MOLECULAR CLONING OF THE HUMAN EOSINOPHIL PEROXIDASE

Evidence for the Existence of a Peroxidase Multigene Family

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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)[1], an abundant protein in the matrix of the eosinophil granule (1), when combined with H_2O_2 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 H_2O_2, 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 A_{485} nm of the reaction of EPO with H_2O_2 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 con-

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[1] *Abbreviations used in this paper:* EPO, eosinophil peroxidase; MNC, mononuclear cells; MPO, myeloperoxidase; TPO, thyroid peroxidase.

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 NH_2 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-[^{32}P]ATP (E. I. duPont 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-A as described before (15). Cells were cultured at a density of 2×10^6/ml at 37°C, in humidified incubators with 5% CO_2, 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% H_2O_2, 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 (10^9) were lysed on day 7–8 of culture, and the total cellular RNA was isolated by the guanidine-isothiocyanate/$CsCl_2$ method (International Biotechnologies Inc., New Haven, CT and Boehringer Manheim 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 (8×10^5) in the cord blood cell library were screened by plaque hybridization using the ^{32}P-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 ^{32}P-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 NH_2-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.

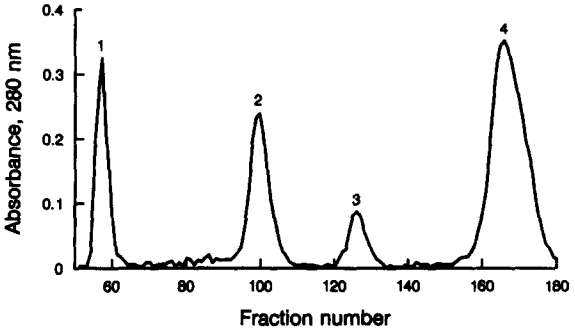

FIGURE 1. Gel filtration of EPO on Sepharose 4B equilibrated with 6 M guanidine hydrochloride. Peaks *1* and *4* are the molecular weight markers, blue dextran (M_r 2 × 10^6) and DNP-lysine (M_r 248), respectively; peaks *2* and *3* correspond to the EPO H and L chain, respectively.

```
                                                  10
EPO-H:  V  D  C/R  E  R  T  C/R  A  Q     L     P  P
                                  20
        C/R  F  P  I  K  I  P  P  N     D  P  R
                 30
        I  K  N  Q  A/R  D  C  I  P  F  T/F  C/S/R
        S  A

                                        10
EPO-L:  R  C/R  S  D  K  Y  R  T  I  T  G  R
                              20
        C/R  N  N  K  R  R  P  L  L  G  A  C
                       30
        N  Q  A  L  A  R  W  L  P  A  E  Y
                 40
        E  D  G  L  S  L  P  F  G  W  T  P
           50
        S/C  R
```

FIGURE 2. Amino acid sequence of the NH_2 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 NH_2 terminus. Amino acid 24 in the EPO-L is boxed. Regions chosen for the construction of the oligonucleotides are underlined.

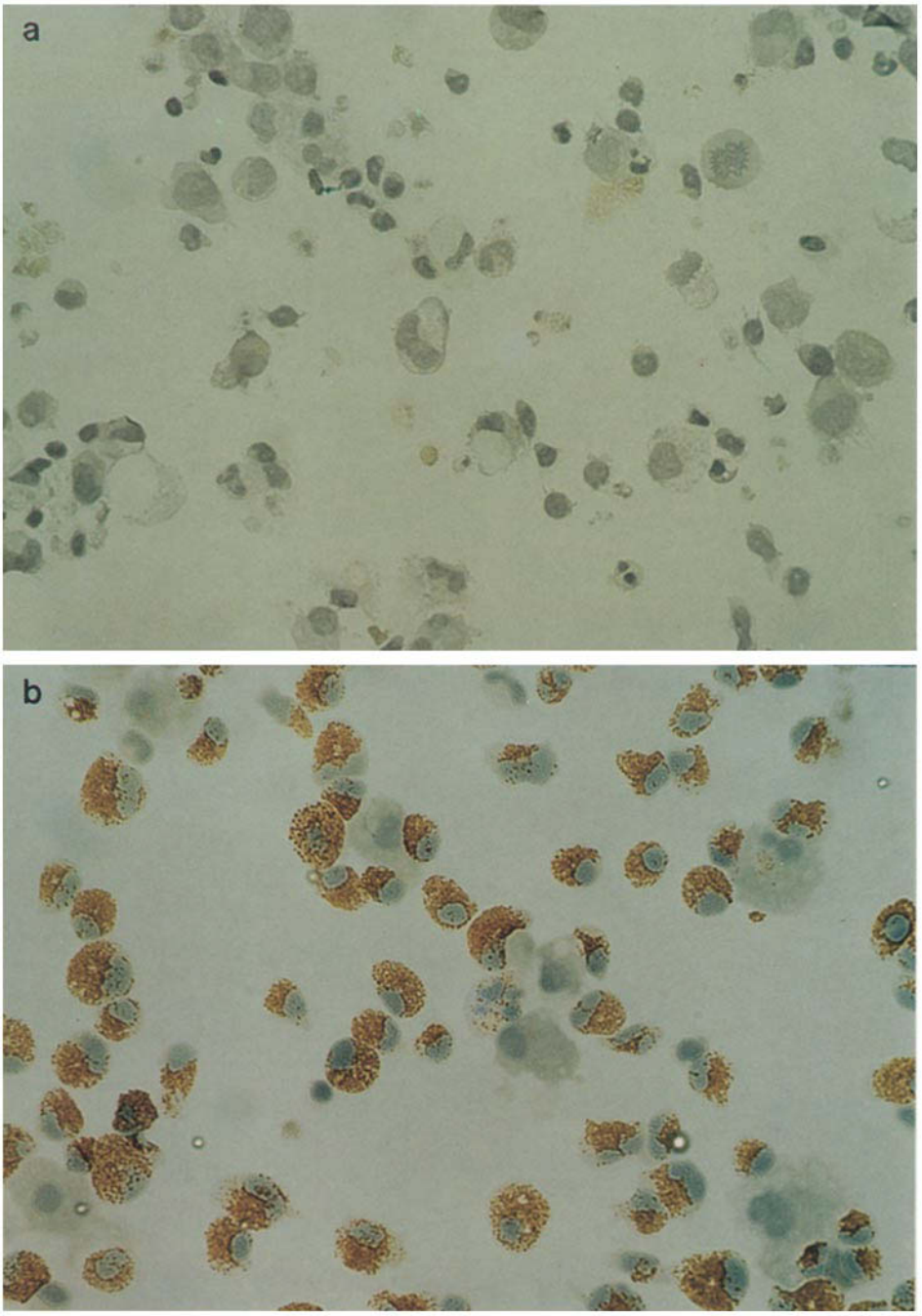

FIGURE 3. Continued on following page.

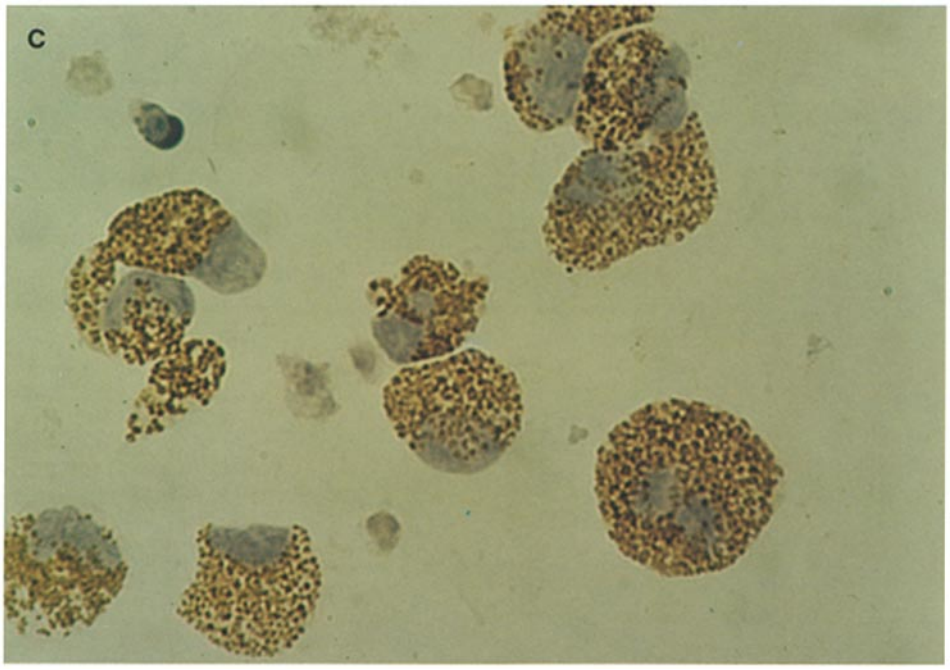

FIGURE 3. Cyanide-resistant peroxidase staining of cord blood MNC. (*a*) Uninduced (original magnification, 400); (*b*) induced with T cell supernatant for 25 d (× 400); and (*c*) same as in B (× 1,000).

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

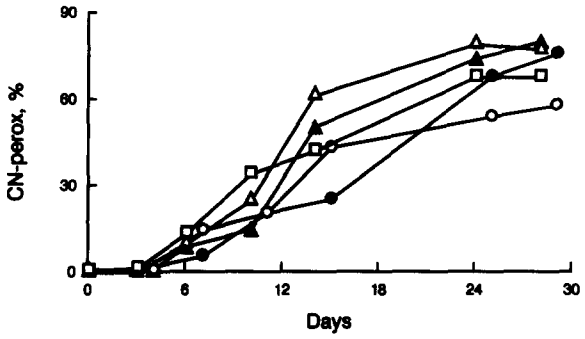

FIGURE 4. Induction of umbilical cord blood MNC by T cell supernatant. Each line represents the results of a single culture. The *x*-axis represents the days in culture, and the *y*-axis represents the percent of cells that stained positive for cyanide-resistant peroxidase.

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 8×10^5 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).

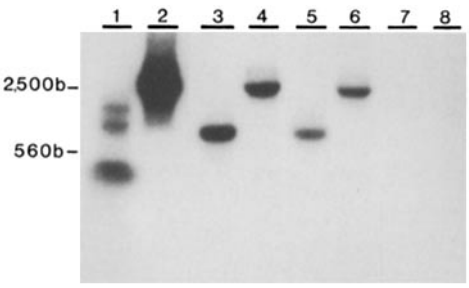

FIGURE 5. Southern blot analysis of a cDNA clone from the cord blood cDNA library. Lanes *1*, *3*, *5*, and *7* digested with Sau-3A; lanes *2*, *4*, *6*, and *8* digested with Eco RI. The cDNA clone was analyzed by hybridization using oligonucleotides derived by reverse translation of the amino acid sequences of EPO H chain, one probe, lanes *1* and *2*; EPO L chain, two probes, lanes *3* and *4* and lanes *5* and *6*, respectively; and ECP, lanes *7* and *8*.

TABLE I

Nucleotide and Amino Acid Sequence Homology of EPO and Other Peroxidases

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)
Chrysosporium	Ligninase (31)	49.7	18 (0.6)
Nicotiana tabacum	Lignin-forming peroxidase (32)	40.3	18.5 (0.5)
Caldariomyces	Chloroperoxidase (33)	I§	16.4 (0.4)

* Reference to the nucleotide sequences of the corresponding peroxidase.

‡ Z values calculated by the Lipman-Pearson method (34); values >10 are considered significant.

§ Insufficient homology for alignment.

TABLE II

Nucleotide and Amino Acid Sequence Homology of EPO Compared with MPO and hTPO

Area of the molecule	Nucleotide similarity index*		Amino acid similarity index	
	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

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GAA TTC CGT GGC CAG GAC CCC TGC CAG GGC ACT GAC CCA GCC TCC CCT GGG GCA GTG GAG   60
 e  f  r  g  q  d  p  c  q  g  t  d  p  a  s  p  g  a  v  e
ACC TCG GTC CTG CGA GAC TGC ATA GCA GAG GCC AAG TTG CTG GTG GAT GCT GCC TAC AAT  120
 t  s  v  l  r  d  c  i  a  e  a  k  l  l  v  d  a  a  y  n
TGG ACC CAG AAG AGC ATC AAG CAG CGG CTT CGC AGC GGT TCA GCC AGC CCC ATG GAC CTC  180
 w  t  q  k  s  i  k  q  r  l  r  s  g  s  a  s  p  m  d  l
CTG TCC TAC TTC AAA CAA CCG GTA GCA GCC ACC AGG ACA GTT GTT CGG GCC GCA GAT TAT  240
 l  s  y  f  k  q  p  v  a  a  t  r  t  v  v  r  a  a  d  y
ATG CAT GTG GCT TTG GGG CTG CTT GAA GAG AAG TTA CAA CCC CAG CGG TCC GGA CCC TTC  300
 m  h  v  a  l  g  l  l  e  e  k  l  q  p  q  r  s  g  p  f
ATT GTC ACT GAT GTG CTA ACA GAA CCA CAG CTG CGG CTG CTG TCC CAG GCC AGT GGC TGT  360
 n  v  t  d  v  l  t  e  p  q  l  r  l  l  s  q  a  s  g  c
GCT CTC CGG GAC CAG GCC GAG CGC TGC AGC GAC AAG TAC CGC ACC ATC ACT GGA CGG TGC  420
 a  l  r  d  q  a  e  r  c  s  d  k  y  r  t  i  t  g  r  c
AAC AAC AAG AGG AGA CCC TTG CTA GGG GCC TCC AAC CAG GCT CTG GCT CGC TGG CTG CCC  480
 n  n  k  r  r  p  l  l  g  a  s  n  q  a  l  a  r  w  l  p
GCC GAG TAT GAG GAT GGG CTG TCG CTC CCC TTC GGC TGG ACC CCC AGC AGG AGG CGC AAT  540
 a  e  y  e  d  g  l  s  l  p  f  g  w  t  p  s  r  r  r  n
GGC TTC CTT CTC CCT CTT GTC CGG GCT GTC TCC AAC CAG ATT GTG CGC TTC CCC AAT GAG  600
 g  f  l  l  p  l  v  r  a  v  s  n  q  i  v  r  f  p  n  e
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 r  l  t  s  d  r  g  r  a  l  m  f  m  q  w  g  q  f  i  d
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TGT GAG AGG ACC TGC GCC CAG CTG CCC CCC TGC TTT CCC ATC AAG ATC CCA CCC AAT GAC  780
 c  e  r  t  c  a  q  l  p  p  c  f  p  i  k  i  p  p  n  d
CCC CGC ATC AAG AAC CAG CGT GAC TGC ATC CCT TTC TTC CGC TCG GCA CCC TCA TGC CCC  840
 p  r  i  k  n  q  r  d  c  i  p  f  f  r  s  a  p  s  c  p
CAA AAC AAG AAC AGA GTC CGC AAC CAG ATC AAC GCG CTC ACC TCC TTT GTG GAC GCC AGC  900
 q  n  k  n  r  v  r  n  q  i  n  a  l  t  s  f  v  d  a  s
ATG GTG TAT GGC AGT GAG GTC TCC CTC TCG CTG CGG CTC CGC AAC CGG ACG AAC TAC CTG  960
 m  v  y  g  s  e  v  s  l  s  l  r  l  r  n  r  t  n  y  l
GGG CTG CTG GCC ATC AAC CAG CGC TTT CAA GAC AAC GGC CGG GCC CTG CTG CCC TTC GAC 1020
 g  l  l  a  i  n  q  r  f  q  d  n  g  r  a  l  l  p  f  d
AAC CTG CAC GAT GAC CCC TGT CTC CTC ACC AAC CGC TCG GCG CGC ATC CCC TGC TTC CTG 1080
 n  l  h  d  d  p  c  l  l  t  n  r  s  a  r  i  p  c  f  l
GCA GGT GAC ACC CGA TCA ACG GAA ACC CCC AAA CTG GCA GCC ATG CAC ACC CTC TTT ATG 1140
 a  g  d  t  r  s  t  e  t  p  k  l  a  a  m  h  t  l  f  m
CGA GAG CAC AAC CGG CTG GCC ACC GAG CTG AGA CGC CTG AAT CCC CGG TGG AAT GGA GAC 1200
 r  e  h  n  r  l  a  t  e  l  r  r  l  n  p  r  w  n  g  d
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FIGURE 6. Continued on following page.

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AAA CTG TAC AAT GAG GCT CGG AAG ATC ATG GGG GCC ATG GTC CAG ATC ATC ACC TAC CGA 1260
 k  l  y  n  e  a  r  k  i  m  g  a  m  v  q  i  i  t  y  r
GAC TTT CTG CCC CTG GTT CTG GGC AAG GCC CGG GCC AGG AGA ACC CTG GGG CAC TAC AGG  1320
 d  f  l  p  l  v  l  g  k  a  r  a  r  r  t  l  g  h  y  r
GGG TAC TGC TCC AAT GTG GAC CCA CGG GTG GCC AAT GTC TTC ACC CTG GCC TTC CGC TTT 1380
 g  y  c  s  n  v  d  p  r  v  a  n  v  f  t  l  a  f  r  f
GGC CAC ACA ATG CTC CAG CCC TTC ATG TTC CGC TTG GAC AGT CAG TAC CGG GCC TCC GCA 1440
 g  h  t  m  l  q  p  f  m  f  r  l  d  s  q  y  r  a  s  a
CCC AAC TCG CAT GTC CCA CTT AGC TCT GCC TTC TTT GCC AGC TGG CGG ATC GTG TAT GAA 1500
 p  n  s  h  v  p  l  s  s  a  f  f  a  s  w  r  i  v  y  e
GGG GGC ATC GAC CCC ATC CTC CGG GGC CTC ATG GCC ACC CCT GCC AAG CTG AAC CGT CAG 1560
 g  g  i  d  p  i  l  r  g  l  m  a  t  p  a  k  l  n  r  q
GAT GCC ATG TTA GTG GAT GAG CTC CGG GAC CGG CTG TTT CGG CAA GTG AGG AGG ATT GGG 1620
 d  a  m  l  v  d  e  l  r  d  r  l  f  r  q  v  r  r  i  g
CTG GAC CTG GCA GCT CTC AAC ATG CAA CGA AGC CGG GAC CAC GGC CTT CCA GGG TAC AAT  1680
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GCT TGG AGG CGC TTC TGT GGG CTC TCC CAG CCC CGG AAT TTG GCA CAG CTT AGC CGG GTG  1740
 a  w  r  r  f  c  g  l  s  q  p  r  n  l  a  q  l  s  r  v
CTG AAA AAC CAG GAC TTG GCA AGG AAG TTC CTG AAT TTG TAT GGA ACA CCT GAC AAC ATT 1800
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TAC CCT CGG GGC TTT GTG AAC TGC AGC CGT ATC CCC AGG TTG AAC CTA TCA GCC TGG CGA 2100
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CTACTGTTTCCCAGCCCTCCCTCCCCTCCCTCACCATGACTAAGAGACCACTCGGTCCTAGCCTCCAGACACCCCACAA 2493
TACTCCTCTGAGCCTGAGGCCAGGCAGCATGCTCTGCTTCTACCAATAAAGCACTGCCGGAATTC              2558
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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 NH_2-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.

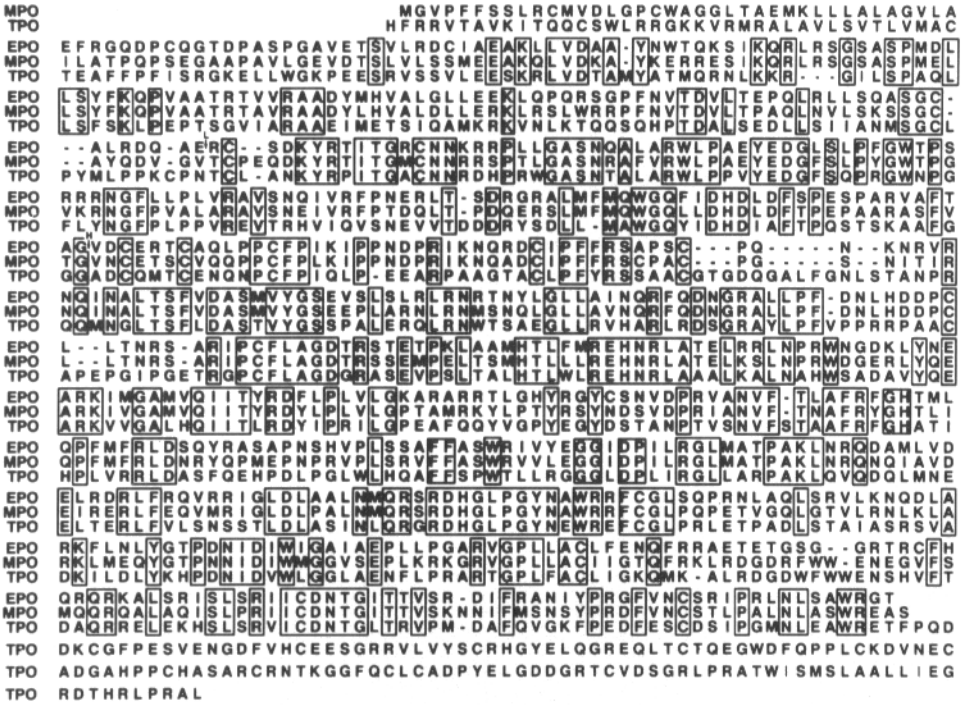

FIGURE 7. Amino acid sequence comparison of EPO, MPO, and hTPO. Amino acids that are identical to all three are boxed. L and H indicate the beginning of the EPO L and H chains, respectively. References for the nucleotide sequences of MPO and hTPO are 27 and 28, respectively.

common. 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 to 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 NH_2 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 NH_2-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.

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