JCI The Journal of Clinical Investigation

Antibiotic proteins of human neutrophils.

J K Spitznagel

J Clin Invest. 1990;86(5):1381-1386. https://doi.org/10.1172/JCI114851.

Research Article

Antibiotic Proteins of Human Neutrophils

John K. Spitznagel
Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, Georgia 30322

Introduction

Despite strenuous opposition, Ilya Metchnikov convinced the scientific world 110 years ago that phagocytosis has a major role in immunity to infection (1).[1] Metchnikov conjectured that phagocytes destroy microbes with their enzymes, or cytases as he called them, but proof of this had to wait for new tools. The field moved when Cohn and Hirsch (1) revealed the role of degranulation in phagocytosis. Hirsch described phagocytin (1). Spitznagel and Chi (1) and Zeya and Spitznagel (1) showed that phagocytin is not one but an array of antibacterial proteins, uniquely cationic, associated with cytoplasmic granules, and coated on bacteria during phagocytosis. Klebanoff described the myeloperoxidase (MPO),[2] H_2O_2, Cl^- antimicrobial system (1), and Gray described the oxidative defect in neutrophils and monocytes of children with X-linked chronic granulomatous disease (2). The duality of the killing mechanisms, oxygen independent and oxygen dependent, and the association of many of their components with cytoplasmic granules became accepted. I deal here with one aspect of research on oxygen-independent mechanisms, the antibiotic proteins of human neutrophils. These proteins comprise at least three structurally different families that share the capacity to kill bacteria independent of oxygen and evidently, contrary to Metchnikov's conjecture, to do so independent of enzymic action.

Current definition of antibiotic proteins

Seven proteins associated with the azurophil granules of human neutrophils are currently in center stage owing to their potent antibioticlike actions (see Table I). In ascending order according to their apparent molecular weights, they are: defensins, HNP-1, HNP-2, and HNP-3,[3] HNP-4 (3); cathepsin G (CatG) (1); CAP37/azurocidin (4–6); and BPI/CAP57/BP (1, 4, 7). That there may be more is reflected in the recent work of Gabay et al. (6) who described HNP-4, a recent addition to the defensins (3), and p29b, a newly described serine proteinase. I shall not deal with lactoferrin or lysozyme because both have been reviewed (1). The seven proteins will be dealt with in three major groups: defensins, CatG and CAP37/azurocidin, and BPI/CAP57/BP. Differences within groups will probably be resolved in the future or be confirmed and their significance clarified.

Antimicrobial action: range and specific activities

Considered as a group, the antibiotic proteins have a wide range, being active against gram positive and gram negative bacteria, viruses, and fungi. Their specific activities vary depending on the protein, the pH, ionic strength, temperature, the presence of nutrients in the test medium, and the species and strain of microbe (1). Table II shows ranges and approximate activities for the most studied antibiotic proteins (3, 5, 7, 8, 9, 10, 27).

Structure and molecular biology

The human defensins comprise a group of four small, strikingly similar cyclic peptides (see Fig. 1). The first three differ only in their NH_2-terminal amino residues, appear unglycosylated, and bear strong homology to defensins of other species (11). Each has six cysteines, is relatively rich in arginine and aromatic amino acids (3, 27). Their cyclic structure is unique and depends on disulfide bonds formed between cysteines (11). HNP-4 is similar, has a similar arrangement of cysteines as do the other defensins, but only 11 of the 33 amino acid positions are alike (3). Two crystalline forms of a human defensin, HNP-1, have been reported (12).

Human defensin cDNA clones show the predicted proteins are produced as 94 residue precursors that are placed in the cell membrane system due to hydrophobic NH_2-terminal signal peptides and are eventually processed to the 30–33 residue proteins found in the neutrophil cytoplasmic granules (13). Interestingly only cDNA coding for HNP-1 and HNP-3 were cloned from the HL60 library used. Thus, HNP-2 may be actually HNP-1 or HNP-3 with its NH_2-terminal amino acid cleaved off. Normal bone marrow cells, but not peripheral blood cells, expressed defensin mRNA.

Cathespin G (Fig. 2) shows strong homologies with elastase, CAP37/azurocidin, and several inflammatory proteinases. CAP37 and azurocidin are probably identical, although

Address reprint requests to John K. Spitznagel, M.D., Professor and Chairman, Dept. of Microbiology and Immunology, Emory University School of Medicine, 3001 Rollins Research Center, Atlanta, GA 30322.

Received for publication 3 April 1990 and in revised form 6 July 1990.

1. Owing to space constraints, this and other citations in print 1984 or earlier will be referred to citation 1 in the reference list.

2. *Abbreviations used in this paper:* BP, bactericidal protein; BPI, bactericidal/permeability increasing protein; CAP37, cationic antimicrobial protein of M_r 37,000 D; CAP57, cationic antimicrobial protein of M_r 57,000 D; CatG, cathepsin G; CGD, chronic granulomatous disease; CH, Chediak-Higashi syndrome; HNP, human neutrophil peptides; LBP, lipopolysaccharide binding protein; MPO, myeloperoxidase; SGD, specific granule deficiency.

J. Clin. Invest.

0021-9738/90/11/1381/06 $2.00
Volume 86, November 1990, 1381–1386

3. Owing to space constraints, this and other citations in print 1989 or earlier will be referred to citation 27 in the reference list.

Table I. Attributes of Antibiotic Proteins

	% Total neutrophil protein*	M_r‡	Amino acid residues in mature protein	% Arginine	% Lysine	Ret. time§
Defensins	5.28–8	<4	29–33	4	0	11.3
CatG	.54	28	236	34	16	16.0
CAP37/Azur	.45	29–37	222	9	2	19.5
BPI/CAP57/BP	.26	55–58	456	10	26	26.0

* Crude granule extract is about 16% of total neutrophil protein. ‡ Gram molecular weight × 10^3 estimated by SDS-PAGE. § Time in minutes of elution from C4 RP-HPLC column with acetonitrile in trifluoroacetic acid gradient.

apparent differences in their molecular weights await explanation. CatG cDNA has been cloned (14) and the inferred amino acid sequence shows it is produced as a pre-pro protein. CAP37/azurocidin has recently been cloned (Morgan, J., personal communication) and shows similar structure suggesting it is produced as a pre-pro protein. In it the cysteines seen in CatG are conserved as well.

CAP37/azurocidin is unusual because, although it has 50% or greater homology with elastase, it fails to perform as a serine proteinase (6, 15) due to replacement of its catalytic His-41 residue (see Fig. 2) by a serine residue (15). Moreover, it contains several constituent peptides not heretofore described in any serine proteinase (Morgan, J., personal communication). The conservation in the neutrophil of a molecule of this family with antibacterial and other properties (see below) yet incapable of serine proteinase activity suggests that it has a unique role in host defense.

A predicted structure of BPI, determined by microsequencing of its NH_2-terminal 27 residues and inferred from sequencing of its cDNA (16, 17), reveals a high proportion of basic and hydrophobic residues among its NH_2-terminal 27 amino acids (see Fig. 3). Rabbit BPI and rabbit lipopolysaccharide binding protein (LBP), described by Tobias and Ulevitch (18), share with human BPI substantial homologies and are closely related to each other but not to other known proteins. We have recently sequenced the NH_2-terminal residues of CAP57, an antibiotic protein (19). Its first 27 NH_2-terminal residues are identical with those of BPI (see Fig. 3). We found that our monoclonal and polyclonal antibodies to CAP57 (20) in Western blots and ELISA were completely reactive with BPI kindly furnished by Randy Scott (Invitron Corp., Redwood City, CA). We believe CAP57 is identical to BPI. BP, reported by Hovde and Gray (7), has an amino acid composition close to that of BPI and its NH_2-terminal residues are 100% homologous with those of BPI (Gray, B. H., personal communication).

Ooi et al. (21) have found that antimicrobial capacity of BPI/CAP57/BP is confined to the NH_2-terminal 25-kD domain of BPI. That domain is cationic and amphipathic (17). That antimicrobial action is confined to one domain or structure of BPI/CAP57/BP is further supported by our discovery of a monoclonal antibody, P1G8, that specifically binds and inhibits the antimicrobial action of the protein, whereas another one, P2A5, binds but fails to inhibit (20). Near the NH_2-terminus of CatG, Bangalore et al. (21a) have identified two small peptides with broad antibacterial action in vitro.

The COOH-terminal nonantibiotic region of BPI/CAP57/BP appears to be relatively anionic and very hydrophobic and may be an anchor that holds the holo-protein in the membrane of the granule (17). Northern blot technique has shown that BPI mRNA is only expressed in cells of the granulocyte series.

Antibacterial action of the proteins, binding, membrane damage, replication

It was clear from Hirsch's early studies that "phagocytin" became bound to bacteria and Modrzakowski showed that this effect probably was due in *S. typhimurium* to binding to LPS (1). Mannion et al. (22) extended this view in studies with BPI/CAP57/BP and *E. coli*. Shafer and Onunka (23) showed that CatG binds to *S. aureus* teichoic acids. According to Farley, binding of BPI/CAP57/BP to *S. typhimurium* was specific and saturable (24). The impaired binding of protein by strains rendered resistant to the antibiotic protein by mutation that blocks the strong negative charges of the lipid A phosphoryl groups suggested that those negative charges are binding sites for the positively charged basic protein. The superior capacity of polymyxin B to block binding of protein to *Salmonella* compared to the weak blocking power of the deacylated poly-

Table II. Antibiotic Range and Activity

	Defensins	CatG	CAP37/Azur	BPI/CAP57/BP
Range	GN, GP, Y, V*	GN	GN	GN
Activity‡	1–50 μg/ml	0.1–50 μg/ml	1–5 μg/ml	0.1–5 μg/ml

* GN, gram negatives, primarily rough enterics; GP, *S. aureus* and *S. faecalis;* Y, yeasts; V, *Herpesviridae.*
‡ Activities vary considerably. Only defensin HNP1 is effective at 1 μg/ml. Only against gonococci is CatG effective at 0.1 μg/ml. Some smooth enterics resist 30 μg BPI/CAP57/BP but *P. aeruginosa* are sensitive to 20–30 ng/ml.

```
                    10          20          30
HNP-1   ACYCRIPACIAGERRYGTCIYQGRLWAFCC
HNP-2    CYCRIPACIAGERRYGTCIYQGRLWAFCC
HNP-3   DCYCRIPACIAGERRYGTCIYQGRLWAFCC
HNP-4   VCSCRLVFCRRTELRVGNCLIGGVSFTYCC
```

Figure 1. NH_2-terminal amino acid sequences of human defensins (3, 27).

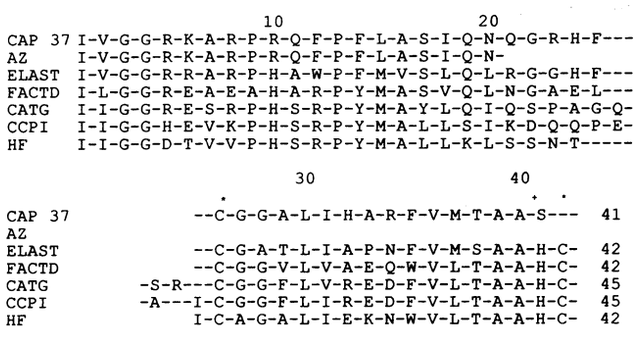

Figure 2. Composite alignment of the amino acid sequences of CAP37 with several inflammatory cell serine proteinases. ELAST (elastase), FACTD (complement factor, D), CATG (cathepsin G), CCPI (cytotoxic T cell protease, I), HF (cytotoxic T cell protease, H factor), AZ (azurocidin). *Position of disulfide bonds. ‡Expected location of 'his' residue to the serine protease catalytic triad (15).

myxin B nonapeptide suggested that hydrophobic bonds were of equal or greater importance than ionic bonds in stabilizing the bound protein in the outer membrane (24). The capacity of the antibiotic proteins to insert in hydrophobic membranes is further suggested by the elegant demonstration by Kagan et al. (25) that defensins form voltage-dependent ion-permeable channels in lipid bilayers. Shafer has shown that CatG binds to the penicillin binding protein 2 of gonococci, an inner membrane protein, thus providing another binding mechanism and directing attention to the cytoplasmic membrane (26). The binding may be lethal due to its capacity to inhibit the transpeptidase reaction needed for peptidoglycan synthesis. It is reasonable to conclude, from available evidence, that membrane damage is a principal effect of the antibiotic proteins (1, 27–30). It appears that in gram negatives the outer membrane is the principal defensive organ and rough mutants are more sensitive than smooth parents as Rest has shown (1), but ultimately it is damage to the cytoplasmic membrane that kills the microbe. In gram positives the capsules, thick peptidoglycan and acidic molecules such as teichoic acids, are defensive: the cytoplasmic membrane is the target of lethal action. Further study is needed to discover the subtle differences between the actions of the different classes of proteins.

All of the antibiotic proteins in their effective concentrations are rapidly bactericidal (1, 4, 27). Bacterial death correlates closely with cytoplasmic membrane damage (27).

Other actions

So much attention has been focused on the antibiotic properties of the granule proteins that the possibility they serve some other physiological function has remained relatively unexplored. However, a few unexpected, interesting, and possibly important functions have been identified. Defensins for example are cytotoxic for tumor cells (27), further evidence of their capacity to damage membranes. The enzymic activity of CatG has been recognized (1) for some time and is consistent with its primary serine proteinaselike structure (14). Although the antimicrobial property of CatG is independent of its enzymic action, its proteolytic action is likely to contribute to digestion of killed microbes.

CAP37/azurocidin is a potent chemotactic agent specific for monocytes and may conceivably have a substantial role in the influx of monocytes in inflammation (31). Its activity is 80–100% that of FMLP (31). Defensins have been reported to be chemotactic (32), but at levels 10–20% that of FMLP.

BPI/CAP57/BP has been established as a member of a family of proteins that bind lipopolysaccharides (22). No catalytic activity has been ascribed to this protein. It has been reported recently that it inhibits the LPS-induced upregulation of CR1 and CR3 on neutrophil surfaces, suggesting that BPI/CAP57/BP modulates or moderates the effects of LPS on the activities of neutrophils (18, 33).

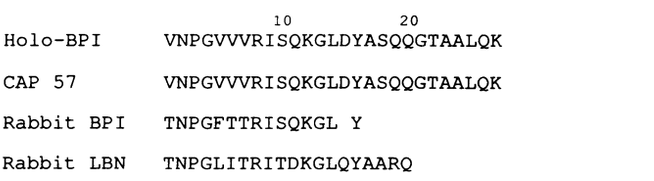

Figure 3. NH_2-Terminal amino acid residues of BPI, CAP57, and LBP (18, 19, 21).

Location and translocation

How can substances as reactive as antibiotic proteins be delivered in effective antimicrobial form to microbes in host tissues? It is possible because they are packaged and carried in the azurophil granules of PMN. The work of Cohn and Hirsch, Spitznagel, Rest and others has shown how this is done (1). The contents of the azurophil granules enter phagolysosomes during phagocytosis, transiently appear bound to microbes entering nascent phagosomes at the neutrophil surface, and quickly are sealed in phagolysosomes (1).

Human defensins are confined to neutrophils where they comprise 30–50% of the granule proteins (6, 27) and are stored in a heavy subset of the azurophil granules (27). They are translocated to phagolysosomes during phagocytosis and deposited there through degranulation, achieving high concentrations. < 8% may be released from the cell during phagocytosis (34). This meager release makes it seem unlikely they exercise an extracellular function. Their optimum antimicrobial activity is said to be pH ~ 7.0, so they probably find a hydrogen ion concentration optimum for their activity, at least in the early phase of phagolysosome formation (1). CatG may comprise as much as 18% of the azurophil granule membrane protein. It is confined to neutrophils and to the azurophil granules (1, 6). Consistent with this, CatG appears in the promyelocytes of the bone marrow and is present in myelocytes and other more mature forms (1). Owing to the segregation of azurophil granules during cell division, the more mature cells contain less CatG than the promyelocytes and myelocytes. Although direct evidence is lacking, it is likely that CatG also enters the phagolysosome during phagocytosis and degranulation, as does myeloperoxidase (1). Its optimum hydrogen ion concentration for antimicrobial action is broad against gonococci (9) but narrow against *S. aureus* (1). The pH should be suitable throughout the life of phagolysosomes.

CAP37/azurocidin also is a component of the azurophil granules (5, 35) with a concentration of ~ 11 fg per cell (31). It is expressed first in promyelocytes. In the mature neutrophil it comprises ~ 17% of proteins extracted from azurophil granule membranes (6). Its antimicrobial activity is maximal at pH 5.7, so it may become increasingly active as phagolysosomes mature (1). 80% or more of CAP37/azurocidin is released from

neutrophils during phagocytosis (15 and see above). Extracellular release of this chemotactic protein may account for the influx of monocytes into inflammatory foci infiltrated by neutrophils (15).

BPI/CAP57/BP is expressed in the neutrophil granulocyte line (17, 19, 36). It is synthesized and stored in the azurophil granules during the promyelocyte and early myelocyte stages of the neutrophil. In mature neutrophils it comprises 2–4% of the granule proteins. Spitznagel et al. (1) showed with density gradient centrifugation and Rice et al. (27) confirmed that azurophil granules are heterogeneous in density and component proteins. BPI/CAP57/BP is concentrated in the least dense azurophil granules according to Percoll sedimentation (19). The localization of different antibiotic proteins in different subpopulations of granules suggests different pathways, different timing, or both, for packaging in and perhaps for release from granules. BPI/CAP57/BP is only sparingly released from neutrophils during phagocytosis (31, 36).

Intraphagosomal events

What actually happens in the phagolysosome? The hydrogen ion concentration in the neutrophil phagolysosome drops to low levels concomitant with its formation. It is clear from available publications that the ranges of pH optima determined for the granule proteins fall within even the narrow estimates of phagolysosome pH. Shafer has pointed out that one or another of the contents, including the most powerfully antibacterial ones, would potentially be optimally active at some point in the history of the phagolysosome (5). It is important to note here that several investigators have gone to some trouble to show that oxygen-independent killing actually occurs (1). Most recently *E. coli* has been shown to be killed both by anaerobic normal neutrophils and by chronic granulomatous disease (CGD) neutrophils. The question was, and is, what killed them. Stinavage took advantage of a mutant, BPI/CAP57/BP-resistant *S. typhimurium* and its isogenic parent to show that the resistant mutant had a clear advantage and survived in larger numbers than the sensitive parent when phagocytized by anaerobic neutrophils (37). This was even true under aerobic conditions, suggesting that intraneutrophilic *Salmonella* are largely killed by the oxygen-independent pathways (37, 38) even when oxygen is available. Unfortunately the experiment fails to give the answer as to which antibiotic protein should get the credit because this strain of *S. typhimurium* is also resistant to CAP37/azurocidin (5). In any event, cationic antimicrobial proteins of neutrophils seem to have an antibiotic role in the phagolysosome.

Genetics and role in disease

It is truly exciting to find we now have knowledge of neutrophil antimicrobial proteins that places us at the threshold of clinical uses. Perhaps the cumulative work reviewed above affords us ways to study the pathophysiology and genetics of disorders of antibiotic proteins as we were afforded ways to study the disorders in oxidative killing by Holmes' discoveries in CGD (39).

Ganz et al. (40), describing antibiotic protein deficiencies have provided the first view of clinical possibilities and have helped to define practical problems. For example, normal values need to be established and this implies a need to establish methods to assure 100% recoveries of the cell proteins of interest. Ganz et al. reported the screening of 50 persons, 17 normal volunteers and 33 patients with various diseases, mostly genetically determined disorders involving the neutrophils. These people were screened for neutrophil levels of defensins, CatG, elastase, lactoferrin, and lysozyme. Severely depressed defensin levels were found in neutrophils of two patients with specific granule deficiency (SGD). Unfortunately the pathogenesis and genetics of SGD are not well defined. The first case of SGD reported was clearly acquired (1), and that 40-yr-old patient was Philadelphia chromosome positive. Most of the subsequently reported cases were children born of nonconsanguineous unions (39). In any event, Ganz's results suggest that the vulnerability of SGD sufferers to infection (39) may be due, at least in part, to defensin deficiency. The molecular genetics of the deficiency were not defined. Nevertheless, the results are stimulating and show the need for far more extensive studies. Studies on patients with disorders of antibiotic proteins will not, however, be straightforward. There is presently no equivalent to the relatively simple screening tests applicable to CGD. Moreover, there are several different antibiotic proteins, each coded by a different gene. As far as is known, the antibiotic proteins act in parallel in vivo and not in series as do the proteins in an interdependent enzyme pathway.

CAP37/azurocidin and BPI/CAP57/BP for example are substantially more potent than the defensins, but the defensins are abundant. Because it is unlikely that mutation would affect all proteins at once, vulnerability to infection may not be a dramatic indicator of deficiency. In Ganz's paper the methodology hinged on complex and expensive technology. In the future, highly specific oligonucleotide probes will be coming on line (13, 14, 17, 41) that will find a place in in situ hybridization and other molecular diagnostic techniques. Such approaches have already shown that defensin genes are localized to human chromosome 8p23 (41). The availability of such techniques will permit highly specific screening in a variety of modalities but the investigations will still be costly.

The three patients with Chediak-Higashi (CH) syndrome had severely depressed elastase and CatG levels (40). as have animals with CH (39). CH is inherited by an autosomal recessive mechanism. Disorders involving deficiency of this enzyme appear to be confined at present to the CH syndrome where the deficiency is one of a constellation of phenotypic abnormalities. None of these can be unequivocally assigned to the manifestations of the disease.

Disorders involving deficiency of CAP37/azurocidin or BPI/CAP57/BP have not yet been reported. The genetic control of these proteins is unknown.

Conclusions

In the closing century the work of Metchnikov has culminated, with the help of many investigators and advances in protein chemistry, molecular biology, microbial pathogenesis, and cell biology, in solid evidence for the existence and O_2-independent function of cationic antibiotic granule proteins in neutrophils of humans and other species. These proteins are delivered to microbes in the tissues by neutrophils where they bind to the microbial cells and disrupt complex membrane functions in the microbes. Much remains to be learned: Are there disorders that involve excesses or absence of antibiotic proteins? What are the molecular genetics of these proteins? What other functions do they mediate beyond microbicidal effects? The accumulated research, purified proteins, cDNA, and anti-

bodies, both polyclonal and monoclonal, all promise, in the words of J. B. Conant, President, Harvard University, 1933-1953, to "reduce the degree of empiricism and to extend the range of hypothesis" in our understanding of the pathophysiology of neutrophil polymorphonuclear granulocytes.

Acknowledgments

I want to express my appreciation to Dr. William Shafer and Dr. Anne Pereira for their help in reviewing this paper and making good suggestions. I also thank Mrs. Polly Worthy for the careful attention she gave to the preparation of this manuscript.

The preparation of this article was supported in part by research grants from the National Institutes of Health (ROl AIl7662 and ROl AI26589).

References

1. Spitznagel, J. K. 1984. Nonoxidative antimicrobial actions of leukocytes. *In* Contemporary Topics in Immunobiology, Regulation of Leukocyte Functions. R. Snyderman, editor. Plenum Publishing Corp., New York. 283–343.

2. Holmes, B., A. R. Page, and R. A. Good. 1967. Studies on the metabolic activity of leukocytes from patients with a genetic abnormality of phagocyte function. *J. Clin. Invest.* 46:1422–1432.

3. Wilde, C. G., J. E. Griffith, M. N. Marra, J. L. Snable, and R. W. Scott. 1989. Purification and characterization of human neutrophil peptide 4, a novel member of the defensin family. *J. Biol. Chem.* 264:11200–11203.

4. Shafer, W. M., L. E. Martin, and J. K. Spitznagel. 1984. Cationic antimicrobial proteins isolated from human neutrophil granulocytes in the presence of diisopropyl fluorophosphate. *Infect. Immun.* 45:29–35.

5. Shafer, W. M., L. E. Martin, and J. K. Spitznagel. 1986. Late intraphagosomal hydrogen ion concentration favors the *in vitro* antimicrobial capacity of a 37-kilodalton cationic granule protein of human neutrophil granulocytes. *Infect. Immun.* 53:651–655.

6. Gabay, J. E., R. W. Scott, D. Campanelli, J. Griffith, C. Wilde, M. N. Marra, M. Seeger, and C. F. Nathan. 1989. Antibiotic proteins of human polymorphonuclear leukocytes. *Proc. Natl. Acad. Sci. USA.* 86:5610–5614.

7. Hovde, C. J., and B. H. Gray. 1986. Characterization of a protein from normal human polymorphonuclear leukocytes with bactericidal activity against *Pseudomonas aeruginosa. Infect. Immun.* 541:142–148.

8. Greenwald, G. I., and T. Ganz. 1987. Defensins mediate the microbicidal activity of human neutrophil granule extract against acinetobacter calcoaceticus. *Infect. Immun.* 55:1365–1368.

9. Shafer, W. M., V. C. Onunka, and L. E. Martin. 1986. Antigonococcal activity of human neutrophil cathepsin G. *Infect. Immun.* 54:184–188.

10. Shafer, W. M., S. A. Engle, L. E. Martin, and J. K. Spitznagel. 1988. Killing of *Proteus mirabilis* by polymorphonuclear leukocyte granule proteins: evidence for species specificity by antimicrobial proteins. *Infect. Immun.* 56:51–53.

11. Selsted, M. E., and S. S. Harwig. 1989. Determination of the disulfide array in the human defensin HNP-2. A covalently cyclized peptide. *J. Biol. Chem.* 264:4003–4007.

12. Stanfield, R. L., E. M. Westbrook, and M. E. Selsted. 1988. Characterization of two crystal forms of human defensin neutrophil cationic peptide 1, a naturally occurring antimicrobial peptide of leukocytes. *J. Biol. Chem.* 12:5933–5935.

13. Daher, K. A., R. I. Lehrer, T. Ganz, and M. Kronenberg. 1988. Isolation and characterization of human defensin cDNA clones. *Proc. Natl. Acad. Sci. USA.* 85:7327–7331.

14. Salvesen, G., D. Farley, J. Shuman, A. Przybyla, C Reilly, and J. Travis. 1987. Molecular cloning of human cathepsin G: structural similarity to mast cell and cytotoxic T lymphocyte proteinases. *Biochemistry.* 26:2289–2293.

15. Pereira, H. A., J. K. Spitznagel, J. Pohl, D. E. Wilson, J. Morgan, I. Palings, and J. W. Larrick. 1990. CAP 37, a 37 KD neutrophil granule cationic protein shares homology with inflammatory proteinases. *Life Sci.* 46:189–196.

16. Weiss, J., C. E. Ooi, and P. Elsbach. 1986. Structural and immunological dissection of highly conserved bactericidal/permeability-increasing proteins. *Clin. Res.* 34:537a. (Abstr.)

17. Gray. P. W., G. Flaggs, S. R. Leong, R. J. Gumina, J. Weiss, C. E. Ooi, and P. Elsbach. 1989. Cloning of the cDNA of a human neutrophil bactericidal protein. Structural and functional correlations. *J. Biol. Chem.* 264:9505–9509.

18. Tobias, P. S., G. C. Mathison, and R. J. Ulevitch. 1988. A family of lipopolysaccharide binding proteins involved in responses to gram negative sepsis. *J. Biol. Chem.* 263:13479–13481.

19. Pereira, H. A., J. K. Spitznagel, E. F. Winton, W. Shafer, L. E. Martin, G. S. Guzman, J. Pohl, R. W. Scott, M. M. Marra and J. M. Kinkade, Jr. 1990. The ontogeny of a 57kD cationic antimicrobial protein of human polymorphonuclear leukocytes: localization to a novel granule population. *Blood.* In press.

20. Spitznagel, J. K., H. A. Pereira, L. E. Martin, G. S. Guzman, and W. M. Shafer. 1987. A monoclonal antibody that inhibits the antimicrobial action of a 57 kD cationic protein of human polymorphonuclear leukocytes. *J. Immunol.* 139:1291–1296.

21. Ooi, C. E., J. Weiss, P. Elsbach, B. Frangione, and B. Mannion. 1987. A 25-kDa NH_2-terminal fragment carries all the antibacterial activities of the human neutrophil 60-kDa bactericidal/permeability increasing protein. *J. Biol. Chem.* 262:14891–14894.

21a. Bangalore, N., J. Travis, V. C. Onunka, J. Pohl, and W. M. Shafer. 1990. Identification of the primary antimicrobial domains in human neutrophil cathepsin G. *J. Biol. Chem.* 266:13584–13588.

22. Mannion. B. A., I. S. Kalatzis, J. Weiss, and P. Elsbach. 1989. Preferential binding of the neutrophil cytoplasmic granule-derived bactericidal/permeability increasing protein to target bacteria. *J. Immunol.* 142:2807–2812.

23. Shafer, W. M., and V. C. Onunka. 1989. Mechanism of staphylococcal resistance to non-oxidative antimicrobial action of neutrophils:importance of pH and ionic strength in determining the bactericidal action of cathepsin G. *H. Gen. Microbiol.* 135:825–830.

24. Farley, M. M., W. M. Shafer, and J. K. Spitznagel. 1988.Lipopolysaccharide structure determines ionic and hydrophobic binding of a cationic antimicrobial neutrophil granule protein. *Infect. Immun.* 56:1589–1592.

25. Kagan, B. L., M. E. Selsted, T. Ganz, and R. I. Lehrer. 1990. Antimicrobial defensin peptides form voltage-dependent ion-permeable channels in planar lipid bilayer membranes. *Proc. Natl. Acad. Sci. USA.* 87:210–214.

26. Shafer, W. M., V. C. Onunka, M. Jannoun, and L. W. Huthwaite. 1990. Molecular mechanism for the antigonococcal action of cathepsin G. *Mol. Microbiol.* In press.

27. Lehrer, R. I., A. Barton, K. A. Daher, S. S. Harwig, T. Ganz, and M. E. Selsted. 1989. Interaction of human defensins with *Escherichia coli.* Mechanism of bactericidal activity. *J. Clin. Invest.* 84:553–561.

28. Viljanen, P., P. Koski, and M. Vaara. 1988. Effect of small cationic leukocyte peptides (defensins) on the permeability barrier of the outer membrane. *Infect. Immun.* 56:2324–2329.

29. Weiss, J., K. Muello, M. Victor, and P. Elsbach. 1984. The role of lipopolysaccharides in the action of the bactericidal/permeability increasing neutrophil protein on the bacterial envelope. *J. Immunol.* 132:3109–3115.

30. Hovde, C. J., and B. H. Gray. 1986. Physiological effects of a bactericidal protein from human polymorphonuclear leukocytes on *Pseudomonas aeruginosa. Infect. Immun.* 52:90–95.

31. Pereira, H. A., W. M. Shafer, J. Pohl, L. E. Martin, and J. K.

Spitznagel. 1990. CAP37, a human neutrophil-derived chemotactic factor with monocyte specific activity. *J. Clin. Invest.* 85:1468–1476.

32. Territo, M. C., T. Ganz, M. E. Selsted, and R. I. Lehrer. 1989. Monocyte chemotactic activity of defensins from human neutrophils. *J. Clin. Invest.* 84:2017–2020.

33. Marra, M. N., C. G. Wilde, J. E. Snable, J. L. Griffith, and R. W. Scott. 1990. Bactericidal/permeability increasing protein has endotoxin neutralizing activity. *J. Immunol.* 144:662–666.

34. Ganz, T. 1987. Extracellular release of antimicrobial defensins by human polymorphonclear leukocytes. *Infect. Immun.* 55:568–571.

35. Campanelli, D. C., P. A. Detmers, C. F. Nathan, and J. E. Gabay. 1990. Azurocidin and a homologous serine protease from neutrophils: differential antimicrobial and proteolytic properties. *J. Clin. Invest.* 85:904–915.

36. Weiss, J., and I. Olsson. 1987. Cellular and subcellular localization of the bactericidal/permeability-increasing protein of neutrophils. *Blood.* 69:657–659.

37. Stinavage, P., L. E. Martin, and J. K. Spitznagel. 1989. O antigen and lipid A phoshoryl groups in resistance of *Salmonella typhimurium* LT-2 to nonoxidative killing in human polymorphonuclear neutrophils. *Infect. Immun.* 57:3894–3900.

38. Weiss, J., L Kao, M. Victor, and P. Elsbach. 1985. Oxygen-independent intracellular and oxygen-dependent extracellular killing of *Escherichia coli* S15 by human polymorphonuclear leukocytes. *J. Clin. Invest.* 76:206–212.

39. Rotrosen, D., and J. I. Gallin. 1987. Disorders of phagocyte function. *In* Ann. Rev. Immunol. W. E. Paul, C. G. Fathman, and H. Metzger, editors. Annual Reviews Inc; Palo Alto. 5:127–150.

40. Ganz, T., J. A. Metcalf, J. I. Boxer, L. A. Gallin, and R. I. Lehrer, 1988. Microbicidal/cytotoxic proteins of neutrophils are deficient in two disorders: Chediak-Higashi syndrome and "specific" granule deficiency. *J. Clin. Invest.* 82:552–556.

41. Sparkes, R. S., M. Kronenberg, C. Heinzmann, K. A. Daher, I. Klisak, T. Ganz, and T. Mohandas. 1990. Assignment of defensin gene(s) to human chromosome 8p23. *Genomics.* 5:240–244.