Cationic defensins arise from charge-neutralized propeptides: a mechanism for avoiding leukocyte autocytotoxicity?

David Michaelson, John Rayner, Marcelo Couto, and Tomas Ganz

Will Rogers Institute Pulmonary Research Laboratory at UCLA, Department of Medicine, UCLA School of Medicine, Los Angeles, California

Abstract: Defensins, small cationic polypeptides with antimicrobial and cytotoxic properties, are among the principal constituents of cytoplasmic granules of mammalian neutrophils and certain macrophages. To identify conserved structural features of defensin precursors that may be important for their targeting to cytoplasmic granules or for prevention of autocytotoxicity, we isolated and sequenced three neutrophil-specific rabbit defensin cDNAs that code for preproprotein precursors to the mature defensins NP-3a, NP-4, and NP-5. The preprodefensins NP-3a, NP-4, and NP-5, like the previously characterized preprodefensins, lack consensus sequences for N-linked glycosylation, suggesting that defensins are targeted to lysosome-like granules by a mechanism not dependent on the mannose-6-phosphate receptor. Analysis of all seven known myeloid prodefensins revealed a structure wherein an anionic propiece neutralizes the cationicity of the mature peptide. Because defensins apparently require cationic epitopes for cell membrane permeabilization and cytotoxicity, charge neutralization of mature peptides by their anionic propieces may prevent autocytotoxicity during defensin synthesis and processing. *J. Leukoc. Biol.* 51: 634-639; 1992.

Key Words: *neutrophil · macrophage · lysosomal granules*

INTRODUCTION

Phagocytic leukocytes employ a variety of molecules, including reactive oxygen intermediates and granule polypeptides, to effect their microbicidal and cytotoxic functions. Many of these effector molecules act indiscriminately and have the potential to injure or kill the phagocytes. Little is known about the mechanisms that prevent such damage to the phagocyte.

Defensins are small, variably cationic peptides that have microbicidal, chemotactic, and endocrine-regulatory activity; permeabilize biological membranes; and are cytotoxic to mammalian cells in vitro [1]. Three of the four human defensins, human neutrophil peptides HNP1-3, make up about 5% of the protein content of human neutrophils; a fourth peptide, HNP-4, is about a 100-fold less abundant [1, 2]. The six known rabbit defensins, neutrophil peptides NP-1, 2, 3a, 3b, 4, and 5 [3], constitute at least 15% of the protein content of rabbit neutrophils. Two defensins, macrophage cationic peptides MCP-1 and 2, are also abundant constituents of rabbit alveolar macrophages; these peptides are chemically identical to NP-1 and 2, respectively [3, 4]. In human and rabbit polymorphonuclear leukocytes (PMNs) and rabbit alveolar macrophages (AMs), defensins are packaged in membrane-bound cytoplasmic granules [2-6]. In the granules, these peptides colocalize with anionic mucopolysaccharides that may keep the defensins in a latent state [6]. However, the mechanisms that prevent autocytotoxicity during defensin synthesis and intracellular transport are not known.

Previously characterized defensin cDNAs correspond to the most cationic known defensins, MCP-1 and 2 [7], and the least cationic defensins, HNP-1 and 3 [8]. All are synthesized as larger precursors with an amino-terminal signal sequence for targeting to the endoplasmic reticulum [9, 10], an anionic propiece, and the mature defensin at the carboxyl terminus. In pulse-chase studies of human defensin processing in immature human myeloid cells [11], the 94-amino-acid preprodefensin was converted to the mature 29- to 30-amino-acid form over 6-24 h by at least three proteolytic steps that sequentially removed the amino-terminal portions of the polypeptide. We reasoned that this relatively complex maturation pathway may play a role in the maintenance of defensin latency and that the anionic propiece serves an important function that involves interaction with the cationic portion of the prodefensin molecule. In this communication we report the cloning and sequencing of three cDNAs corresponding to defensins whose cationic charges are intermediate between the extremes of HNP-3 and MCP-1, analyze the conserved structural features of all the preprodefensins characterized to date, and propose that defensin precursors may be inactivated by their anionic propieces during processing and intracellular transport.

MATERIALS AND METHODS

Strains and Reagents

A lambda gt10 rabbit bone marrow library, produced in collaboration with Dr. Patrick W. Gray, Genentech, South San Francisco, CA, was introduced into *Escherichia coli* strain C600hflA. Plaque screening was performed with Genescreen Plus nylon membranes (NEN-DuPont) according to the manufacturer's specifications. Sequencing reagents were from USB's Sequenase version 2.0 kit or Pharmacia's T7 polymerase kit. M13 strains mp18 and mp19 were grown in *E. coli* strains JM101, JM109, and XL1blue. Restriction endonucleases, T4 polynucleotide kinase, T4 ligase, and the Klenow fragment of DNA polymerase I were purchased from BRL, USB, and Pharmacia.

Abbreviations: AM, alveolar macrophage; HNP, human neutrophil peptide; MBP, major basic protein; MCP, macrophage cationic peptide; NP, neutrophil peptide; PMN, polymorphonuclear leukocyte.

The nucleotide sequences of NP-3a, 4, and 5 cDNAs, and the NP-3a gene have been deposited with Genbank, accession numbers M64600, M64601, M64602, and M64599.

Reprint requests: Tomas Ganz, Department of Medicine, CHS 37-055, UCLA School of Medicine, Los Angeles, CA 90024-1736.

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cDNA Cloning and Sequencing

Plaque lifts of the cDNA library were screened using a variety of probes (Table 1). Hybridizations were done according to the NEN Genescreen protocol except that in hybridization and prehybridization solutions 0.5% (final concentration) powdered nonfat milk was used in place of Denhardt's solution. Oligonucleotide probes were end labeled using T4 polynucleotide kinase and double-stranded DNA probes by the random oligonucleotide priming method [12].

Hybridizing cDNA clones were plaque purified and their lambda phage DNA digested with *Eco*RI for subcloning into M13 mp18 or mp19 [12]. Sequencing was done by the Sanger method [13] on both DNA strands.

Charge Calculations and Sequence Alignments

Protein charge at pH 7 was estimated using the PC-GENE Chargpro program (Intelligenetics, Palo Alto, CA). Nucleotide and protein sequence alignments were performed with the help of PC-GENE Clustal, Nalign and Palign programs.

RESULTS

cDNA cloning

Reasoning that the highly conserved signal sequence common to the cDNAs for human defensins HNP-1 and 3 and rabbit defensins MCP-1 and 2 [7] may also be present in other members of the defensin family, we initially screened the rabbit bone marrow cDNA library using a 30-bp oligonucleotide probe against this sequence (Table 1). The hybridizing clones distinct from MCP-1 and 2 coded for a precursor of the defensin NP-3a. Clones for NP-5 cDNA were identified by hybridization with a degenerate 20-bp oligonucleotide probe (20-mer, Table 1) derived from amino acid residues 1–7 of the mature NP-5 protein [3]. Because the NP-4 and NP-5 defensins differ in only 5 of 33 amino acids, we searched for the NP-4 cDNA with the *Bam*HI-*Xho*I fragment that contains all but the most 3' end of the NP-5 cDNA. To avoid recloning the highly abundant NP-5 cDNA, we counterscreened with a 16-mer that hybridizes to a region of NP-5 that differs from NP-4 in three amino acid residues. As expected, all the clones hybridizing to the NP-5 probe but not to the 16-mer coded for an NP-4 precursor.

cDNA Sequencing

The sequences of NP-3a, 4, and 5 cDNAs are shown in Figure 1, aligned to emphasize their homology. MCP-1 and 2 sequences, previously published [7], are included for comparison. The NP-3a cDNA sequence was confirmed by cloning the NP-3a gene from an EMBL4 rabbit sperm genomic library provided generously by Dr. Katherine Knight, University of Chicago, Chicago, IL. A typical TATA box consensus located in a position homologous to those of the MCP-1 and MCP-2 genes is found 38 bp upstream of the 5' terminus of the NP-3a cDNA clone, suggesting that the 5' terminus of the NP-3a cDNA is nearly complete.

Homology Among Members of the Defensin Family

Homologies among the cDNA sequences of the three newly cloned defensins NP-3a, NP-4, and NP-5, as well as the two human and two rabbit defensins cloned previously, are diagrammed in Figure 2.

Structure of Preprodefensins

The translations of the open reading frames of the cDNA sequences and a proposed defensin precursor structure are shown in Figure 3. The signal sequences and the indicated signal cleavage sites conform to the known consensus [10]. Charged and conserved residues are emphasized. These putative defensin precursors, as well as those of HNP-1 and 3 [8] and MCP-1 and 2 [7], all have a tripartite structure with a typical signal peptide [9, 10, 14] and an anionic propiece, both of which must be cleaved during granule maturation to generate the mature peptide. There is striking conservation of the signal peptide sequence, of several negatively charged glutamate/aspartate residues in the propiece, and of the positively charged lysine near the mature peptide cleavage site. Interestingly, despite substantial variation in the cationicity of mature defensins, each propiece is anionic to a degree that nearly neutralizes the positive charge of the mature peptide (Fig. 4).

DISCUSSION

We cloned and sequenced the cDNAs for three rabbit neutrophil defensins, NP-3a, NP-4, and NP-5. One of these, NP-3a, has been reported to act as a "corticostatin," a negative regulator of cortisol production by adrenal cells in culture [15]. The homology relationships among the various defensin cDNAs, and especially the occurrence of highly homologous pairs with less homology to the remaining members of the defensin family, constitute further evidence that defensins evolved by repeated gene duplication [7]. Overall, the divergence between human and rabbit defensin cDNA sequences of about 40% over the 80 million years since

TABLE 1. Screening of cDNA Library

Probe	Origin	Specificity	Sequence	Reference
30-mer	30 bp from signal peptide	General defensin	CTGCAGGGCCACCAG GAGAATGGCAGCAAG	[1, 3]
MCP	Central *Pst*I fragment of MCP-2 cDNA	MCP-1 and 2 (NP-1 and 2)		[3]
3'-NP-3a	3' *Eco*RI fragment of NP-3a cDNA	NP-3a	Figure 1	
5'-NP-3a	5' *Eco*RI fragment of NP-3a cDNA	NP-3a	Figure 1	
20-mer	Degenerate oligonucleotide, amino acids 1–7 of NP-5	NP 5	CC(TAG)CGACANGTAC AAAANAC	
Xho-Bam	5' *Xho*I fragment of NP-5 cDNA	NP-4 and 5	Figure 1	
16-mer	Synthetic oligonucleotide	NP-5	GATTCCTTTGTGGGTC	

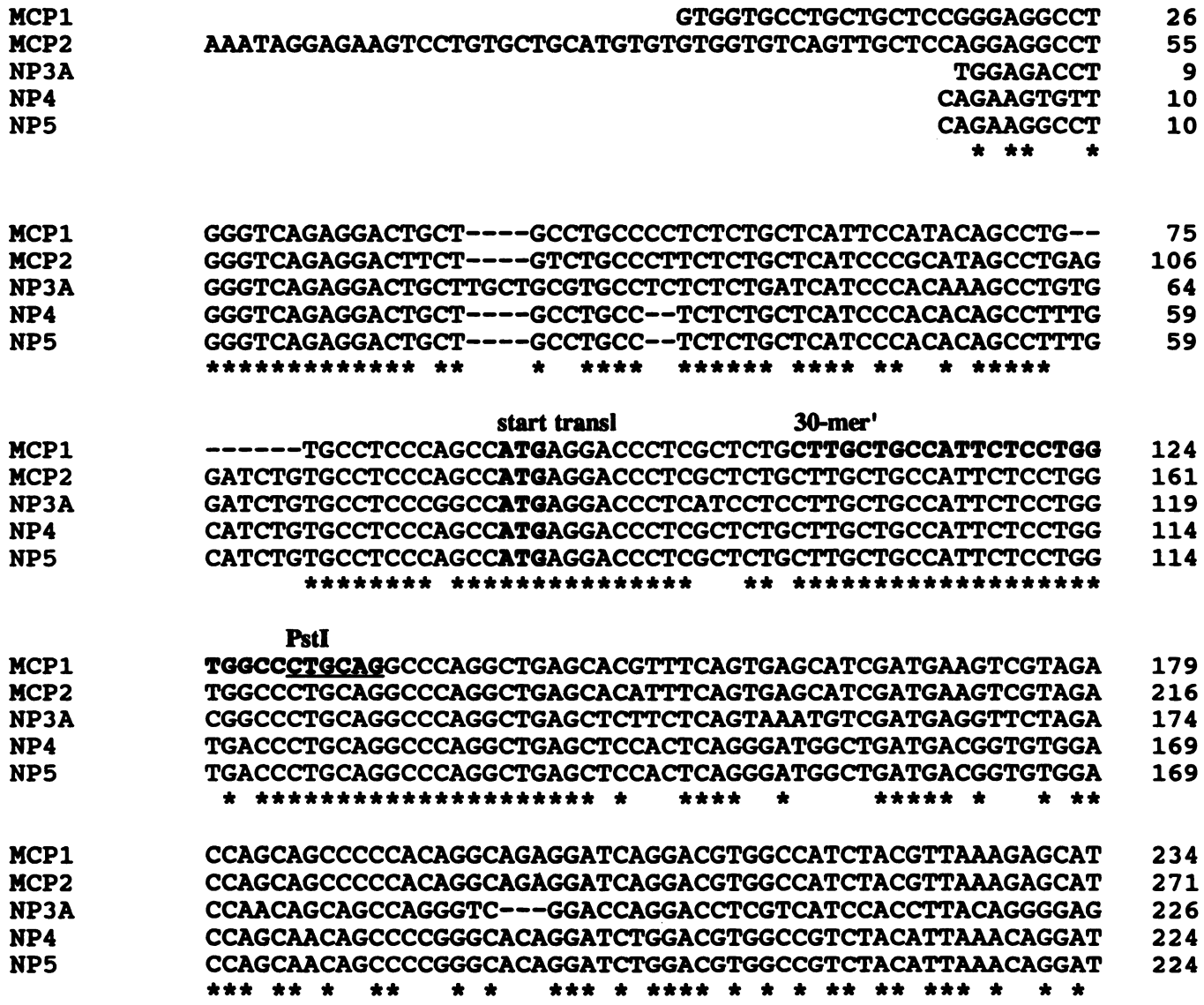

Fig. 1. The cDNA sequences of defensins NP-3a, NP-4, and NP-5 aligned with those of MCP-1 and MCP-2 [7]. Conserved bases are marked with an asterisk. Restriction sites used for generating probe fragments are boldface and underlined. Other features of interest are boldface: sequences corresponding to synthetic oligonucleotide probes 16-mer and 30-mer and codons corresponding to translation start, amino terminus of mature defensins, and translation stop.

mammalian speciation is approximately equal to the rate of divergence of sequences subject to little evolutionary selection [16]. However, several regions of defensin cDNAs show remarkable conservation both between human and rabbit [7] and between various rabbit genes (Fig. 1). The strictly conserved regions (Figs. 1 and 3) correspond to the 5' untranslated region, most of the signal sequence, four anionic amino acid residues in the propiece, a lysine near the junction of the propiece and mature peptide, and the 11 amino acid residues that form the characteristic defensin motif in the mature peptide [1].

The 1–2 amino acid changes in the signal peptide of myeloid defensins since the divergence of rabbit and human correspond to a 1% rate of change per 8–16 million years of evolution. This rate is 4- to 8-fold lower than that for the signal sequences of lysozyme and insulin, the latter about average for signal peptides [17, 18]. The apparent selective conservation of the signal peptide in myeloid defensins from human and rabbit suggests that this part of the defensin precursor or the corresponding region of the mRNA or genomic DNA may have as yet unknown functions that impose additional constraints on evolutionary variation. Cloning of myeloid defensin cDNAs from other species should rule out the possibility that the apparent conservation of the signal sequence is due to chance alone. FASTA search of the Swiss Protein 17.0 and GenPept 64.3 databases [19] for protein sequences homologous to the 19-amino-acid signal peptide of MCP-1 defensin revealed that all proteins with >50% identity in this region belonged to the defensin-cryptdin-corticostatin family [1]. Unlike myeloid defensins, the murine peptide cryptdin, contained in intestinal (nonmyeloid) Paneth cells [20], manifested the expected evolutionary divergence between its signal peptide and that of MCP-1 (only 9 of 19 aa identical).

The nascent defensin peptide must be targeted to the appropriate intracellular compartment, a lysosome-like storage

Fig. 1 Continued.

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                                                                  begin mature def
MCP1    GAGAGCTCCGCTCTTGAAGCTTTAGGTGTAAAGGCAGGTGTGGTCTGTGCGTGCA   289
MCP2    GAGAGCTCCGCTCTTGAAGCTTTAGGTGTAAAGGCAGGTGTGGTCTGTGCGTGCA   326
NP3A    GAAAGCTCTGCTCTTCAAGTCC------CAGATACAAAGGGCATCTGTGCTTGCA   275
NP4     GAAACCTCCCCTCTTGAAGTTTTGGGTGCAAAGGCAGGCGTGTCCTGTACTTGCA   279
NP5     GAAACCTCCCCTCTTGAAGTTTTGGGTGCAAAGGCAGGCGTGTTCTGTACTTGCA   279
        ** * ***  ***** ***          * *  **   *    **** * ****

                16-mer          EcoRI                    PstI
MCP1    GACGAGCCCTCTGTTTGCCTCGGGAACGTCGTGCTGGGTTCTGCAGAATCCGTGG   344
MCP2    GACGAGCCCTCTGTTTGCCTCTGGAACGTCGTGCTGGGTTCTGCAGAATCCGTGG   381
NP3A    GAAGACGCTTTTGCCCGAATTCTGAACGCTTTTCGGGGTACTGCAGAGTCAATGG   330
NP4     GACGATTCAGTTGTGGGTTTGGGGAACGCGCCTCTGGGTCCTGCACAGTCAATGG   334
NP5     GAGGATTCCTTTGTGGGTCTGGGGAACGTGCCTCTGGGTCCTGCACAATCAATGG   334
        ** **  *   **   *  *   *****     * **** ***** * **  ***

                                          stop     stop
MCP1    AAGAATCCACCCACTCTGCTGCCGCCGCTAAGCATGAAAAGCAGAAAAAAAAGCA   399
MCP2    AAGAATCCACCCACTCTGCTGCCGCCGCTAAGCATGAAAAGCAGAAAAAAAAGCA   436
NP3A    AGCCCGCTATGTACGCTGTTGCAG---CAGAAGATGAAGAACAGAAAAA----CG   378
NP4     AGTCCGCCACACACTCTGCTGCCGCCGCTGAGCATCAAAAACAGAAAAAAAAG--   387
NP5     AGTCCGCCACACACTCTGCTGCCGGCGCTGAGCATCAAAAACA-AAGAAAAAG--   386
        *      * *   ** *** *** *   *  *  ** ** * ** ** **

                                  XhoI
MCP1    AGTTCCTTATTTGCCTTGAAACCTCAAGTGAACATGTTTTGACTACTGAGCCT-T   453
MCP2    AGTTCCTTATTTGCCTTGAAACCTCAAGTGAACATGTTTTGACTACTGAGCCT-T   490
NP3A    GATTCCTTATTTGCTTTTACAATTCAAGGGAAACTGTTTCCACTCCTCTGCTATG   433
NP4     AGTTCCTTATTTTCTTGGAGACCTCGAGGGAAAACATTATTACT-CTTTGCCTCA   441
NP5     AGTTCCTTATTTGCGTGGAGACCTCGAGGGAAGATGCTAT-ACC-CTTTGCCTCA   439
          ********** * *  * *  ** ** ***     *   **  **  **

MCP1    CCCCATCCTTTCCTATCCTAG-TAAAGTGCTTGTAATGAAGAG    495
MCP2    CCCCATCCTTTCCTATCCTAG-TAAAGTGCTTGTAATGAAGAG    532
NP3A    TCCTTTGCTTTCCTACCCCAAATAAAATTCTTGAAG           469
NP4     TCTTTTCCTTTCCCACCCTAAATAAAGAGCTTGTAGTC         479
NP5     TCTTTTCCTTTCCCATCCTAAATAAAGAGCTTGTAGTC         477
         *   * ****** * ** *  ****   **** *
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granule [2-6]. The best-characterized signal for lysosomal targeting is the mannose-6-phosphate motif on mannose-rich carbohydrates attached to the Asn-X-Ser/Thr consensus for N-linked glycosylation [21, 22]. Most defensin precursors lack Asn altogether, and none sequenced so far contain the Asn-X-Ser/Thr consensus [7, 8]. Thus it is unlikely that the conventional mannose-6-phosphate receptor mechanism directs the subcellular transport of defensins. Although preprodefensins may contain a recognition patch that marks them for delivery to the cytoplasmic granules, the nature of this motif is not apparent from the comparison of linear amino acid sequences.

At concentrations of 10-100 μg/ml, several members of the defensin family are cytotoxic to mammalian cells [23] and permeabilize artificial lipid bilayers [24]. Because the intracellular concentration of defensins in human PMNs and rabbit PMNs and AMs greatly exceeds their cytotoxic and membrane-permeabilizing concentrations [1], mechanisms that mitigate defensin cytotoxicity must be operative during peptide biosynthesis and in storage compartments (cytoplasmic granules). Similar considerations apply to biosynthetic pathways of proteases (e.g., cathepsin D), where it has been suggested that a short propiece acts as an "activation peptide" that prevents protease activity until the inhibitory propiece is proteolytically removed [25, 26]. We propose that during defensin processing the peptide propiece acts to inhibit the membrane permeabilizing and cytotoxic activity of the mature portion of the peptide by neutralizing the latter's cationic charges. Although the cationic charge of the mature defensins is not the sole determinant of cytotoxicity, the cytotoxic and microbicidal activities of defensins in each mammalian species in general increase with their positive charge [2, 3, 23]. Further, defensin-mediated cytotoxicity is inhibited by polyanionic heparin [23]. Current models of defensin structure based on crystallographic and two-dimensional nuclear magnetic resonance data postulate an amphiphilic molecule with one positively charged face [27]. The insertion of defensins into biological membranes is

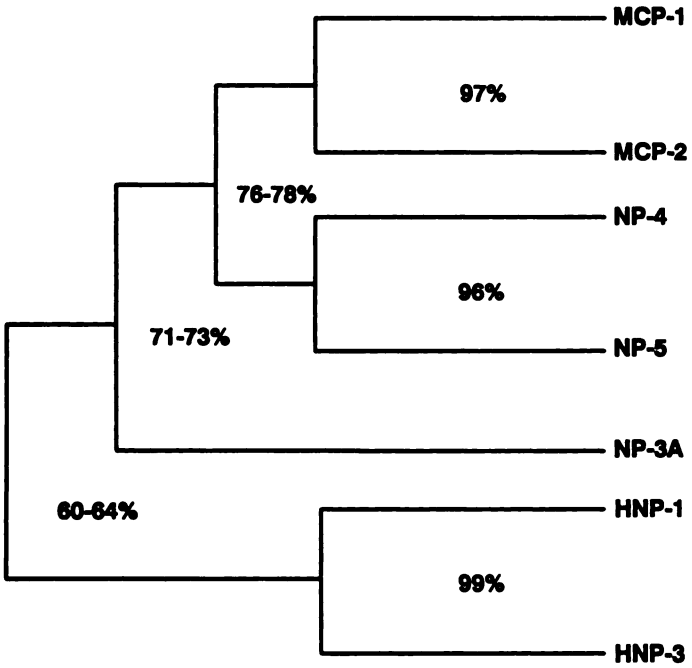

Fig. 2. Homology tree of the seven known myeloid defensin cDNAs. After optimal alignment, homology in the overlap region was calculated for each pair of proteins as homology (%) = (length of overlap − mismatches − gaps)/(length of overlap) × 100%. When one of the two branches in the comparison contains more than one protein, the minimum and maximum homologies between members of the two branches are shown.

thought to be driven by interaction of the transmembrane electromotive force with the cationic aspect of the defensin molecule [24, 27]. All defensin propieces, including that of the less closely related nonmyeloid murine cryptdin [20], have a negative charge that can neutralize the positive charge of the mature peptide. Moreover, anionic residues in the propiece are disproportionately conserved (Fig. 3), suggesting that they serve an important function. Although the three-dimensional structure of the propieces is not yet known, we anticipate that the coiled propiece wraps around the mature portion in such a way that its anionic pockets interact with the cationic regions of the mature protein. This arrangement would block the cationic residues important for cytotoxicity, thus avoiding potential damage to the myeloid cell during defensin processing and intracellular transport. A similar proposal was made by Barker et al. [28] based on their sequencing of the cDNA for human eosinophil granule major basic protein (MBP), a cytotoxic molecule stored in cytoplasmic granules of eosinophilic leukocytes. These workers have shown [29] that polymeric anionic amino acids but not their monomeric counterparts inhibited the cytotoxicity of MBP at concentrations that neutralized the charge of the cationic protein.

The case for the neutralizing function of the anionic propiece of defensins is now strengthened by the correlation between the cationic charge of the mature peptide and the anionic charge of the propiece in the seven defensin precursors described to date. Direct demonstration that charge balance functions to keep defensin propeptides inactive

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SIGNAL SEQUENCE
                1         10
HNP1P           MRTLAILAAILLVALQAQA
HNP3P           MRTLAILAAILLVALQAQA
MCP1P           MRTLALLAAILLVALQAQA
MCP2P           MRTLALLAAILLVALQAQA
NP3AP           MRTLILLAAILLAALQAQA
NP4P            MRTLALLAAILLVTLQAQA
NP5P            MRTLALLAAILLVTLQAQA
                **** .****** .. *****

PROPIECE
                20        30        40        50        60
HNP1P           EPLQARADEVAAAPEQIAADIPEVVVSLAWDESLAPKHPGSRKNM
HNP3P           EPLQARADEVAAAPEQIAADIPEVVVSLAWDESLAPKHPGSRKNM
MCP1P           EHVSVSIDEVVDQQPPQAEDQ-DVAIYVKEHESSALEALGV-KAG
MCP2P           EHISVSIDEVVDQQPPQAEDQ-DVAIYVKEHESSALEALGV-KAG
NP3AP           ELFSVNVDEVLDQQQP-GSDQ-DLVIHLTGEESSALQVPDT-K
NP4P            ELHSGMADDGVDQQQPRAQDL-DVAVYIKQDETSPLEVLGA-KAG
NP5P            ELHSGMADDGVDQQQPRAQDL-DVAVYIKQDETSPLEVLGA-KAG
                *       *.  ... . . *  .... .  .*. . .  . .*

MATURE PROTEIN
                      70        80        90
HNP1P           -ACYCRIPACIAGERRYGTCIYQGRLWAFCC---
HNP3P           -DCYCRIPACIAGERRYGTCIYQGRLWAFCC---
MCP1P           VVCACRRALCLPRERRAGFCRIRGRIHPLCC-RR
MCP2P           VVCACRRALCLPLERRAGFCRIRGRIHPLCC-RR
NP3AP           GICACRRRFCPNSERFSGYCRVNGARYVRCCSRR
NP4P            VSCTCRRFSCGFGERASGSCTVNGVRHTLCC-RR
NP5P            VFCTCRGFLCGSGERASGSCTINGVRHTLCC-RR
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Fig. 3. Amino acid sequences of preprodefensins NP-3a, NP-4, and NP-5 aligned with those of MCP-1 and 2 [7] and HNP-1 and 3 [8]. Conserved residues are marked with an asterisk, partly conserved residues with a dot. Cationic amino acids (R,K) are boldface, anionic amino acids (E,D) are underlined. Sequences were aligned with the PC-GENE Clustal program (Intelligenetics, Palo Alto, CA).

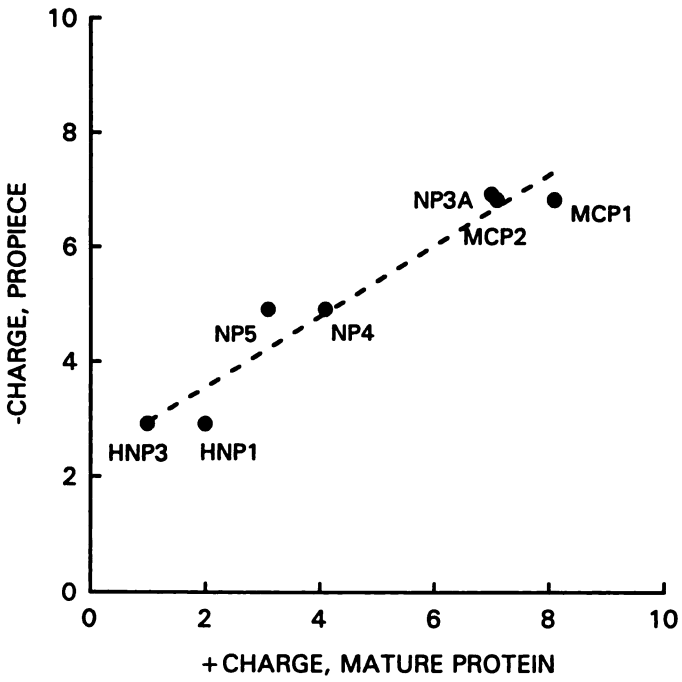

Fig. 4. Plot of the cationic charge of each mature defensin versus the anionic charge of the corresponding propiece for each of the seven known myeloid preprodefensins. The charges are calculated for the intact proprotein where the propiece is joined to the mature peptide.

awaits the purification or synthesis of these propeptides and the preparation of charge variants by mutagenesis or peptide synthesis.

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REFERENCES

1. Ganz, T., Selsted, M.E., and Lehrer, R.I. Defensins. *Eur. J. Haematol.* **44,** 1, 1990.
2. Ganz, T., Selsted, M.E., Szklarek, D., Harwig, S.S., Daher, K., Bainton, D.F., and Lehrer, R.I. Defensins, natural peptide antibiotics of human neutrophils. *J. Clin. Invest.* **76,** 1427, 1985.
3. Selsted, M.E., Brown, D.M., DeLange, R.J., Harwig, S.S., and Lehrer, R.I. Primary structures of six antimicrobial peptides of rabbit peritoneal neutrophils. *J. Biol. Chem.* **260,** 4579, 1985.
4. Selsted, M.E., Brown, D.M., DeLange, R.J., and Lehrer, R.I. Primary structure of MCP-1 and MCP-2, natural peptide antibiotics of rabbit lung macrophages. *J. Biol. Chem.* **258,** 14485, 1983.
5. Patterson-Delafield, J., Martinez, R.J., and Lehrer, R.I. Microbicidal cationic proteins in rabbit alveolar macrophages: a potential host defense mechanism. *Infect. Immun.* **30,** 180, 1980.
6. Rice, W.G., Ganz, T., Kinkade, J.M., Selsted, M.E., Lehrer, R.I., and Parmley, R.T. Defensin-rich granules of human neutrophils. *Blood* **70,** 757, 1987.
7. Ganz, T., Rayner, J.R., Valore, E.V., Tumolo, A., Talmadge, K., and Fuller, F. The structure of the rabbit macrophage defensin genes and their organ-specific expression. *J. Immunol.* **143,** 1358, 1989.
8. Daher, K.A., Lehrer, R.I., Ganz, T., and Kronenberg, M. Isolation and characterization of human defensin cDNA clones. *Proc. Natl. Acad. Sci. USA* **85,** 7327, 1988.
9. Blobel, G., and Dobberstein, B. Transfer of proteins across membranes. I. Presence of proteolytically processed and unprocessed nascent immunoglobulin light chains on membrane-bound ribosomes of murine myeloma. *J. Cell Biol.* **67,** 835, 1975.
10. Verner, K., and Schatz, G. Protein translocation across membranes. *Science* **241,** 1307, 1988.
11. Valore, E.V., and Ganz, T. Posttranslational processing of defensins in immature human myeloid cells. *Blood* **79,** 1538, 1992.
12. Sambrook, J., Fritsch, E.F., and Maniatis, T. *Molecular Cloning.* New York: Cold Spring Harbor Laboratory Press, 1989.
13. Sanger, F., Niklen, S., and Coulson, A.R. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74,** 5463, 1977.
14. von Heine, G. The signal peptide. *J. Membr. Biol.* **115,** 195, 1990.
15. Zhu, Q.Z., Hu, J., Mulay, S., Esch, F., Shimasaki, S., and Solomon, S. Isolation and structure of corticostatin peptides from rabbit fetal and adult lung. *Proc. Natl. Acad. Sci. USA* **85,** 592, 1988.
16. Wu, C.-I., and Li, W.-H. Evidence for higher rates of nucleotide substitution in rodents than in man. *Proc. Natl. Acad. Sci. USA* **82,** 1741, 1985.
17. Li, W., Wu, C., and Luo, C. A new method for estimating synonymous and nonsynonymous rates of nucleotide substitution considering the relative likelihood of nucleotide and codon changes. *Mol. Biol. Evol.* **2,** 150, 1985.
18. Weisman, L.S., Krummel, B.M., and Wilson, A.C. Evolutionary shift in the site of cleavage of prelysozyme. *J. Biol. Chem.* **261,** 2309, 1986.
19. Pearson, W.R., and Lipman, D.J. Improved tools for biological sequence comparison. *Proc. Natl. Acad. Sci. USA* **85,** 2444, 1988.
20. Oullette, A.J., Greco, R.M., James, M., Frederick, D., Naftilan, J., and Fallon, J.T. Developmental regulation of cryptdin, a corticostatin/defensin precursor in mouse small intestinal crypt epithelium. *J. Cell Biol.* **108,** 1687, 1989.
21. Dahms, N.M., Lobel, P., and Kornfeld, S. Mannose 6-phosphate receptors and lysosomal enzyme targeting. *J. Biol. Chem.* **264,** 12115, 1989.
22. Kornfeld, S. Trafficking of lysosomal enzymes. *FASEB J.* **1,** 462, 1987.
23. Lichtenstein, A.K., Ganz, T., Selsted, M.E., and Lehrer, R.I. In vitro tumor cell cytolysis mediated by peptide defensins of human and rabbit granulocytes. *Blood* **68,** 1407, 1986.
24. Kagan, B.L., Selsted, M.E., Ganz, T., and Lehrer, R.I. Antimicrobial defensin peptides form voltage-dependent ion-permeable channels in planar lipid bilayer membranes. *Proc. Natl. Acad. Sci. USA* **87,** 210, 1990.
25. Erickson, A.H. Biosynthesis of lysosomal endopeptidases. *J. Cell. Biochem.* **40,** 31, 1989.
26. Erickson, A.H., Conner, G.E., and Blobel, G. Biosynthesis of a lysosomal enzyme. *J. Biol. Chem.* **256,** 11224, 1981.
27. Hill, C.P., Yee, J., Selsted, M.E., and Eisenberg, D. Crystal structure of defensin HNP-3, an amphiphilic dimer. *Science* **251,** 1481, 1991.
28. Barker, R.L., Gundel, R.H., Gleich, G.J., Checkel, J.L., Loegering, D.A., Pease, L.R., and Hammann, K.J. Acidic poly amino acids inhibit human eosinophil granule major basic protein toxicity. *J. Clin. Invest.* **88,** 798, 1991.
29. Barker, R.L., Gleich, G.J., and Pease, L.R. Acidic precursor revealed in human eosinophil granule major basic protein cDNA. *J. Exp. Med.* **168,** 1493, 1988.