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Potential Therapeutic Role of Cationic Peptides in Three Experimental Models of Septic Shock

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The therapeutic efficacies of buforin II, indolicidin, and KFFKFFKFF were investigated in three rat models of septic shock: (i) rats injected intraperitoneally with 10 μg of *Escherichia coli* O111:B4 lipopolysaccharide, (ii) rats given an intraperitoneal injection of 2×10^{10} CFU of *Escherichia coli* ATCC 25922, and (iii) rats in which intra-abdominal sepsis was induced via cecal ligation and single puncture. All animals were randomized to receive parenterally isotonic sodium chloride solution, 1 mg of buforin II per kg of body weight, 1 mg of indolicidin per kg, 1 mg of KFFKFFKFF per kg, and 20 mg of imipenem per kg. The main outcome measures were bacterial growth in abdominal exudate and plasma, endotoxin and tumor necrosis factor alpha (TNF-α) concentrations in plasma, and lethality. Treatment with all peptides resulted in significant reductions in plasma endotoxin and TNF-α concentrations compared with those resulting from the imipenem and saline treatments. On the other hand, imipenem treatment significantly reduced the levels of bacterial growth compared with the reductions achieved with the peptide and saline treatments. All compounds reduced the rates of death compared to that for the controls. Although the peptides demonstrated lower levels of antimicrobial activity than imipenem, they exhibited the dual properties of antimicrobial and antiendotoxin agents.

Severe sepsis and septic shock are major causes of morbidity and mortality in neutropenic individuals, hospitalized patients, and all immunocompromised subjects (3, 26, 28). The lipopolysaccharides (LPSs) associated with the cell membranes of gram-negative bacteria, known as endotoxin, activate host effector cells through stimulation of receptors on their surfaces (13). These target cells secrete large quantities of inflammatory cytokines, such as tumor necrosis factor (TNF), interleukin-1 (IL-1), IL-6, IL-8, platelet-activating factor, arachidonic acid metabolites, erythropoietin, and endothelin (1, 2, 13, 17, 27). LPS is composed of an O-polysaccharide chain, a core sugar, and a lipophilic fatty acid, lipid A (1, 13). Lipid A produces the signal responsible for the induction of cytokine genes. Many methods have been used to treat septic shock, including treatments with monoclonal antibodies to endotoxin, IL-1 receptor antagonists, and antioxidants and various anti-inflammatory therapies, but no treatment has produced clinically effective results (1–3, 13, 17, 26–28).

Antimicrobial peptides are positively charged molecules isolated from a wide variety of animals and plants. Among these compounds, buforin II (which is isolated from the stomach of an Asian toad named *Bufo bufo gargarizans*), indolicidin (a 13-residue peptide isolated from cytoplasmic granules of bovine neutrophils), and KFFKFFKFF (a novel synthetic cationic peptide) have broad-spectrum antibacterial activities and outer membrane permeability-increasing properties (5, 19, 25). In addition, cationic peptides have received increasing attention in recent years as they also possess antiendotoxin activities (7, 8, 12, 15). Actually, there is a growing evidence that their role in the host defense against infections is as important to the host as antibodies, immune cells, and phagocytes (12). In addition, these compounds bind to LPS by electrostatic interactions involving the negatively charged phosphoryl groups and by hydrophobic interactions involving the acyl chains of lipid A and so have antiendotoxin activities, in contrast to other antibiotics, which can induce endotoxinemia (4, 21, 23).

Many animal models of sepsis have been described, but none has proved superior (20). The present experimental study was performed to assess the antimicrobial and antiendotoxin activities of buforin II, indolicidin, and KFFKFFKFF compared to those of clinically used antibiotics in three different rat models of septic shock.

MATERIALS AND METHODS

Animals. Adult male Wistar rats (weight range, 250 to 300 g) were used for all experiments. All animals had access to chow and water ad libitum throughout the study. The study was approved by the Animal Research Ethics Committee of the Istituto Nazionale Riposo e Cura Anziani Istituto di Ricovero e Cura a Carattere Scientifico, University of Ancona, Ancona, Italy.

Organisms and reagents. A commercially available quality control strain of *Escherichia coli*, strain ATCC 25922, was used. Endotoxin (*E. coli* serotype O111:B4; Sigma-Aldrich S.r.l., Milan, Italy) was prepared in sterile saline, aliquoted, and stored at −80°C for short periods.

Agents. Buforin II and KFFKFFKFF were synthesized (Faculty of Chemistry, Gdansk, Poland) with an automatic synthesizer (Plus Pep; Milligen 9050; Millipore Corporation, Burlington, Vt.) and were purified by reversed-phase (Vydac C_{18}; 16 by 250 mm) high-pressure liquid chromatography (HPLC) on a Knauer two-pump Well-Chrom K1001 system or on a Beckman System Gold chromatograph. The products were analyzed by HPLC, chemical analysis, and matrix-assisted laser desorption ionization mass spectrometry (MALDI). Indolicidin

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was obtained from Sigma-Aldrich. The peptides were dissolved in distilled H_2O at 20 times the required maximal concentrations. For the in vitro studies, serial dilutions of the peptides were successively prepared in 0.01% acetic acid containing 0.2% bovine serum albumin in polypropylene tubes, while for the in vivo experiments, they were diluted in physiological saline. Imipenem (Merck Sharp & Dohme, Milan, Italy) powder was diluted in accordance with the recommendations of the manufacturer. Solutions were made fresh on the day of assay.

Susceptibility testing. Susceptibility testing was performed by the broth microdilution method by the procedures outlined by the National Committee for Clinical Laboratory Standards (18). However, since cationic peptides bind to polystyrene, 96-well polypropylene plates (Sigma-Aldrich) were substituted for polystyrene plates (6). The MIC was taken as the lowest antibiotic concentration at which observable growth was inhibited. Experiments were performed in triplicate.

Experimental design. Septic shock was induced by three different experimental methods: (i) by intraperitoneal administration of LPS (model i), (ii) by induction of *E. coli* peritonitis (model ii), and (iii) by cecal ligation and puncture (model iii).

(i) Model i. Five groups, each containing 20 animals, were anesthetized by intramuscular injection of ketamine (30 mg/kg of body weight) and injected intraperitoneally with 1.0 mg of *E. coli* serotype O111:B4 LPS in a total volume of 500 μl of sterile saline. Immediately after injection, the animals in the five groups received intraperitoneally isotonic sodium chloride solution (control group C_0), 1 mg of buforin II per kg, 1 mg of indolicidin per kg, 1 mg of KFFKFFKFF per kg, and 20 mg of imipenem per kg, respectively.

(ii) Model ii. *E. coli* ATCC 25922 was grown in brain heart infusion broth. When the bacteria were in the log phase of growth the suspension was centrifuged at 1,000 × *g* for 15 min, the supernatant was discarded, and the bacteria were resuspended and diluted in sterile saline. All animals (five groups, each containing 20 animals) were anesthetized as described above. The abdomen of each animal was shaved and prepared with iodine. The rats received an intraperitoneal inoculum of 1 ml of saline containing 2×10^{10} CFU of *E. coli* ATCC 25922. Immediately after bacterial challenge, the animals in the five groups received intraperitoneally isotonic sodium chloride solution (control group C_1), 1 mg of buforin II per kg, 1 mg of indolicidin per kg, 1 mg of KFFKFFKFF per kg, and 20 mg of imipenem per kg, respectively.

(iii) Model iii. All animals (five groups, each containing 20 animals) were anesthetized as described above. The abdomen of each animal was shaved and prepared with iodine. Through a midline laparotomy, the cecum was filled with feces by milking the stools back from the descending colon, and the cecum was then ligated just below the ileocecal valve with a 3-0 silk ligature. The antimesenteric cecal surface was punctured twice with a 23-gauge needle below the ligature, the bowel was placed back into the peritoneal cavity, and the abdomen was closed in two layers. The operative procedure was done under aseptic conditions. For administration of antibiotics, a catheter was placed into the jugular vein and was sutured to the back of the rat. The drugs were given immediately after the surgical procedure. The animals in the five groups received isotonic sodium chloride solution (control group C_2), 1 mg of buforin II per kg, 1 mg of indolicidin per kg, 1 mg of KFFKFFKFF per kg, and 20 mg of imipenem per kg, respectively.

Evaluation of treatment. On the basis of the kind of experiment, at the end of the study the rate of blood culture positivity, the quantities of bacteria in the intra-abdominal fluid, the rate of lethality, and plasma endotoxin and TNF alpha (TNF-α) levels were evaluated. The animals were monitored for the subsequent 72 h.

The surviving animals (models ii and iii) were killed with chloroform, and blood samples for culture were obtained by aseptic percutaneous transthoracic cardiac puncture. In addition, to perform quantitative evaluations of the bacteria in the intra-abdominal fluid, 10 ml of sterile saline was injected intraperitoneally, samples of the peritoneal lavage fluid were serially diluted, and a 0.1-ml volume of each dilution was spread onto blood agar plates. The limit of detection was ≤1 $\log_{10}$ CFU/ml. The plates were incubated both in air and under anaerobic conditions at 35°C for 48 h.

For determination of endotoxin and TNF-α levels in plasma, 0.2-ml blood samples were collected from the jugular vein 0, 2, 6, and 12 h after injection. During this time, a catheter was placed into the vein and sutured to the back of the rat.

Endotoxin concentrations were measured by the commercially available *Limulus* amebocyte lysate test (E-TOXATE; Sigma-Aldrich). Plasma samples were serially diluted twofold with sterile endotoxin-free water and were heat treated for 5 min in a water bath at 75°C to destroy inhibitors that can interfere with the activation. The endotoxin content was determined as described by the manufacturer. Endotoxin standards were tested in each run, and the concentrations of endotoxin in the text samples (in endotoxin units [EU] per milliliter) were calculated by comparison with the standard curve. TNF-α levels were measured by a commercially available solid-phase sandwich enzyme-linked immunosorbent assay (Nuclear Laser Medicine, S.r.l., Settala, Italy) by the protocol supplied by the manufacturer. The standards and samples were incubated with a TNF-α antibody-coated 96-well microtiter plate. The wells were washed with buffer and then incubated with biotinylated anti-TNF-α antibody conjugated to streptavidin-peroxidase. This was washed away and the color was developed in the presence of a chromogen (tetramethylbenzidine) substrate. The intensity of the color was measured in a microplate reader (MR 700; Dynatech Laboratories, Guernsey, United Kingdom) by reading the absorbance at 450 nm. The results for the samples were compared with the standard curve to determine the amount of TNF-α present. All samples were run in duplicate. The lower limit of sensitivity for TNF-α by this assay was 0.05 ng/ml. The intra-assay and interassay coefficients of variation were 6.9 and 8.7%, respectively.

TABLE 1. Plasma endotoxin and TNF-α levels in a rat model 6 h after intraperitoneal administration of 1.0 mg of *E. coli* serotype O111:B4 LPS

Treatment (dose [mg/kg])[a]	Endotoxin concn (EU/ml)[b]	TNF-α concn (ng/ml)[b]
None (control group C_0)	16.13 ± 3.61	94.73 ± 11.28
BUF II (1)	≤0.015 ± 0.0[c,d]	0.15 ± 0.02[c,d]
IND (1)	0.32 ± 0.11[c]	4.23 ± 0.81[c]
KFF (1)	0.50 ± 0.17[c]	6.55 ± 1.43[c]
IMP (20)	17.45 ± 4.92	98.41 ± 13.49

[a] BUF II, buforin II; IND, indolicidin; KFF, KFFKFFKFF; IMP, imipenem.
[b] The values are means ± SDs.
[c] $P < 0.05$ versus control group C_0 and the imipenem-treated group.
[d] $P < 0.05$ versus any other group.

Statistical analysis. MICs are presented as the average values obtained in triplicate from three independent measurements. Survival data were compared by the log rank test; qualitative results for blood and intra-abdominal fluid cultures were analyzed by the χ^2 test, Yates' correction, or Fisher's exact test, depending on the sample size. Quantitative evaluations of the bacteria in intra-abdominal fluid cultures are presented as means ± standard deviations (SDs) of the mean; statistical comparisons between groups were made by analysis of variance. Post hoc comparisons were performed by Bonferroni's test. Plasma endotoxin and TNF-α levels were analyzed by the Kruskal-Wallis test; multiple comparisons between groups were performed by the appropriate standard procedure. Each comparison group contained 15 rats. Significance was accepted when the *P* value was ≤0.05.

RESULTS AND DISCUSSION

In vitro susceptibility. *E. coli* ATCC 25922 had different susceptibilities to the peptides tested, as determined by the broth microdilution method: the MICs of buforin II, indolicidin, and KFFKFFKFF were 1.00, 2.00, and 8.00 mg/liter, respectively. The MIC of the control agent (imipenem) was 0.12 mg/liter.

Animal models. (i) Intraperitoneal administration of LPS (model i). Intraperitoneal peptide treatments given immediately after administration of 1.0 mg of *E. coli* serotype O111:B4 LPS were better than no treatment and intraperitoneal treatment with imipenem. In fact, there were significant differences ($P < 0.05$) in plasma endotoxin and TNF-α levels in the peptide-treated groups compared with those in control group C_0 and the imipenem-treated group (Table 1). In particular, treatment with buforin II resulted in the highest levels of antiendotoxin activity, resulting in undetectable endotoxin levels in plasma and the lowest levels of production of TNF-α.

(ii) *E. coli*-induced peritonitis (model ii). The rate of lethality in control group C_1 was 100%. All intraperitoneal antibiotic treatments given immediately after challenge were better than

TABLE 2. Efficacies of intraperitoneal buforin II, indolicidin, KFFKFFKFF and imipenem in a rat model of *E. coli*-induced peritonitis

Treatment (dose [mg/kg])[a]	Lethality[b] (no. of dead rats/ total no. [%])	Qualitative blood culture (no. of positive cultures/ total no.)	Bacterial count (CFU/ml)[c]	Endotoxin concn (EU/ml)[c]	TNF-α concn (pg/ml)[c]
None (control group C_1)	15/15 (100)	15/15	$8.9 \times 10^8 \pm 2.2 \times 10^8$	0.218 ± 0.09	331.7 ± 77.3
BUF II (1)	4/15[d] (26.6)	5/15[d]	$2.7 \times 10^4 \pm 0.7 \times 10^4$[d]	≤0.015 ± 0.0[d,e]	18.5 ± 2.7[d,e]
IND (1)	6/15[d] (40.0)	6/15[d]	$9.3 \times 10^4 \pm 2.4 \times 10^4$[d]	0.096 ± 0.02[d,f]	86.3 ± 11.8[d,f]
KFF (1)	8/15[d] (53.3)	9/15[d]	$5.2 \times 10^5 \pm 1.1 \times 10^5$[d]	0.101 ± 0.02[d,f]	107.4 ± 22.9[d,f]
IMP (20)	2/15[d,g] (13.3)	2/15[d,g]	$4.7 \times 10^2 \pm 1.2 \times 10^2$[e,f]	0.211 ± 0.05	318.3 ± 63.1

[a] The rats were administered *E. coli* (2×10^{10} CFU) intraperitoneally along with 1 ml of sterile saline solution. BUF II, buforin II; IND, indolicidin; KFF, KFFKFFKFF; IMP, imipenem.
[b] Lethality was monitored for 72 h following the challenge.
[c] The values are means ± SDs.
[d] $P < 0.05$ versus control group C_1.
[e] $P < 0.05$ versus any other group.
[f] $P < 0.05$ versus the imipenem-treated group.
[g] $P < 0.05$ versus the peptide-treated groups.

no treatment ($P < 0.05$). Specifically, survival rates were 73.4, 60.0, 46.7, and 86.7% in the groups treated with buforin II, indolicidin, KFFKFFKFF, and imipenem, respectively (Table 2). Bacteriological evaluation showed 100% positive blood and intra-abdominal fluid cultures for control group C_1: the average bacterial count in the peritoneal fluid from dead or surviving animals at 72 h was $8.9 \times 10^8 \pm 2.2 \times 10^8$ CFU/ml. Imipenem had the highest antimicrobial activities and therapeutic efficacies. Similar to the lethality endpoint, there were significant differences in the results for the quantitative bacterial cultures when the data obtained for the imipenem-treated groups were compared with those obtained for the peptide-treated groups ($P < 0.05$). Endotoxin and TNF-α concentrations increased constantly in control group C_1, with mean peak levels achieved at 6 h postinjection. All peptide-treated groups showed significant reductions in plasma endotoxin and TNF-α levels compared to those in the control and the imipenem-treated groups. In contrast, no significant differences in plasma endotoxin and TNF-α concentrations were observed between the imipenem-treated group and control group C_1. The results observed at 6 h postinjection are summarized in Table 2.

(iii) Cecal ligation and puncture (model iii). The rate of lethality in control group C_2 was 100%. All intravenous antibiotic treatments given immediately after challenge were better than no treatment ($P < 0.05$). Specifically, rates of survival were 66.7, 46.7, 33.3, and 80.0% in the groups treated with buforin II, indolicidin, KFFKFFKFF, and imipenem, respectively (Table 3). Bacteriological evaluation showed 100% positive blood and intra-abdominal fluid cultures for control group C_2. Specifically, the peritoneal fluid of the control group demonstrated high bacterial numbers, averaging $2.9 \times 10^9 \pm 0.7 \times 10^9$ CFU/ml. Gram-negative and gram-positive bacteria were simultaneously isolated from more than 90% of the animals. The pathogens isolated from both blood and the abdominal fluid were mainly members of the family *Enterobacteriaceae*, including *Escherichia coli*, *Enterobacter* spp., *Klebsiella* spp., and gram-positive cocci (primarily enterococci). The most frequently isolated anaerobic isolates were *Bacteroides* spp. As in model ii, imipenem had the highest antimicrobial activities and therapeutic efficacies. Among the peptides tested, buforin II was confirmed to be the most active. As shown with the other experimental models, constant increases in plasma endotoxin and TNF-α concentrations were found in the animals in model iii, with mean peak levels achieved at 6 h postinjection. Overall, the peptides produced significant reductions in plasma endotoxin and TNF-α levels compared to the levels in the controls, while imipenem produced significant increases. The results are summarized in Table 3.

Although several papers have been devoted to the study of animal models of septic shock, there continues to be a lack of a reliable model system. For this reason, extrapolation of results obtained with animal models to human pathologies

TABLE 3. Efficacies of intravenous buforin II, indolicidin, KFFKFFKFF, and imipenem in a rat model of cecal ligation and puncture induced peritonitis

Treatment (dose [mg/kg])[a]	Lethality[b] (no. of dead rats/ total no. tested [%])	Qualitative blood culture (no. positive cultures/ total no.)	Bacterial count (CFU/ml)[c]	Endotoxin concn (EU/ml)[c]	TNF-α concn (pg/ml)[c]
None (control group C_1)	15/15 (100)	15/15	$2.9 \times 10^9 \pm 0.7 \times 10^9$	0.255 ± 0.08	132.3 ± 37.5
BUF II (1)	5/15[d] (33.3)	6/15[d]	$5.6 \times 10^4 \pm 1.4 \times 10^4$[d]	0.021 ± 0.00[d,e]	9.4 ± 1.6[d,e]
IND (1)	8/15[d] (53.3)	8/15[d]	$8.8 \times 10^4 \pm 2.3 \times 10^4$[d]	0.079 ± 0.02[d,f]	36.3 ± 8.7[d,f]
KFF (1)	10/15[d] (66.7)	11/15[d]	$6.6 \times 10^5 \pm 1.3 \times 10^5$[d]	0.097 ± 0.02[d,f]	50.5 ± 12.8[d,f]
IMP (20)	3/15[d,f] (20.0)	3/15[d,g]	$5.9 \times 10^2 \pm 1.5 \times 10^2$[d,g]	0.240 ± 0.06	129.1 ± 33.7

[a] BUF II, buforin II; IND, indolicidin; KFF, KFFKFFKFF; IMP, imipenem.
[b] Lethality was monitored for 72 h following the challenge.
[c] The values are means ± SDs.
[d] $P < 0.05$ versus control group C_1.
[e] $P < 0.05$ versus any other group.
[f] $P < 0.05$ versus the imipenem-treated group.
[g] $P < 0.05$ versus the peptide-treated groups.

should be regarded with caution. Peritonitis models have been proposed as the "gold standard"; nevertheless, studies with a single dose of endotoxin and studies with a single inoculum of one gram-negative species have been the models of sepsis most often used for the screening of antiendotoxin and antimicrobial drugs. However, these models are not representative of the situation during clinical infections: humans are usually exposed not to only one species of gram-negative bacteria but to gram-positive organisms as well and are exposed in different ways. For these reasons, the animal model of cecal ligation and puncture, which resembles the clinical situation of bowel perforation and mixed bacterial infection of intestinal origin, seems to be the more realistic model of sepsis with a clear resemblance to the clinical situation. Analysis of the data from our three experiments showed that the different models did not have different essential effects on the parameters evaluated. Actually, the only clear difference in the models concerned the plasma TNF-α concentration. In particular, in agreement with previous results, model iii, in which intra-abdominal sepsis was induced via cecal ligation and a single puncture, TNF-α appeared slightly later and its levels were lower, which was different from the results obtained with the other two models. Nevertheless, it was evident that the efficacies of the compounds were not affected by the animal models used and that these were retained regardless of the system used.

In the present study the effects of buforin II, indolicidin, and KFFKFFKFF were evaluated by the use of different endpoints. Since severe infections caused by gram-negative organisms involve the release of the endotoxic component responsible for the increase in cytokine levels in plasma and this phenomenon is thought to be a pathogenic element of sepsis (2, 16, 17, 27), we were interested in investigating compounds that could neutralize endotoxins. Current treatments for septic shock caused by gram-negative bacteria rely on antibiotics to control the infection and intensive-care support to correct dysfunctions of the cardiovascular, endocrine, and other organ systems. Nevertheless, it is known that many antibiotics used clinically can be harmful when administered for the treatment of severe infections cause by gram-negative bacteria, in that they can stimulate the release of endotoxin and thus increase the rates of occurrence of symptoms and life-threatening complications (4, 21, 24). Recent reports have described a large collection of antimicrobial peptides that comprise a widespread effector arm of the immune system (14). Actually, they have been identified in organisms as diverse as bacteria, protozoa, plants, insects, and vertebrates. Use of the antimicrobial polycationic peptides might offer the opportunity to inhibit not only bacterial growth but also the biological activity of the endotoxin (7, 9–11, 22). Actually, these cationic molecules act on gram-negative bacteria by initially binding to their surface polyanionic LPS, followed by self-promoted uptake across the outer membrane. By virtue of this affinity for LPS, the cationic peptides operate as antiendotoxin agents and inhibit the production of cytokines such as TNF-α by macrophages stimulated with LPS (8, 12).

The results of this study have demonstrated that single intraperitoneal and intravenous doses of buforin II, indolicidin, and KFFKFFKFF produced significant reductions in plasma endotoxin and TNF-α levels compared to the levels in the control and the carbapenem-treated groups. Specifically, buforin II showed much stronger antiendotoxin activity than indolicidin and KFFKFFKFF in all models evaluated in the study. These differences in the antiendotoxin potentials between the peptides, although statistically not significant, can be ascribed in part to the differences in their mechanisms of action. As mentioned above, the killing mechanism detected for most polycationic peptides consists of attacks on the outer and inner membranes, ultimately resulting in lysis of the organisms (12). Otherwise, buforin II kills bacteria without cell lysis, suggesting the possibility that its target is not the cell membranes (19). On the other hand, the two models (models ii and iii) that also evaluated the antimicrobial roles of the compounds showed that the peptides lack the antibiotic potency of imipenem. In fact, our data demonstrated that, despite the high plasma endotoxin and TNF-α levels, the strong antimicrobial activity of this clinically used antibiotic resulted in the lowest rate of lethality by inhibition of bacterial growth at the highest levels. The relevance of the pivotal role of antimicrobial activity was pointed out by the third experimental model, in which various gram-negative and gram-positive bacterial species were involved in the pathogenesis of the sepsis. In fact, lethality data demonstrated that the strong activity and the broad antimicrobial spectrum of imipenem prevailed against the peptides' antiendotoxin activities exclusively directed against the LPS component of the membranes of the gram-negative bacteria. Nevertheless, on the basis of our results, the use of cationic peptides might have certain potential advantages. First, the use of single doses of peptides resulted in significant inhibition of bacterial growth compared with that in the control groups. In addition, they produced significant reductions in plasma endotoxin levels compared to those achieved in the imipenem-treated groups, confirming their double antimicrobial and antiendotoxin activities, while the administration of imipenem significantly increased the plasma endotoxin and TNF-α levels compared with those in the control groups and the peptide-treated animals. Finally, there is a substantial interest in identifying novel strategies to overcome not only infections but also the underlying conditions. For this reason, agents that can modulate the effects of endotoxin and the inflammatory response, in addition to having direct antimicrobial activity, would be advantageous.

REFERENCES

1. **Bone, R. C.** 1991. Pathophysiology of sepsis. Ann. Intern. Med. **115:**457–469.
2. **Bone, R. C.** 1993. Gram-negative sepsis: a dilemma of modern medicine. Clin. Microbiol. Rev. **6:**57–68.
3. **Brun-Buisson, C., F. Doyan, J. Carlet, P. Dellamonica, F. Gouin, A. Lepoutre, J. C. Mercier, G. Offenstadt, and B. Regnier.** 1995. Incidence, risk factors, and outcome of severe sepsis and septic shock in adults. JAMA **274:**968–974.
4. **Cohen, J., and J. S. McConnell.** 1985. Antibiotic-induced endotoxin release. Lancet **ii:**1069–1070.
5. **Falla, T. J., and R. E. W. Hancock.** 1997. Improved activity of a synthetic indolicidin analog. Antimicrob. Agents Chemother. **41:**771–775.
6. **Giacometti, A., O. Cirioni, F. Barchiesi, M. S. Del Prete, M. Fortuna, F. Caselli, and G. Scalise.** 2000. In vitro susceptibility tests for cationic peptides: comparison of microbroth dilution methods for bacteria that grow aerobically. Antimicrob. Agents Chemother. **44:**1694–1696.
7. **Giacometti, A., O. Cirioni, R. Ghiselli, C. Viticchi, F. Mocchegiani, A. Riva, V. Saba, and G. Scalise.** 2001. Effect of mono-dose intraperitoneal cecropins in experimental septic shock. Crit. Care Med. **29:**1666–1669.
8. **Gough, M., R. E. W. Hancock, and N. M. Kelly.** 1996. Antiendotoxin activity of cationic peptide antimicrobial agents. Infect. Immun. **64:**4922–4927.
9. **Hancock, R. E. W.** 1997. Peptides antibiotics. Lancet **349:**418–422.
10. **Hancock, R. E. W.** 1998. Therapeutic potential of cationic peptides. Expert. Opin. Investig. Dis. **7:**167–174.
11. **Hancock, R. E. W., and D. S. Chapple.** 1999. Peptide antibiotics. Antimicrob. Agents Chemother. **43:**1317–1323.

12. **Hancock, R. E. W., and M. G. Scott.** 2000. The role of antimicrobial peptides in animal defenses. Proc. Natl. Acad. Sci. USA **97:**856–861.
13. **Hardaway, R. M.** 2000. A review of septic shock. Am. Surg. **66:**22–29.
14. **Huttner, K. M., and C. L. Bevins.** 1999. Antimicrobial peptides as mediators of ephithelial host defense. Pediatr. Res. **45:**785–794.
15. **Iwagaki, A., M. Porro, and M. Pollack.** 2000. Influence of synthetic antiendotoxin peptides on lipopolysaccharide (LPS) recognition and LPS-induced proinflammatory cytokine responses by cells expressing membrane-bound CD14. Infect. Immun. **68:**1655–1663.
16. **Mendez, C., A. A. Kramer, K. F. Salhab, G. A. Valdes, J. G. Norman, K. J. Tracey, and L. C. Carey.** 1999. Tolerance to shock: an exploration of mechanism. Ann. Surg. **229:**843–850.
17. **Mira, J. P., A. Cariou, F. Grall, C. Delclaux, M. R. Losser, F. Heshmati, C. Cheval, M. Monchi, J. L. Teboul, F. Riché, G. Leleu, L. Arbibe, A. Mignon, M. Delpech, and J. F. Dhainaut.** 1999. Association of TNF2, a TNFα promoter polymorphism, with septic shock susceptibility and mortality. JAMA **282:**561–568.
18. **National Committee for Clinical Laboratory Standards.** 1997. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A4. National Committee for Clinical Laboratory Standards, Wayne, Pa.
19. **Park, C. B., K. S. Yi, K. Matsuzaki, M. S. Kim, and S. C. Kim.** 2000. Structure-activity analysis of buforin II, a histone H2A-derived antimicrobial peptide: the proline hinge is responsible for the cell-penetrating ability of buforin II. Proc. Natl. Acad. Sci. USA **97:**8245–8250.
20. **Parker, S. J., and P. E. Watkins.** 2000. Experimental models of gram-negative sepsis. Br. J. Surg. **88:**22–30.
21. **Prins, J. M., E. J. Kuijper, M. L. C. M. Mevissen, P. Speelman, and S. J. H. van Deventer.** 1995. Release of tumor necrosis factor alpha and interleukin 6 during antibiotic killing of *Escherichia coli* in whole blood: influence of antibiotic class, antibiotic concentration, and presence of septic serum. Infect. Immun. **63:**2236–2242.
22. **Scott, M. G., H. Yan, and R. E. W. Hancock.** 1999. Biological properties of structurally related α-helical cationic antimicrobial peptides. Infect. Immun. **67:**2005–2009.
23. **Shenep, J. L., R. P. Barton, and K. A. Mogan.** 1985. Role of antibiotic class in the rate of liberation of endotoxin during therapy for experimental gram-negative bacterial sepsis. J. Infect. Dis. **151:**1012–1018.
24. **Tracey, K. J., Y. Fong, D. Hesse, K. R. Manogue, A. T. Lee, G. C. Kuo, S. F. Lowry, and A. Cerami.** 1987. Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia. Nature (London) **330:**662–664.
25. **Vaara, M., and M. Porro.** 1996. Group of peptides that act synergistically with hydrophobic antibiotics against gram-negative enteric bacteria. Antimicrob. Agents Chemother. **40:**1801–1805.
26. **Wheeler, A. P., and G. R. Bernard.** 1999. Treating patients with severe sepsis. N. Engl. J. Med. **340:**207–214.
27. **Zervos, E., J. Norman, D. Denham, L. C. Carey, D. Livingstone, and A. S. Rosemurgy.** 1997. Cytokine activation through sublethal hemorrhage is protective against early lethal endotoxic challenge. Arch. Surg. **132:**1216–1221.
28. **Ziegler, E. J., C. J. Fisher, and C. L. Sprung.** 1991. Treatment of gram-negative bacteremia and septic shock with HA-1A human monoclonal antibody against endotoxin. N. Engl. J. Med. **324:**429–436.