A new family of cysteine-rich antimicrobial peptides from bovine neutrophils was isolated and characterized. Thirteen structurally homologous peptides were purified to homogeneity from a granule-rich cytoplasmic fraction of purified blood neutrophils. The complete sequences of the peptides were determined by a combination of enzymatic digestion, Edman degradation, and additional biochemical characterization of the carboxyl termini. The peptides are characterized by a highly cationic 38-42-residue chain which includes 6 invariantly spaced cysteines which form three disulfides. They share a highly conserved consensus sequence which is also found in a recently described epithelial antimicrobial peptide from bovine trachea. The in vitro antibacterial activities of the 13 neutrophil peptides, determined in assays using Staphylococcus aureus and Escherichia coli as test organisms, demonstrated that each peptide possessed antimicrobial activity, and that several were as active as the most potent neutrophil defensin, rabbit NP-1. Though the structural and functional attributes of the bovine neutrophil peptides are similar to those of defensins, the two peptide families are distinguished by their unique consensus sequences and additionally by differing tri-disulfide motifs. We therefore propose that this new defensin-like antimicrobial peptide family be named β-defensins.

The cytoplasmic granules of polymorphonuclear leukocytes (neutrophils, PMN) contain numerous antimicrobial poly-

peptides which equip these cells to inactivate ingested microbial targets by mechanisms considered to be "oxygen-independent" (1-3). These granule proteins constitute an antimicrobial arsenal which includes defensins, a family of broad spectrum antibiotic peptides which are released into the phagosome during phagolysosome fusion (1, 4). To date, members of the defensin family have been isolated from neutrophils of human (5), rabbit (6), rat (7, 8), and guinea pig origin (9, 10), and most recently from the Paneth cells of mouse small intestine (11, 12). The current investigation was initiated to determine whether defensins may contribute to the antimicrobial activity of ruminant neutrophils, leukocytes which possess a class of morphologically unique cytoplasmic granules previously shown to contain potent bactericidal polypeptides (13).

The unique features of ruminant granulocytes were first described by Gennaro and Baggiolini and coworkers (14, 15) who demonstrated that neutrophils of cattle, goats, sheep, and ibex are endowed with large numbers of unusually large cytoplasmic granules which are distinct from the classical azurophil and specific granules. Subsequent studies (13) established that the majority of the antibacterial polypeptides of bovine neutrophils are contained in these unique organelles. Romeo and Gennaro and coworkers (16-18) have demonstrated that the large granules of bovine neutrophils contain potent microbicidal peptides which are structurally distinct from defensins. These include three arginine-rich peptides, termed bacterecins, which efficiently kill several Gram-positive and Gram-negative bacteria in vitro (16, 18). Recently, we reported the isolation and characterization of a novel tridecapeptide amide from bovine neutrophils. Termined indolicitin, this cationic peptide was shown to be unusually rich in tryptophan and to have potent bactericidal activity against Escherichia coli and Staphylococcus aureus (19).

In this report, we describe the results of studies which were initiated to determine the presence and biologic role of defensins in bovine neutrophils. The strategy for detection and isolation of bovine defensins was based on the isolation of low molecular weight, cysteine-containing antimicrobial peptides extracted from granule-rich lysates of purified neutrophils. Using gel filtration and reversed-phase HPLC, 13 structurally related peptides were purified to homogeneity, tested for antibacterial activity, and fully sequenced. Though possessing some features of defensins, namely their similar size, cationicity, and the presence of three intramolecular disulfides, the bovine peptides differ significantly in structure from defensins, and thus represent a new class of host defense proteins.
peptides. To distinguish them from classical defensins, we propose that this novel peptide family be termed \( \beta \)-defensins.

**MATERIALS AND METHODS**

**Bovine Neutrophils**—Polymorphonuclear leukocytes (PMN) were purified from 1-liter batches of fresh citrated bovine blood. Following sedimentation for 40 min at 700 \( \times \) g and 37 \( ^\circ \)C, the erythrocyte column was subjected to 7's of hypotonic lysis, after which isotonicity was restored using 5 \( \times \) phosphate-buffered saline. The leukocyte-rich suspension was then sedimented at 120 \( \times \) g (4 \( ^\circ \)C, 15 min). Residual erythrocytes were lysed by repeating this procedure one or two times. Aliquots were removed for quantification by hemocytometry and differential counts. Preparations obtained by this procedure contained an average of 4 \( \times \) 10\(^6\) cells per liter of whole blood that 97% \( \pm \)3% were neutrophils. Half of the preparations were treated with 2 mM diisopropylfluorophosphate (DFP (20)). Neutrophil preparations were then cooled to 4 \( ^\circ \)C for 20 min and disrupted by nitrogen cavitation in a Parr bomb (21). The cavitate was centrifuged at 800 \( \times \) g for 20 min and sedimented at 12,000 \( \times \) g for 40 min and stored at -80 \( ^\circ \)C.

PMN Granule Extracts—Preparations of frozen granules from 1-5 \( \times \) 10\(^6\) PMN were extracted with 5 ml of ice-cold 10% acetic acid per 1 \( \times \) 10\(^6\) cell equivalents. After stirring on ice for 18 h, the suspension was clarified by centrifugation at 27,000 \( \times \) g for 20 min at 4 \( ^\circ \)C and the supernatant was lyophilized and stored at -80 \( ^\circ \)C.

Size Exclusion Chromatography—Low molecular weight peptides were dissolved in 10% acetic acid at a concentration of approximately 1 \( \times \) 10\(^6\) cells per ml, clarified by centrifugation, and loaded onto a 4.8- \( \times \) 100-cm column of BioGel P-60 equilibrated in 5% acetic acid. The column was run at 8 \( ^\circ \)C with an elution rate of 2 cm per h, and 1-ml fractions were collected with continuous monitoring at 290 nm.

**Results**

Purification of Bovine Neutrophil Peptides—Previous electrophoretic analyses of the acid-soluble proteins of bovine PMN granules demonstrated that these preparations contain a complex mixture of proteins varying in size from 1000 to 200,000 Da (19). In order to isolate putative defensins from bovine neutrophil granules, 1-3 \( \times \) 10\(^6\) cell equivalents of acid-solubilized granule protein was fractionated on a BioGel P-60 column, and antibacterial activity in pooled eluent fractions was assayed as described in “Materials and Methods.”

As described in a recent report, peak F was predominantly comprised of indolidin, a novel 13-residue antibiotic peptide amide (19).

SDS-PAGE of pooled fractions from the P-60 column indicated that most of the proteins eluting in peak E were approximately 5 kDa (data not shown), and amino acid analysis demonstrated that the overall cysteine content of material in this peak was approximately 15%. Since these are features consistent with the size and composition of defensins, peak E fractions were combined and further purified by HPLC.

The initial RP-HPLC purification of peak E fractions yielded a complex chromatogram (Fig. 2) in which most peaks contained two or more peptides as determined by acid-urea PAGE. One of the earliest peaks (indicated by the asterisk in Fig. 2) contained an antibacterial peptide of approximately 1500 Da. Automated sequence analysis (data not shown) revealed that this peptide was identical to the cyclic dodecapeptide bacteriocin described earlier by Romeo et al. (16).

Subsequent steps in the purification of the 13 peptides described here employed modified gradient conditions and/or use of 0.13% heptfluorobutyric acid as the ion-pairing agent. These steps enabled the purification of 13 unique peptides, each of which was determined to be pure by its homogeneous behavior on analytical RP-HPLC (Fig. 3) and acid-urea PAGE (Fig. 4). As described below, the peptides constitute a family of related peptides (bovine neutrophil \( \beta \)-defensins (BNBDs)) which have been numbered 1-13 based on their increasing retention times on RP-HPLC (Fig. 3 and Table 1). Peptides eluting in unnumbered peaks in Fig. 2 were characterized by amino acid analysis and SDS-PAGE and were either devoid of cysteine or were much larger than BNBD 1-13, indicating that these peptides were unrelated to \( \beta \)-defensins.
The cellular content of β-defensin peptide was estimated by quantitating the amount of homogeneous BNBD 1–13 recovered and correcting for losses at each step in purification. Using acid-urea and SDS-PAGE to assess recovery, we estimated that approximately 80% of the cellular content of β-defensins was extracted from granule-enriched fractions and that recovery from the P-60 column was virtually quantitative. Assuming 75% recovery during RP-HPLC, the quantity of the combined 13 β-defensins, averaged from two complete purifications, was approximately 4.9 mg/10^10 cells, and the quantity of each of the remaining peptides was similarly estimated as summarized in Table I.

Amino Acid Analysis—The composition of each peptide was established by amino acid analysis of native and performic acid-oxidized or S-carboxamidomethylated samples, and each was analyzed at least twice. Absorbance scans of each of the peptides were carried out between 300 and 200 nm, providing an accurate estimate of tyrosine and tryptophan content (26). As summarized in Table I, the 13 peptides contained from 38 to 42 amino acids, 6 of which were half-cystine residues. The native peptides did not react with Ellman’s reagent or iodoacetamide, indicating that the cysteines were most likely present as disulfides. In addition to their high cysteine content, the peptides were generally rich in the basic amino acids arginine and lysine, but tyrosine and alanine were relatively uncommon.

Sequence Analyses—Two cycles of manual Edman degradation allowed the identification of amino-terminal residues of six peptides (BNBDs 1, 2, 8, 11, 12, and 13). The amino termini of the remaining seven peptides were deblocked with pyroglutamate aminopeptidase, demonstrating the presence of a pyroglutamyl residue at the amino terminus of each of these peptides. Automated sequence analysis was carried out on 1–5 nmol of each S-alkylated peptide. Repetitive sequencing yields averaged 93–97%, allowing for unambiguous assignment of 511 of 519 amino acids by automated Edman degradation. The 8 residues requiring additional steps for identification included the carboxyl-terminal tryptophans of BNBDs 1, 2, 3, 6, 11, 12, and 13, and the carboxyl-terminal arginine of BNBD-4. With the exceptions of BNBD-4 and -6, the carboxyl terminus of each of the eight above-mentioned peptides was determined by analysis of carboxypeptidase A-released amino acids. The carboxyl-terminal arginine of BNBD-4 was confirmed by amino acid analysis of a purified chymotryptic peptide composed of residues 33–41 which had the composition Gly (1.27), Arg (2.64), Pro (2.06), Val (0.98),
**β-Defensins: a New Family of Antimicrobial Peptides**

To confirm the carboxyl-terminal tryptophan, the mass of BNBD-6 was determined on a sample of native peptide by fast atom bombardment mass spectrometry. The monoisotopic mass of BNBD-6 was 4814.2 a.m.u., in close agreement with the theoretical mass of 4816, and consistent with the presence of the carboxyl-terminal tryptophan. Furthermore, ultraviolet spectral analysis (26) indicated the presence of a single tryptophan in both the intact peptide and in the carboxyl-terminal chymotryptic fragment containing residues 33–42. The sequences of all 13 peptides were in excellent agreement with their respective amino acid compositions (Table I).

The complete amino acid sequences of BNBD 1–13, shown in Fig. 5, reveal the high degree of primary structural similarity of this peptide family. Like defensins, each peptide is characterized by 6 invariant cysteine residues, 2 of which are sequential and situated near the peptide carboxyl terminus. However, the spacing of the other cysteine residues in the sequence differs from defensins, and the disulfide connectivities, determined in BNBD-12, differ from those of defensins (31). Given these structural distinctions, we have proposed that this new family of peptides be designated β-defensins.

In addition to the conserved cysteines, the β-defensin sequences contain several amino acids that are highly if not absolutely conserved (Fig. 5). By aligning highly conservative substitutions (Ser/Thr; Val/Ile/Leu/Phe) and one position where only Pro or Arg appears in the primary structures of 10 or more β-defensins, a common consensus sequence of 27 amino acids is revealed (Fig. 6).

A sequence similarity search using the BLAST algorithm (32) revealed only a single protein with substantial identity to the β-defensins, this being tracheal antimicrobial peptide (TAP), a peptide isolated by Diamond et al. (33) from bovine tracheal epithelium. The primary structure of TAP contains the 27-residue β-defensin consensus sequence, though it is not identical to any of the neutrophil-derived β-defensins described here (Fig. 6).
TABLE I

| Residue | BNBD-1 | BNBD-2 | BNBD-3 | BNBD-4 | BNBD-5 | BNBD-6 | BNBD-7 | BNBD-8 | BNBD-9 | BNBD-10 | BNBD-11 | BNBD-12 | BNBD-13 |
|---------|--------|--------|--------|--------|--------|--------|--------|--------|--------|----------|----------|----------|----------|
| Cys     | 6.04 (6) | 6.56 (6) | 6.20 (6) | 6.34 (6) | 5.59 (6) | 5.81 (6) | 6.37 (6) | 6.18 (6) | 6.41 (6) | 5.73 (6) | 6.16 (6) | 5.91 (6) | 5.94 (6) |
| Asp     | 3.03 (3) | 2.16 (2) | 2.01 (2) | 1.74 (2) | 2.58 (3) | 0.99 (1) | 1.91 (2) | 1.67 (2) | 2.20 (2) | 2.04 (2) | 0.92 (1) | 0.94 (1) | 0.96 (1) |
| Glu     | 0.85 (1) | 1.08 (1) | 1.93 (2) | 2.88 (3) | 2.70 (3) | 1.99 (2) | 1.90 (2) | 2.04 (2) | 3.12 (3) | 2.20 (2) | 1.01 (1) | 1.02 (1) | 0.93 (1) |
| Ser     | 1.73 (2) | 0.82 (1) | 0.86 (1) | 0.82 (1) | 1.78 (2) | 1.67 (2) | 1.75 (2) | 1.84 (2) | 3.78 (4) |
| Gly     | 3.72 (4) | 4.40 (4) | 5.11 (5) | 3.73 (4) | 3.87 (4) | 6.52 (6) | 4.96 (5) | 3.96 (4) | 5.10 (5) | 5.32 (5) | 6.23 (6) | 6.40 (6) | 7.30 (7) |
| His     | 1.99 (2) | 0.84 (1) | 0.83 (1) | 0.91 (1) | 0.89 (1) | 0.98 (1) | 0.95 (1) |
| Arg     | 4.57 (4) | 8.01 (6) | 7.97 (8) | 7.83 (8) | 5.24 (5) | 8.47 (8) | 7.92 (8) | 6.57 (7) | 7.29 (7) | 7.02 (7) | 6.06 (6) | 5.71 (5) | 5.36 (5) |
| Thr     | 0.98 (1) | 2.61 (3) | 2.60 (3) | 1.07 (1) | 0.91 (1) | 2.56 (3) | 1.86 (2) | 1.92 (2) | 1.84 (2) | 1.12 (1) | 1.12 (1) | 0.91 (1) | 0.98 (1) |
| Ala     | 0.93 (1) | 1.00 (1) |
| Pro     | 2.76 (3) | 2.82 (3) | 2.73 (3) | 3.80 (4) | 4.70 (5) | 3.08 (3) | 2.94 (3) | 2.90 (3) | 3.06 (3) | 2.16 (2) | 5.04 (5) | 4.86 (5) | 5.05 (5) |
| Tyr     | 0.93 (1) | 1.08 (1) |
| Val     | 0.85 (1) | 2.78 (3) | 2.61 (3) | 3.56 (4) | 3.58 (4) | 3.86 (4) | 2.81 (3) | 2.90 (3) | 2.98 (3) | 2.12 (2) | 1.66 (2) | 2.56 (3) | 2.93 (3) |
| Met     | 0.72 (1) | 1.45 (2) | 2.13 (2) | 1.23 (1) | 1.03 (1) | 0.93 (1) | 0.89 (1) |
| Ile     | 3.19 (4) | 2.65 (4) | 3.64 (4) | 1.83 (2) | 3.01 (3) | 3.06 (3) | 3.82 (4) | 3.70 (4) | 3.84 (4) | 2.00 (2) | 2.91 (3) | 3.01 (3) | 4.03 (4) |
| Leu     | 1.07 (1) | 1.00 (1) | 1.00 (1) | 1.19 (1) | 1.39 (1) | 4.09 (4) | 1.06 (1) | 1.13 (1) | 1.07 (1) |
| Phe     | 2.00 (2) | 2.00 (2) | 2.00 (2) | 1.82 (2) | 1.00 (1) | 2.10 (2) | 1.92 (2) | 1.86 (2) | 1.85 (2) | 1.00 (1) | 1.18 (1) | 1.00 (1) |
| Lys     | 0.90 (1) | 0.78 (1) | 0.74 (2) | 1.00 (1) | 0.90 (1) | 1.00 (1) | 1.00 (1) | 0.75 (1) | 0.91 (1) |
| Trp     | 1.10 (3) | 1.09 (1) | 1.07 (1) | 1.09 (1) | 1.07 (1) |
| Total   | 28 | 40 | 42 | 41 | 40 | 42 | 40 | 38 | 40 | 40 | 38 | 38 | 42 |
| MW*     | 4273 | 4643 | 4809 | 4760 | 4443 | 4816 | 4550 | 4354 | 4522 | 4504 | 4458 | 4101 | 4445 |
| HPLC RT (min)* | 18.25 | 18.32 | 18.32 | 19.99 | 20.03 | 20.81 | 20.87 | 21.72 | 21.94 | 22.30 | 22.72 | 24.30 | 24.83 |
| AU-PAGE order** | 9 | 3 | 4 | 8 | 13 | 6 | 1 | 2 | 5 | 10 | 7 | 11 | 12 |
| mg/10⁶ cells*** | 0.10 | 0.27 | 2.17 | 0.17 | 0.06 | 0.25 | 0.12 | 0.17 | 0.15 | 0.43 | 0.27 | 0.70 | 0.06 |

* Cys (cysteic acid) analyzed after performic acid oxidation.
* Cysteic acid residues identified by UV spectrophotometry and release by carboxypeptidase A.
* Cysteic acid residues identified by UV spectrophotometry and release by carboxypeptidase Y treatment, and confirmed by mass spectrometry.
* Molecular weights calculated from amino acid sequences (Fig. 5).
* HPLC retention time (see Fig. 3 for HPLC conditions).
* Relative order of migration on acid urea-PAGE with 1 being the highest Rf and 13 the lowest Rf value (see Fig. 4).
* Approximate content of each peptide based on recovery as described under “Results.”

FIG. 5. Amino acid sequences of bovine neutrophil β-defensins. The primary structures of BNBD 1-13 are shown in single letter code. The sequences are aligned to demonstrate the most conserved amino acids which have been outlined. The numbering of residues is indexed to the longest of the β-defensin peptides.
Antimicrobial Activity of β-Defensins—The antibacterial activity of each β-defensin was evaluated using S. aureus 502A and E. coli ML35 as test organisms. Using a sensitive radial diffusion assay, each peptide was tested against two bacterial organisms with β-defensin concentrations ranging from 10 to 300 µg/ml. The data presented in Fig. 7 reveal the dose-dependent activity of each peptide as measured by the size of the clear zone surrounding the sample well. In most cases, the log of the peptide concentration was linearly related to the diameter of the growth-free zone. Though the relative potencies of peptides differed, all 13 were active against E. coli, and all but BNBD-1 and BNBD-5 were active against S. aureus in the range of concentrations tested. In most cases, the zone of clearing was greater against E. coli than S. aureus.

The antibacterial activities of the β-defensins were compared with those of three previously characterized antimicrobial peptides: rabbit neutrophil defensin NP-1, the most potent of the classical defensins (24), indolicidin (19), and the cyclic dodecapeptide bactenecin described above (16). Like the β-defensins, the latter two peptides were purified from bovine neutrophil granules. As shown in Fig. 7, the antistaphylococcal activity of rabbit NP-1 was the greatest of any of the β-defensins, the latter two peptides were purified from cyclic dodecapeptide bactenecin described above (16). Like the β-defensins, the latter two peptides were purified from bovine neutrophil granules. As shown in Fig. 7, the antistaphylococcal activity of rabbit NP-1 was the greatest of any of the β-defensins, the latter two peptides were purified from cyclic dodecapeptide bactenecin described above (16). Like the β-defensins, the latter two peptides were purified from bovine neutrophil granules. As shown in Fig. 7, the antistaphylococcal activity of rabbit NP-1 was the greatest of any of the β-defensins, the latter two peptides were purified from cyclic dodecapeptide bactenecin described above (16). Like the β-defensins, the latter two peptides were purified from cyclic dodecapeptide bactenecin described above (16). Like the β-defensins, the latter two peptides were purified from cyclic dodecapeptide bactenecin described above (16). Like the β-defensins, the latter two peptides were purified from cyclic dodecapeptide bactenecin described above (16). Like the β-defensins, the latter two peptides were purified from cyclic dodecapeptide bactenecin described above (16). Like the β-defensins, the latter two peptides were purified from cyclic dodecapeptide bactenecin described above (16).

DISCUSSION

Defensins were the first antimicrobial peptides isolated from leukocytes and, until this report, were the only phagocyte-derived molecules known which contain a conserved tridisulfide structural motif. Though we did not detect classical defensins in bovine neutrophils, the search for them led to the discovery of a new class of distinct but related peptide antibiotics. The β-defensins constitute a highly conserved family of at least 13 neutrophil peptides which are characterized by a disulfide motif different from that of the defensin family (31, 34). In the accompanying paper (31), we report the determination of the disulfide structure in BNBD-12 and discuss possible structural relationships between classical and β-defensins which emerge from a comparison of the respective cysteine connectivities.

Unlike classical defensins which have free amino termini, seven of the 13 β-defensins were found to be blocked at the amino terminus with a pyroglutamyl residue which results from the enzymatic cyclization of amino-terminal glutamine (35). The significance of the pyroglutamyl amino termini is not known, but may confer some aspect of functional specificity (see below), or may provide these particular β-defensins with increased resistance to endogenous leukocyte proteases.

Three of the β-defensins appear to be amino-terminal procressing variants of corresponding peptides which are slightly longer. The sequences of BNBD-2 and -8 are identical to BNBD-3 and -9 respectively, except that the latter two peptides each have a pyroglutamyl-glycine dipeptide extension. In addition, BNBD-12 and -13 have identical sequences except that BNBD-13 has a Ser-Gly-Ile-Ser amino-terminal tetrapeptide (Fig. 5). The occurrence of highly related sequences varying in structure at only the amino terminus raises the possibility that the smaller peptides might be generated by proteolytic degradation during purification or, alternatively, that these minor sequence variations reflect genetic polymorphisms expressed in individual animals. The first possibility is unlikely since all steps in the β-defensin purification up to RP-HPLC were carried out at 0–8 °C at pH < 2.3. In addition, we compared the yield of β-defensins purified from cells which were treated with DFP, a permeant serine protease inhibitor, with those from untreated cells and found no difference in the total and relative yield of the 13 peptides described here. The possibility that the sequence differences reflect genetic polymorphism in the β-defensin locus is excluded since all 13 β-defensins described were isolated from the cells of individual Hereford cows. Therefore, it is most likely that each pair of amino-terminal analogs is produced by differential processing of a common precursor. The proteolytic processing might be a regulated process for producing the observed mixture of β-defensin analogs. Alternatively, the amino-terminal analogs may reflect incomplete or asynchronous post-translational processing which results as granulopoiesis ceases in the bone marrow.

The relative ratios of the three β-defensin pairs, estimated from yields obtained during several purifications are approximately 1:8 for BNBD-2:BNBD-3, 1:1 for BNBD-9:BNBD-8, and 10:1 for BNBD-12:BNBD-13. Of the first pair, BNBD-3 has the dipeptidyl extension and is much more abundant. We speculate that the small amount of BNBD-2 may represent the fraction of BNBD-3 in which the amino-terminal glutamine was not converted to pyroglutamate, thereby allowing for additional proteolytic processing to occur. As measured by our in vitro studies, BNBD-2 and -3 have equivalent antibacterial activity against both test organisms. Similarly, the antibacterial activities of BNBD-12 and -13 are approximately equal, though BNBD-12 is by far the more abundant of this pair. This suggests that, like the BNBD-2 and -3 pair, BNBD-13 may be represent an incompletely processed precursor of BNBD-12. In contrast, the BNBD-8 and -9 pair differ in antibacterial potency, with the more abundant BNBD-9 having the greater in vitro activity (Fig. 7). This difference in activity reveals that the amino terminus is a structural determinant of function, much as the amino terminus of human defensins plays a role in antimicrobial potency (5).

The methods used for purifying β-defensins were chosen to minimize processing which might occur if the neutrophils were activated. Frank et al. (17) have shown that the arginine/proline-rich bactenecins are converted from inactive precursors...
BNBD-9

FIG. 7. Antibacterial activities of purified β-defensins. Nutrient agar plates seeded with *E. coli* ML35 (●) or *S. Aureus* 502A (○) were used to assess antibacterial activity of 13 neutrophil β-defensins in addition to rabbit defensin NP-1, bactenecin dodecapeptide, and indolicidin. Activity is expressed as the diameter of clearing (millimeters) resulting from the application of 5 µl of peptide at the concentrations shown.

sors to actively antimicrobial mature peptides when bovine neutrophils are stimulated with various secretagogues. Interestingly, we have found no evidence for such an inducible processing pathway for activation of β-defensins. Furthermore, the yield of β-defensin peptides in these studies was unaffected by treatment with DFP or the temperature used for cell extraction, suggesting that these peptides reside in granules as fully processed molecules.

Like the neutrophil β-defensins, the epithelial peptide TAP is reported to be active in vitro against *E. coli* and *S. aureus* as well as *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (33). Because the antibacterial assay method used in this study differed from that employed by Diamond et al. (33), it is not possible to directly compare our antimicrobial potency data with the former study. It is interesting to note that the deduced TAP precursor has a glutamine in the position corresponding to the pyroglutamyl residue in the seven amino-blocked β-defensins.

TAP is the first example of an antimicrobial peptide to be isolated from tracheal epithelium, and its discovery suggests an important role for natural peptide antibiotics in host defense of epithelial surfaces. Recent reports from Ouellette et al. (11) and Selsted et al. (12) have extended this observation in studies which characterized defensins from the epithelium of the small intestine. The presence of β-defensins in myeloid elements and tracheal epithelium suggests the possibility that members of this peptide family may be expressed and function in other tissues as well.
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