-zap vector (22, 26). The complete sequence was compared against other peroxidases in the GeneBank™ database. The DNAstar computer program (DNAstar, Madison, WI) was used for the nucleotide and protein analyses and for the sequence comparison.
Results

Purification of EPO Polypeptide Chains and Their Partial Amino Acid Sequence. EPO was purified from the eosinophil granules of patients with hypereosinophilic syndrome. After reduction and carboxymethylation, the molecular weight of the two EPO subunits was determined by gel filtration (Fig. 1). The results of eight experiments gave a molecular mass for the H and L chains of 57,000 (± 4,200) and 11,000 (± 1,500) daltons, respectively. The NH₂-terminal 38 and 50 amino acids of the H and L chains, respectively, were determined (Fig. 2) and three degenerate 17 mer oligonucleotide probes (one for the H and two for the L chain) were synthesized from the most unique parts of the molecule, as determined by comparison to sequences in the PIR™ database.

![Figure 1](image1.png)

**Figure 1.** Gel filtration of EPO on Sepharose 4B equilibrated with 6 M guanidine hydrochloride. Peaks 1 and 4 are the molecular weight markers, blue dextran (M, 2 x 10⁶) and DNP-lysine (M, 248), respectively; peaks 2 and 3 correspond to the EPO H and L chain, respectively.

![Figure 2](image2.png)

**Figure 2.** Amino acid sequence of the NH₂ terminus of the EPO H (EPO-H) and L (EPO-L) chains in the one-letter code, as determined by amino acid sequence analysis. When more than one amino acid was possible at one position, it is indicated with more than one letter separated by slashes. Every 10th amino acid is numbered above the corresponding letter, starting from the NH₂ terminus. Amino acid 24 in the EPO-L is boxed. Regions chosen for the construction of the oligonucleotides are underlined.
Figure 3. Continued on following page.
Construction of a cDNA Library from Human-induced Cord Blood MNC. Attempts to identify EPO cDNA clones in a HL-60 cDNA library were unsuccessful and we were also not able to identify any other cell line consistently producing EPO. Therefore, MNC from the umbilical cord blood of five donors were cultured for 4 wk in the presence of the T cell supernatant. A sample of each culture was stained for cyanide-resistant peroxidase (specific for EPO) every 3-10 d to monitor the differentiation of cord blood cell precursors to eosinophils (15) (Fig. 3). As shown in Fig. 4, the cells became positive for EPO after 4-6 d in culture, and the percentage of positive...

**Figure 3.** Cyanide-resistant peroxidase staining of cord blood MNC. (a) Uninduced (original magnification, 400); (b) induced with T cell supernatant for 25 d (x 400); and (c) same as in B (x 1,000).

**Figure 4.** Induction of umbilical cord blood MNC by T cell supernatant. Each line represents the results of a single culture. The x-axis represents the days in culture, and the y-axis represents the percent of cells that stained positive for cyanide-resistant peroxidase.
cells increased up to 80%, after 4 wk. Cells (10^9) were pooled from the five cultures and RNA was isolated.

A cDNA library was constructed from poly(A)^+ RNA and 8 x 10^5 independent recombinant clones were screened by hybridization procedures with the three oligonucleotide probes described above. A single clone was found to hybridize with the three probes in both plaque hybridization and Southern blot analysis (Fig. 5).

Nucleotide Sequence of EPO cDNA. After restriction of the cDNA clone with Eco RI, a 2.5-kB insert was subcloned in M13mp10 and the DNA sequence of both strands was determined. As shown in Fig. 6, the cDNA was composed of 2,558 nucleotides, 2,106 of which were in an open reading frame. By comparison to the known partial EPO subunit amino acid sequences, the cDNA nucleotide sequence was divided in a 381-bp prosequence, a 333-bp sequence corresponding to the coding region of the EPO-L chain, a 1,392-bp sequence that codes for the EPO-H chain, and a 452-bp untranslated region at the 3' end containing the AATAAA polyadenylation signal. The partial amino acid sequence (Fig. 2) was identical to the amino acid sequence predicted from the nucleotide sequence of the EPO clone, except for the amino acid 24 of the L chain, which was cysteine instead of serine.

The molecular mass of the precursor protein, calculated from the predicted amino acid sequence, was 79,551 daltons, with an isoelectric point of 10.22. The L and H chains correspond to 12,712- and 53,011-dalton proteins, with isoelectric points of 10.8 and 10.7, respectively.

Comparison of the EPO nucleotide sequence to other peroxidases (Table I) revealed a 69.5% overall similarity index to the neutrophil myeloperoxidase (MPO), with 76% similarity of the H chains and 73% of the L chains. The similarity to other peroxidases was 40-60%. Comparison of EPO amino acid sequence to other peroxidases revealed that EPO and MPO have 68.3% identity, being 69.4% and 70% identical at the L and H chains, respectively (27) (Table II). Thyroid peroxidase (TPO) is the second most similar peroxidase to EPO (28) (Tables I and II). To determine whether or not a conserved sequence suggestive of active site exists in all peroxidases, the predicted amino acid sequences of EPO, MPO, and human TPO were aligned (Fig. 7). The homology was striking throughout the entire molecules, especially at the L and H chains. Therefore, no conclusions can be drawn about active sites. Comparison with the amino acid sequence of other peroxidases gave very poor alignment (not shown) indicating no significant homology (Table I).
TEN ET AL.

Table I

| Source | Peroxidase                          | Similarity index |
|--------|-------------------------------------|------------------|
|        |                                     | Nucleotide  | Amino acid |
|        |                                     | %            | %          |
| Human  | Myeloperoxidase (27)*               | 69.5        | 68.3 (109) |
| Porcine| Thyroid peroxidase (29)             | 60.4        | 43.6 (13)  |
| Human  | Thyroid peroxidase (28)             | 59          | 43.5 (67)  |
| Human  | Glutathione peroxidase (30)         | 51.3        | 36.4 (0.6) |
| Chryso sporium | Ligninase (31)         | 49.7        | 18 (0.6)   |
| Nicotiana tabacum | Lignin-forming peroxidase (32) | 40.3        | 18.5 (0.5) |
| Caldariomyces | Chloroperoxidase (33)     | 15          | 16.4 (0.4) |

* Reference to the nucleotide sequences of the corresponding peroxidase.
1 Z values calculated by the Lipman-Pearson method (34); values >10 are considered significant.
2 Insufficient homology for alignment.

Table II

| Area of the molecule | Nucleotide similarity index MPO | Amino acid similarity index MPO |
|----------------------|---------------------------------|---------------------------------|
|                      | MPO    | hTPO | MPO    | hTPO |
|                      | %      | %    | %      | %    |
| Total                | 69.5   | 59   | 68.3   | 43.5 |
| L chain              | 73     | 65.4 | 69.4   | 51.4 |
| H chain              | 75.8   | 58.7 | 70     | 45.1 |
| UTR†                 | 44.8   | 50.7 |        |      |

* References for the nucleotide sequences of MPO and hTPO are 27 and 28, respectively.
† UTR = 3' untranslated region.

Discussion

We have identified a cDNA clone corresponding to the mRNA of the human EPO. The sequence of the clone confirms the existence of a unique mRNA that codes for a large precursor that contains both the L and the H chains (10). Furthermore, this mRNA also contains a prosequence at the 5' end that codes for a peptide similar to the prosequence of the neutrophil MPO (27). However, a typical leader sequence was not included in the clone and we were unable to do complete comparisons of the EPO and MPO preprosequence. Comparison of the EPO sequence with other peroxidases shows a striking homology, both at the nucleotide and the amino acid level, suggesting the existence of a peroxidase multigene family that evolved by gene duplication. The study of EPO and other eosinophil granule proteins has been hampered by the difficulty in obtaining sufficient numbers of eosinophils, because these cells exist in the blood in very low numbers, and patients with eosinophilia are not
Figure 6. Continued on following page.
FIGURE 6. Nucleotide sequence of the EPO clone from the cord blood cell cDNA library. The predicted amino acid sequence is displayed below the corresponding nucleotides in the one letter code. The NH2-terminal amino acids of the L and H subunits are underlined. Numbers indicate the nucleotide position. These sequence data have been submitted to the EMBL/GenBank Data Libraries under the accession number X14346.
The promyelocytic leukemia cell line, HL-60, expresses several eosinophil granule proteins (35), but we were unable to find a clone corresponding to EPO in a cDNA library from uninduced HL-60 cells. The establishment of a method to induce the differentiation of eosinophils from umbilical cord blood MNC allowed us to obtain a sufficient number of cells to construct a cDNA library that presumably contains the sequences that code for eosinophil products. This library has been helpful to isolate not only a cDNA clone corresponding to EPO, but also another clone that corresponds to the eosinophil cationic protein (manuscript in preparation), and can be further used for the study of other eosinophil products.

The cDNA library was constructed from human umbilical cord leukocytes induced by a PHA-A-stimulated T cell supernatant. This conditioned media was depleted of IL-2, to avoid the induction of lymphocyte differentiation, and presumably contains IL-5, shown to induce eosinophil differentiation from bone marrow and umbilical cord blood cells (15). The mRNA for the construction of the library was obtained after the cells began to express granules that stained for cyanide-resistant peroxidase, assuming that the level of mRNA would be maximum at that point. The predicted amino acid sequence of EPO was identical at the NH₂ terminus of the subunits to the sequence obtained by amino acid sequence analysis, except for one amino acid in the L chain. The codon corresponding to that amino acid differed in a single base in both sequences (TCC vs. TGC). This amino acid sequence difference suggests the possibility of peroxidase polymorphism among different individuals.
The predicted amino acid sequence of EPO shows a 79,551-dalton precursor protein containing a preprosequence at the NH₂-terminus end that presumably is cleaved to give rise to a 65,723-dalton molecule that is further cleaved into 12,712- and 53,011-dalton subunits. These predicted molecular masses agree with the results obtained by gel chromatography by us and others (9), suggesting that the mature molecule is not glycosylated even though there are N-linked oligosaccharide acceptor sites in the H chain.

EPO and MPO have similar nucleotide and amino acid sequences and also show similar protein subunit organization. These results suggest that they have similar functions in eosinophils and neutrophils during inflammatory reactions. The study of the molecular biology of EPO should be helpful in understanding the complete and partial EPO deficiencies reported in some populations (36, 37).

Summary

Human eosinophil peroxidase (EPO) was purified from eosinophil granules derived from the peripheral blood of patients with eosinophilia. The molecular mass of the H and L subunits was determined by gel filtration to be 57,000 and 11,000 daltons, respectively. The partial amino acid sequences of both subunits were used to construct oligonucleotides for the screening of several cDNA libraries, including one derived from human-induced umbilical cord mononuclear cells. A cDNA clone was isolated corresponding to EPO. The nucleotide sequence revealed an open reading frame of 2,106 bp, corresponding to a prosequence, L chain, and H chain, in this order. Comparison of the EPO nucleotide sequence with other peroxidases, such as myeloperoxidase, suggests the existence of a multigene family.

We thank Dr. E. Weiben for his help in the RNA in vitro translation and his valuable comments throughout all this work; Dr. T. Ishizaka for her help in the cell cultures; R. Horton, Dr. K. Hamann, D. Loegering, Dr. R. Barker, and Dr. D. Singer for helpful discussion; and L. Arneson for the skillful preparation of the manuscript.

Received for publication 12 December 1988 and in revised form 12 January 1989.

References

1. Gleich, G. J., and C. R. Adolphson. 1986. The eosinophilic leukocyte: structure and function. Adv. Immunol. 39:177.
2. Jong, E. C., A. A. Mahmoud, and S. J. Klebanoff. 1981. Peroxidase-mediated toxicity to schistosomula of Schistosoma mansoni. J. Immunol. 126:468.
3. Jong, E. C., and S. J. Klebanoff. 1980. Eosinophil mediated mammalian tumor cell cytotoxicity: role of the peroxidase system. J. Immunol. 124:1949.
4. Henderson, W. R., E. Y. Chi, and S. J. Klebanoff. 1980. Eosinophil peroxidase-induced mast cell secretion. J. Exp. Med. 152:265.
5. Goetzl, E. J. 1982. The conversion of leukotriene C4 to isomers of leukotriene B4 by human eosinophil peroxidase. Biochem. Biophys. Res. Commun. 106:270.
6. Motojima, S., E. Frigas, D. A. Loegering, and G. J. Gleich. 1989. Toxicity of eosinophil cationic proteins for guinea pig tracheal epithelium in vitro. Am. Rev. Respir. Dis. 139:801.
7. Hamann, K. J., R. L. Barker, J. L. Checkel, J. W. McCall, and G. J. Gleich. 1987. Eosinophil peroxidase-mediated toxicity to microfilariae of Brugia pahangi and Brugia malayi. Fed. Proc. 46:615.
8. Wever, R., M. N. Hamers, R. S. Weening, and R. Roos. 1980. Characterization of the peroxidase in human eosinophils. *Eur. J. Biochem.* 108:491.

9. Ben, G., J. M. Bolscher, H. Plat, and R. Wever. 1984. Some properties of human eosinophil peroxidase, a comparison with other peroxidases. *Biochim. Biophys. Acta.* 784:177.

10. Olsson, I., A.-M. Persson, K. Stromberg, I. Winqvist, P.-C. Tai, and C. J. F. Spry. 1985. Purification of eosinophil peroxidase and studies of biosynthesis and processing in human marrow cells. *Blood.* 66:1143.

11. Carlson, M. G. Ch., C. G. B. Peterson, and P. Venge. 1985. Human eosinophil peroxidase: purification and characterization. *J. Immunol.* 134:1875.

12. Fish, W. W., K. G. Mann, and C. Tanford. 1969. The estimation of polypeptide chain molecular weights by gel filtration in 6M guanidine hydrochloride. *J. Biol. Chem.* 244:4989.

13. Wasmoen, T. L., M. P. Bell, D. A. Loehering, G. J. Gleich, F. G. Prendergast, and D. J. McKean. 1988. Biochemical and amino acid sequence analysis of human eosinophil granule major basic protein. *J. Biol. Chem.* 263:12559.

14. Matteucci, M. D., and M. H. Caruthers. 1981. Synthesis of deoxyoligonucleotides on a polymer support. *J. Am. Chem. Soc.* 103:3185.

15. Saito, H., H. Kiyohiko, A. M. Dvorak, K. M. Leiferma, A. D. Donnenberg, N. Arai, K. Ishizaka, and T. Ishizaka. 1988. Selective differentiation and proliferation of hematopoietic cells induced by recombinant human interleukins. *Proc. Natl. Acad. Sci. USA.* 85:2288.

16. Zucker-Franklin, D., and G. Grusky. 1976. The identification of eosinophil colonies in soft-agar cultures by differential staining for peroxidase. *J. Histochem. Cytochem.* 24:1270.

17. Chirgwin, J., A. Przybyla, R. MacDonald, and W. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry.* 18:5294.

18. Aviv, H., and P. Leder. 1972. Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. *Proc. Natl. Acad. Sci. USA.* 69:1408.

19. Jackson, R. J., and T. Hunt. 1983. Preparation and use of nuclease-treated rabbit reticuloocyte lysates for the translation of eukaryotic messenger RNA. *Methods Enzymol.* 96:50.

20. Short, J. M., J. M. Fernandez, J. A. Sorge, and W. D. Huse. 1988. Lambda ZAP: a bacteriophage lambda expression vector with in vivo excision properties. *Nucleic Acids Res.* 16:7583.

21. Benton, W. D., and R. W. Davis. 1977. Screening lambda-gt recombinant clones by hybridization to single plaques in situ. *Science (Wash. DC).* 196:190.

22. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 382-387.

23. Gleich, G. J., D. A. Loehering, M. P. Bell, J. L. Checkel, S. J. Ackerman, and D. J. McKean. 1986. Biochemical and functional similarities between human eosinophil cationic protein: homology with ribonuclease. *Proc. Natl. Acad. Sci. USA.* 83:3146.

24. Messing, J. 1983. New M13 vectors for cloning. *Methods Enzymol.* 101:10.

25. Sanger, F., S. Nickelen, and A. R. Coulson. 1977. DNA sequencing with chain termination inhibitors. *Proc. Natl. Acad. Sci. USA.* 74:5463.

26. Johnson, K. R., W. M. Naussef, A. Care, M. J. Wheelock, S. Shane, S. Hudson, H. P. Kocher, M. Selsted, C. Miller, and G. Rovera. 1987. Characterization of cDNA clones for human myeloperoxidase: predicted amino acid sequence and evidence for multiple mRNA species. *Nucleic Acids Res.* 15:2013.

27. Kimura, S., T. Kotani, O. W. McBride, K. Umeki, K. Hirai, T. Nakayama, and S. Ohtaki. 1987. Human thyroid peroxidase: complete cDNA and protein sequence, chromosome mapping and identification of two alternatively spliced mRNAs. *Proc. Natl. Acad. Sci. USA.* 88:5555.
29. Magnusson, R. P., J. Gestautas, A. Taurog, and B. Rapoport. 1987. Molecular cloning of the structural gene for porcine thyroid peroxidase. *J. Biol. Chem.* 262:13885.

30. Sukenaga, Y., K. Ishida, T. Takeda, and K. Takagi. 1987. cDNA sequence coding for human glutathione peroxidase. *Nucleic Acids Res.* 15:7178.

31. Tien, M., and C.-P. Tu. 1987. Cloning and sequencing of a cDNA for a ligninase from phanerochaete chrysosporium. *Nature (Lond.)* 326:520.

32. Lagrimini, L. M., W. Burkhart, M. Moyer, and S. Rothstein. 1987. Molecular cloning of complementary DNA encoding the lignin-forming peroxidase from tobacco: molecular analysis and tissue-specific expression. *Proc. Natl. Acad. Sci. USA.* 84:7542.

33. Fang, C., P. Kenigsberg, M. J. Axley, M. Nuell, and L. P. Hagler. 1986. Cloning and sequencing of chloroperoxidase cDNA. *Nucleic Acids Res.* 14:8061.

34. Lipman, D. J., and W. R. Pearson. 1985. Rapid and sensitive protein similarity searches. *Science (Wash. DC.)* 227:1435.

35. Fischkoff, S. A., A. Pollak, G. J. Gleich, J. R. Testa, S. Misawa, and T. Rober. 1984. Eosinophilic differentiation of the human promyelocytic leukemia cell line, HL-60. *J. Exp. Med.* 160:179.

36. Presentey, B., and H. Joshua. 1982. Peroxidase and phospholipid deficiency in human eosinophilic granulocytes. A marker in population genetics. *Experientia (Basel).* 38:628.

37. Hoffman, J. J. M. L., and A. G. R. M. Tielens. 1987. Partial deficiency of eosinophil peroxidase. *Blut.* 54:167.