The eosinophil cationic protein (ECP) is one of several small, distinct arginine-rich proteins that have been isolated from the eosinophil's large specific granule. The molecular mass of human ECP has been estimated at 18-21 kD (1-3), depending on the degree of glycosylation (3). ECP has been shown to possess a wide variety of biological activities, including the ability to stimulate factor XII-dependent coagulation pathways (4), to neutralize the anticoagulant effects of heparin (5), and to inhibit lymphocyte proliferation induced by PHA or mixed lymphocyte reactions (6). ECP is a potent cytotoxin; ECP-containing supernatants of activated eosinophils have been shown to be toxic to isolated myocardial cells in vitro (7), and both ECP and the eosinophil granule major basic protein (MBP) were found in the endothelial and endomyocardial lesions characteristic of the hypereosinophilic syndrome (8). Furthermore, ECP is also a potent helminthotoxin; destruction of schistosomula of Schistosoma mansoni was reported at concentrations as low as 10^{-7} M (8-10-fold more active than MBP) (9, 10). ECP has also been shown to kill trypomastigote and amastigote stages of Trypanosoma cruzi (11). Both ECP and the related granule protein, eosinophil-derived neurotoxin (EDN), induce the neurotoxic effect known as the Gordon phenomenon (12) when injected into the cerebrospinal fluid of experimental animals (3, 13-15). Although the molecular mechanism(s) by which ECP carries out these varied biologic activities is not clear, Young et al. (16) have suggested that ECP might damage cells by a colloid-osmotic process, as they have shown that it can introduce non-ion selective pores in both cellular and synthetic membranes.

Gleich et al. (3) published the NH₂-terminal sequences of ECP and the related granule protein, EDN; the NH₂-terminal sequence of ECP showed 67% identity to that of EDN, and 26% identity to the sequence of human pancreatic ribonuclease.
MOLECULAR CLONING OF HUMAN EOSINOPHIL CATIONIC PROTEIN (HPR), suggesting that ECP and EDN might belong to the ribonuclease multigene family. ECP and EDN were subsequently found to have ribonuclease activity, with ECP showing 100-fold less activity than EDN against a yeast RNA substrate (17, 18). We have previously isolated and sequenced a full-length cDNA clone for EDN (19); the complete cDNA-derived amino acid sequence was identical to a human nonsecretory ribonuclease isolated from urine (HNSR) (19, 20). In addition, there was 32% sequence identity between EDN and HPR, including conservation of the structural cysteine and catalytic lysine and histidine residues. We now report the isolation of a full-length cDNA clone for ECP. The predicted amino acid sequence of mature ECP shows 66% sequence identity with EDN/HNSR, but has 12 more arginine residues and a higher net positive charge. While EDN is expressed in a wide variety of hematopoietic cells and cell lines (19), ECP appears to be expressed only in eosinophils and their precursors.

Materials and Methods

Purification of RNA from Peripheral Blood Cells. Peripheral blood granulocytes obtained by leukopheresis of a patient with the hypereosinophilic syndrome were purified as described (19). After two washes in cold HBSS and lysis of RBC by brief suspension in cold lysis buffer (100 mM potassium carbonate, 150 mM ammonium chloride, and 0.1 mM EDTA, pH 7.2), the resuspended cell pellet was underlayered with a cushion of Ficoll-Hypaque (1.077 g/ml; Pharmacia Fine Chemicals, Piscataway, NJ) and centrifuged at 400 g for 30 min at room temperature. The mature granulocytes in the pellet were separated from cells suspended in the Ficoll-Hypaque layer (hypodense granulocytes and some mononuclear cells), and the RNA was extracted and purified by guanidium-isothiocyanate extraction followed by cesium chloride density gradient centrifugation (21). RNA from discarded samples of normal bone marrow cells was purified as described except without cell separation over Ficoll-Hypaque. Monocytes were purified from peripheral blood of normal donors by Ficoll-Hypaque density centrifugation and adherence to plastic. Neutrophil RNA was purified from peripheral blood neutrophils obtained from normal donors. Cells were washed and separated over Ficoll-Hypaque as above; in each case, the pellets contained <5% contaminating eosinophils.

cDNA Library Screening. cDNA clones for ECP were isolated from a λ gt11 library prepared from poly (A)+ RNA purified from the peripheral mononuclear cells of a patient with eosinophilic leukemia, and from a λ gt11 library obtained from Dr. Stuart Orkin (Children's Hospital Medical Center, Boston, MA) prepared from poly (A)+ RNA from HL-60 cells induced for 7 d with DMSO. Screening was done with a 17-base 32-fold degenerate oligonucleotide (Fig. 1) synthesized on a DNA synthesizer (model 381; ABI, Foster City, CA) and radiolabeled on the 5' end using γ[32P]ATP (New England Nuclear, Boston, MA) and T4 polynucleotide kinase (22) or on the 3' end with a threefold molar excess of α[32P]dCTP (New England Nuclear) and terminal deoxynucleotidyl transferase (International Biotechnologies, Inc., New Haven, CT). Prehybridization, hybridization, and washing were done as described (23), except for the washing procedure, which included two washes at room temperature in 6× SSC (22) followed by a 1-min final wash in 6× SSC at 37°C. Positive recombinants were purified and the inserts subcloned into M13 phage forideoxynucleotide sequencing (24). Sequence evaluation was done with the assistance of Bionet (Menlo Park, CA) and DNAstar (Madison, WI) sequence analysis software.

Induction of HL-60 Differentiation. RNA was extracted as described above from cells of the promyelocytic leukemia HL-60 cell line (American Type Culture Collection no. CCL-240) grown in RPMI 1640 medium supplemented with 10% FCS (Gibco Laboratories, Grand Island, NY) both with and without inducing agents, which included 48 h in 5 × 10−7 M vitamin D3 or 48 h in 1.1% DMSO. The monocytic or granulocytic induction of HL-60 cells was verified by morphology as well as mRNA and surface expression of CD11b, as previously described (25). RNA was also extracted from a cloned subline of HL-60 committed to eosinophilic differentiation (HL-60 3 + c− 5 [26]) grown either in the absence of inducing agents or in the presence of 10% BCGF-2/IL-5 (Cellular Products, Inc., Buffalo, NY) for 72 h.
Northern Blotting and Hybridization. Total RNA samples (10 μg each), extracted and purified as described above, were analyzed by agarose formaldehyde gel electrophoresis, ethidium stained to ensure equivalent RNA loading, and blotted onto nylon membranes (ICN Biomedicals, Irvine, CA) in 10× SSC (22). The ECP (bases 1–725) and EDN (bases 171–725) cDNA probes were labeled with α[32P]dCTP by the random hexamer priming method (27). Filters were prehybridized for at least 4 h in a solution containing 5× SSC, 50% formamide, 5× Denhardt’s solution, 0.05 M sodium phosphate, pH 6.3, 1% glycine, 0.1% SDS, and 250 μg/μl sheared denatured salmon sperm DNA at 42°C. Hybridization was done in a solution containing 50% formamide, 5× SSC, 1× Denhardt’s solution, 0.02 M sodium phosphate, pH 6.5, and 10⁶ cpm/ml denatured radiolabeled probe at 42°C for at least 12 h (22). Hybridized filters were washed twice at room temperature in 2× SSC, and for 30 min at 55°C in 0.2× SSC; washed filters were exposed for 48 h to Kodak XAR film with a single intensifying screen at −80°C. RNA size standards (0.16–1.77 kb) were purchased from Bethesda Research Laboratories, Gaithersburg, MD.

Results

Isolation and Sequencing of cDNA Clones. Primary screening of the eosinophil leukemia λ gt11 cDNA library with the 32-fold degenerate oligonucleotide no. 73 (Fig. 1) yielded a 483-bp cDNA clone (nucleotides 243–725). Subsequent screening of this library with this cDNA probe yielded a longer clone (nucleotides 192–725); the full-length (725 bp) cDNA clone was isolated from the DMSO-induced HL-60 λ gt11 cDNA library. Fig. 1 shows the nucleotide sequence of the cDNA for ECP; both the coding and noncoding strands were sequenced to completion. The complete cDNA clone contains a Kozak-like translation initiation sequence at nucleotides 52–58 (28), an open reading frame extending from nucleotides 55–534 (encoding a total of 160 amino acids), and a polyadenylation signal at nucleotides 695–700, with a 15-base spacer preceding the poly A tail. The nucleotide sequence of the cDNA clone for ECP is 89% identical to that reported for the related eosinophil granule protein, EDN (19). There are a total of 81 bp mismatches (including a single three-base gap at nucleotides 487–489), with 72 of the mismatches found within the coding region; 62 (86%) of the mismatches within the coding region result in changes in the encoded amino acid sequence.

The amino acid sequence encoded by the ECP cDNA confirms the identity of the 60 residues previously identified by NH2-terminal sequencing (3). The NH2-terminal arginine residue of granule-extracted ECP is the arginine encoded by nucleotides 136–138; the molecular mass of the protein encoded from nucleotides 136–534 is 15.6 kD. The amino acid sequence contains three Asn-X-Thr/Ser sequences that have been identified as sites of potential N-linked glycosylation (29–31).

From nucleotides 55 to 135, the open reading frame encodes a 27-residue segment that was not previously identified in the NH2-terminal sequence of granule-derived ECP (3). This segment is markedly hydrophobic; 20 of the 27 residues are either hydrophobic or aliphatic. A 27-residue NH2-terminal hydrophobic sequence was also encoded by the cDNA isolated for EDN (19); 24 of the 27 NH2-terminal residues of this ECP leader sequence are identical to those of EDN.

Sequence Comparisons. Fig. 2 a shows the alignment of the cDNA-derived amino acid sequences of mature ECP and EDN, without the aforementioned NH2-terminal hydrophobic sequences. Of the 133 amino acids encoded by ECP cDNA, 88 have identical counterparts in the sequence of EDN (thus, 66% identity between the two sequences). Despite the similarities in amino acid sequence, the calculated isoelectric points of the two peptides differ significantly; the calculated pI of ECP is 10.8,
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Figure 1. Nucleotide sequence of the 725-base coding strand of the ECP cDNA with translation of the open reading frame (160 amino acids), potential N-linked glycosylation sites (underlined), and location of the 32-fold degenerate (no. 73) 17-base oligonucleotide probe used to isolate the cDNA. The Kozak initiation sequence containing the presumptive start codon (ATG) at nucleotides 52-58 is boxed, the NH₂ terminus (R, position +1) of the granule-derived protein is boxed and darkly shaded, and the stop codon (TAA) at nucleotides 535-537 is boxed and lightly shaded. The 3' polyadenylation signal (AATAAA) at nucleotides 695-700 is boldly underlined. The 27-amino acid leader sequence precedes the NH₂-terminal R, and is denoted as positions -27 to -1. These sequence data have been submitted to the EMBL/GenBank Data Libraries under the accession number X15161.
FIGURE 2. (a) Alignment of the cDNA-derived amino acid sequences of ECP and EDN (not including leader sequences). Regions of identical sequence are within the boxes; the cysteine residues are within the shaded boxes. All arginine residues (R) in ECP have been circled (total of 20); arginine residues present in ECP but not in EDN have also been shaded. Numbering is as per the ECP sequence. (b) Alignment of the amino acid sequences of ECP, EDN (identical to HNSR [19, 20]), HLR (32), HPR (33), and ANG (34, 35). Regions of conserved sequence are enclosed in boxes, conserved cysteine residues are lightly shaded, and the putative catalytic histidine and lysine residues are heavily shaded. Dashes represent gaps introduced to permit alignment of cysteines and of the catalytic residues. Numbers above and to the right are as per the sequence of ECP.
while that of EDN is 8.9. The higher calculated pI reflects ECP's higher arginine content. While EDN has eight arginine residues, ECP has a total of 20 arginine residues; at 15 of the 45 residues (33%) at which ECP and EDN differ, there is an arginine residue in the ECP sequence.

Fig. 2 b shows the alignment of the cDNA-derived amino acid sequence of ECP (beginning at the arginine at position 1 of the NH₂ terminus of the granule-extracted protein [3]) with the sequences of EDN (19) (shown to be identical to HNSR [19, 20]), human liver ribonuclease (HLR) (32), HPR (33), and angiogenin (ANG) (34, 35). The sequences of EDN, HNSR, and the 25 NH₂-terminal residues of HLR are identical. While the sequence of ECP shows 66% identity with the sequences of EDN (and thus HNSR and HLR), there is only 31% identity between ECP and HPR, and 25% identity between ECP and ANG.

Northern Analysis. The presence of mRNA encoding ECP was assessed in a number of hematopoietic cells and cell lines. Fig. 3 shows a Northern analysis of various

![Northern Analysis](image)
sources of RNA probed with the 725-bp cDNA for ECP and a 554-bp cDNA for EDN (19). The molecular sizes of the ECP and EDN mRNAs were indistinguishable on the basis of their electrophoretic mobilities; comparison with RNA standards gave an estimate of the molecular size as 970 bases, which is compatible with a short 5' untranslated region and 3' poly A tail. Whereas mRNA for EDN was detected in uninduced cells of the promyelocytic line, HL-60 (Fig. 3 b, lane 1), mRNA for ECP was either absent or present at levels below detectable limits in these cells (Fig. 3 a, lane 1 and Fig. 3 c, lane 1). Likewise, mRNA for ECP was not detected in HL-60 cells induced toward monocytic differentiation with vitamin D3 (Fig. 3 a, lane 2), or in mature monocytes (Fig. 3 a, lane 4).

In contrast, mRNA for EDN was detected in vitamin D3-induced HL-60 cells (Fig. 3 b, lane 2) and in mature peripheral monocytes (Fig. 3 b, lane 4), but at significantly lower levels than in the uninduced HL-60 cells. Despite the fact that ECP mRNA was undetectable in the uninduced HL-60 eosinophilic subline (Fig. 3 c, lane 1), there was ECP mRNA detected in cells induced toward eosinophilic differentiation with BCGF-2/IL-5 (Fig. 3 c, lane 2); EDN mRNA was likewise up-regulated with this induction (Fig. 3 d, lane 2). There was also an up-regulation of EDN mRNA in HL-60 cells induced toward neutrophilic differentiation with DMSO (Fig. 3 b, lane 3); in contrast, mRNA for ECP remained undetectable under these conditions (Fig. 3 a, lane 3), and was not detectable in mRNA from mature neutrophils (not shown). mRNA for both ECP and EDN was detected in total RNA samples prepared from Ficoll-Hypaque fractions of hypodense peripheral blood eosinophil-rich granulocytes (68% eosinophils, 30% neutrophils, 2% mononuclear cells) (Fig. 3 c, lane 3 and Fig. 3 d, lane 3) or from peripheral blood eosinophil-rich granulocytes of normal density (73% eosinophils, 27% neutrophils) (Fig. 3 c, lane 4 and Fig. 3 d, lane 4), both obtained from a patient with the hypereosinophilic syndrome. Neither message was detected in the sample of total RNA from bone marrow cells (Fig. 3 c, lane 5 and Fig. 3 d, lane 5).

Discussion

ECP and EDN are cationic granule proteins with similar amino acid content (5, 36, 37) that are distinguishable on the basis of molecular size, immunogenicity, and biological activities (37). We have isolated full-length cDNA clones for both EDN (19) and ECP. The cDNA sequences of ECP and EDN are 89% identical; the Kozak-like translation initiation sequences, stop codon (TAA), and location of the polyadenylation signal (AATAAA) with respect to the poly A tail are the same in both cDNA sequences.

The cDNA sequence of ECP encodes a 160-amino acid polypeptide, one fewer than the cDNA for EDN, which encodes 161 amino acids (19). Alignment of both the cDNA and the amino acid sequences placed the gap at codon CGA (nucleotides 475-477 of the EDN cDNA) corresponding to the omission in ECP of arginine 118 in EDN. This arginine is not a putative catalytic residue, nor is it conserved among ribonucleases in general (38); its significance with respect to the functional differences between EDN and ECP is unknown.

The 27 NH2-terminal residues encoded by the ECP cDNA are not found in the NH2-terminal sequence of the granule-extracted protein (3). This segment is markedly hydrophobic, with a sequence of 10 uninterrupted hydrophobic/aliphatic
residues, characteristic of a leader sequence (39, 40). The alanine at position -1 and leucine at position -3 are consistent with von Heijne's criteria for signal peptidase cleavage (41). The process (biosynthesis, packaging into granules, or degranulation/secrection) during which the hydrophobic leader sequence is removed from ECP (or EDN) is not known. In the case of ECP, there is some evidence to suggest that the leader sequence may persist to the secretion stage. Tai et al. (42) have described mAbs that can distinguish between storage (granule) and secreted forms of ECP; monoclonal EG-1 interacts with both storage and secreted ECP, whereas EG-2 recognizes only the secreted form. Interestingly, ECP extracted directly from isolated granules is immunologically indistinguishable from the secreted form (recognized by both EG-1 and EG-2) and has no leader sequence. It is conceivable that the leader sequence is cleaved during secretion (or extraction). This cleavage could result in either a local conformational change and/or exposure of a previously masked epitope permitting recognition by the monoclonal EG-2. Alternatively, EG-2 might simply recognize an epitope that is masked while ECP is packaged in the granule, unrelated to the presence or absence of a leader sequence.

The sequence similarities reported by Gleich et al. (3) for the NH2-terminal residues of granule-extracted ECP and EDN also extend through the COOH termini of the polypeptide sequences (Fig. 2a). The mature ECP polypeptide (without leader sequence) contains 133 amino acids, which is one fewer than that found in EDN (discussed above), with a sequence similarity between the two polypeptides of 66%. The calculated molecular mass of mature ECP is 15.6 kD, which is slightly higher than that of EDN (15.5 kD). Despite the sequence similarities, there is a striking difference in their net charges (pI of ECP is 10.8 vs. 8.9 for EDN). The arginine content of ECP is markedly higher than that of EDN (20 arginine residues in ECP and only eight in EDN). It is possible that this high positive charge confers on ECP the cytotoxic and helminthotoxic properties that are lacking in EDN. Young et al. (16) have shown that ECP (but not eosinophil protein X, which may be identical to EDN [37, Gleich, G.J., personal communication]) has perforin-like activity. In their experiments, ECP induced hemolysis and introduced non-ion-selective pores in both cellular and synthetic lipid membranes. By analogy, mellitin, the major component of honeybee venom, is a 26-amino acid cationic polypeptide that also interacts with cell membranes and results in cell lysis (43). It is not clear whether mellitin introduces ion pores (44, 45) or disrupts the hydrophobic bonding of the phospholipid bilayer (46, 47). DeGrado et al. (46) have shown that when the cationic component of mellitin, the COOH-terminal hexapeptide (sequence KRKRQQ), is removed, the remaining polypeptide remains capable of binding to erythrocyte membranes, but there is no cell lysis. Mellitin-mediated cell lysis is clearly dependent on the presence of a highly positively charged segment; the positively charged residues present in the sequence of ECP (but not EDN) might be responsible for ECP's cytotoxic properties.

Gleich et al. (3) noted the sequences similarities between ECP, EDN, and the amino acid sequence of HPR, and suggested that this group comprised a multigene family. HPR belongs to the secretory group of mammalian ribonucleases, which are characterized by alkaline pH optima and immunological crossreactivity (48), and have also been found in kidney, stomach, and saliva (49–53). This group is immunologically
distinct from the neutral, nonsecretory ribonucleases, which have pH optima near 6.5, and have been found in spleen, lung, liver, and leukocytes (49, 50, 53, 54). We have previously shown that EDN is identical to a nonsecretory ribonuclease isolated from human urine (HNSR) (19, 20) and to the known NH2-terminal residues of HLR (32); our previous results also suggested that EDN may have an identical counterpart in neutrophils (19). ECP is clearly more closely related to these nonsecretory ribonucleases than it is to human pancreatic ribonuclease. In addition, ECP shows 25% sequence identity to ANG, a small polypeptide isolated initially from adenocarcinoma cells (55), that has both angiogenic and ribonucleolytic activities (55-57) and an amino acid sequence similar to other members of the ribonuclease multigene family (34, 35).

We previously reported that the complete sequences of EDN, HNSR, and HPR showed conservation of all eight cysteine residues and the putative catalytic histidine and lysine residues characteristic of other ribonucleases (19). The complete sequence of ECP, likewise, contains these same features. Slifman et al. (17) have shown that both EDN and ECP have ribonuclease activity. While the ribonucleolytic activity of EDN is equivalent to that of RNase A, ECP was found to be 100-fold less active against the yeast RNA substrate. We have repeated these experiments with a yeast tRNA substrate, with identical results (Corrette, S. E. and S. J. Ackerman, unpublished observations). As ECP contains both the structurally important cysteine residues, as well as the catalytic histidines and lysine (Fig. 2 b), there should be another explanation for its reduced ribonucleolytic activity. One significant difference between the primary structures of ECP and EDN is, as described above, the highly positive charge of ECP. The electrostatic charge between the positively charged ECP and negatively charged RNA might prevent the substrate from binding in an appropriate conformation in the catalytic site, or may inhibit turnover by hindering release of the negatively charged cleaved products. Angiogenin was also characterized as a poor ribonuclease when tested against standard RNA substrates (56). However, St. Clair et al. (57) have reported that angiogenin effectively abolishes in vitro protein synthesis by specific cleavage of 28S and 18S rRNA; ECP may have a similar substrate specificity.

MBP is a distinct eosinophil cationic protein (molecular mass of 13.9 kD [58, 59]) that comprises the electron-dense crystalloid core of the eosinophils' specific granule (37). ECP and MBP have similar functions; both ECP and MBP damage schistosomula of S. mansoni (9, 10), neutralize heparin (5, 37), and are implicated as mediators of the myocardial, endothelial, and endocardial damage (8), characteristic of prolonged hypereosinophilic states such as the idiopathic hypereosinophilic syndrome (60). Despite these shared activities, the amino acid sequences of ECP and MBP (58, 59) show only insignificant sequence similarity (5%), making a common molecular mechanism of action unlikely. Some activities are clearly not shared; MBP, but not ECP, induces noncytolytic histamine release from human basophils (61, 62). Furthermore, the effectiveness of ECP and MBP against schistosomula of S. mansoni differed on both quantitative and qualitative bases. Whereas ECP killed 100% of schistosomula in vitro at a 3.75 × 10⁻⁶ M concentration, MBP at this concentration was only marginally toxic, killing only 15% (10). Also, MBP resulted in ballooning of the schistosomula tegumental membrane, while ECP caused a more com-
plete fragmentation (10). As the molecular mechanisms by which these two proteins function are likely to be different, their helminthotoxic and cytotoxic activities may be synergistic.

ECP mRNA and EDN mRNA are indistinguishable on the basis of electrophoretic mobility. Despite size and sequence similarities, there is clearly differential regulation of the two mRNA species. EDN mRNA was detectable in uninduced promyelocytic leukemia HL-60 cells, and was clearly up-regulated with induction toward eosinophilic (BCGF-2/IL-5) and, as previously reported (19), toward neutrophilic (DMSO) differentiation. In contrast, ECP mRNA was seen only in an eosinophil-committed subline of HL-60 cells induced toward eosinophil differentiation and in eosinophil-rich preparations of peripheral granulocytes. ECP mRNA was not present in HL-60 cells induced toward neutrophilic (DMSO) differentiation, nor was it present in peripheral blood neutrophils. In support of these findings, induction of HL-60 cells with DMSO leads to a diminution of other eosinophil-specific characteristics, including a decrease in Biebrich scarlet staining (63, 64), and reduced mRNA transcription for (65) and biosynthesis of MBP (63).

In summary, mRNA for ECP was present only in eosinophil-rich granulocyte preparations and HL-60 cells induced toward eosinophilic differentiation; unlike EDN, ECP appears to be an eosinophil-specific protein. These results suggest that ECP and EDN may be subject to different regulatory mechanisms. We hypothesize that ECP may have arisen from the ubiquitous EDN ribonuclease as a gene duplication event, and subsequently acquired mutations (i.e., the substitution of multiple arginine residues) may have tailored ECP to subserve eosinophil-specific helminthotoxic and cytotoxic activities. We are currently attempting to isolate genomic clones for both ECP and EDN, with the hope of identifying discrete sequences responsible for the observed differential regulation of transcription.

Summary

We have isolated a 725-bp full-length cDNA clone for the human eosinophil cationic protein (ECP). ECP is a small, basic protein found in the matrix of the eosinophil's large specific granule that has cytotoxic, helminthotoxic, and ribonuclease activity, and is a member of the ribonuclease multigene family. The cDNA sequence shows 89% sequence identity with that reported for the related granule protein, eosinophil-derived neurotoxin (EDN). The open reading frame encodes a previously unidentified 27-amino acid leader sequence preceding a 133-residue mature ECP polypeptide with a molecular mass of 15.6 kD. The encoded amino acid sequence of ECP shows 66% identity to that of EDN and 31% identity to that of human pancreatic ribonuclease, including conservation of the essential structural cysteine and catalytic lysine and histidine residues. mRNA for ECP was detected in eosinophil-enriched peripheral granulocytes and in a subclone of the promyelocytic leukemia line, HL-60, induced toward eosinophilic differentiation with IL-5. No ECP mRNA was detected in uninduced HL-60 cells, or in HL-60 cells induced toward mononuclear differentiation with vitamin D3 or toward neutrophilic differentiation with DMSO. In contrast, mRNA for EDN was detected in uninduced HL-60 cells and was up-regulated in HL-60 cells induced with DMSO. Despite similarities in sequence and cellular localization, these results suggest that ECP and EDN are subject to different regulatory mechanisms.
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