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(54) **PEPTIDES CATIONIQUES ANTIMICROBIENS**
(54) **ANTIMICROBIAL CATIONIC PEPTIDES**

(57) La présente invention concerne une classe nouvelle de peptides cationiques ayant des propriétés antimicrobiennes. Parmi ces peptides, on peut citer: NH_2-KWKSFIKKLTTAVKKVLTTGLPALIS-COOH (n° d'ident. de séquence: 1) et NH_2-KWKSFIKKLTSAAKKVVTTAKPLISS-COOH (n° d'ident. de séquence: 2). L'invention concerne aussi des procédés d'inhibition de la croissance de bactéries dans lesquels les peptides de l'invention sont utilisés. Ces peptides sont particulièrement utiles pour inhiber l'endotoxémie chez un sujet.

(57) A novel class of cationic peptides having antimicrobial activity is provided. Examples of such peptides include NH_2-KWKSFIKKLTTAVKKVLTTGLPALIS-COOH (SEQ ID NO:1) and NH_2-KWKSFIKKLTSAAKKVVTTAKPLISS-COOH (SEQ ID NO:2). Also provided are methods for inhibiting the growth of bacteria utilizing the peptides of the invention. The peptides are particularly useful for inhibiting endotoxaemia in a subject.

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(57) Abstract

A novel class of cationic peptides having antimicrobial activity is provided. Examples of such peptides include NH_2-KWKSFIKKLTTAVKKVLTTGLPALIS-COOH (SEQ ID NO:1) and NH_2-KWKSFIKKLTSAAKKVVTTAKPLISS-COOH (SEQ ID NO:2). Also provided are methods for inhibiting the growth of bacteria utilizing the peptides of the invention. The peptides are particularly useful for inhibiting endotoxaemia in a subject.

ANTIMICROBIAL CATIONIC PEPTIDES

BACKGROUND OF THE INVENTION

1. *Field of the Invention*

This invention relates generally to antimicrobial peptides and specifically to a new class of antimicrobial cationic peptides referred to as bactolysins.

2. *Description of Related Art*

In 1981, the self-promoted uptake hypothesis was first proposed to explain the mechanism of action of polycationic antibiotics in *Pseudomonas aeruginosa*. According to this hypothesis, polycations interact with sites on the outer membranes of Gram-negative bacteria at which divalent cations cross-bridge adjacent lipopolysaccharide molecules. Due to their higher affinity for these sites, polycations displace the divalent cations and, since the polycations are bulkier than the divalent cations, cause structural perturbations in the outer membrane. These perturbations result in increased outer membrane permeability to compounds such as the β-lactam antibiotic nitrocefin, the eukaryotic non-specific defense protein lysozyme and to hydrophobic substances. By analogy, molecules accessing this pathway are proposed to promote their own uptake.

It has been clearly demonstrated that the outer membranes of Gram-negative bacteria are semipermeable molecular "sieves" which restrict access of antibiotics and host defense molecules to their targets within the bacterial cell. Thus, cations and polycations which access the self-promoted uptake system are, by virtue of their ability to interact with and break down the outer membrane permeability barrier, capable of increasing the susceptibility of Gram-negative pathogenic bacteria to antibiotics and host defense molecules. Hancock and Wong demonstrated that a broad range of such compounds could overcome the permeability barrier and coined the name "permeabilizers" to describe them (Hancock and Wong, *Antimicrob. Agents Chemother.*, 26:48, 1984). While self-promoted uptake and permeabilizers were first described for *P. aeruginosa*, they have now been described for a variety of Gram-negative bacteria.

Over the past decade, non-specific defense molecules have been described in many animals, including insects and humans. One subset of these molecules have in common the following features: (a) they are small peptides, usually 15-35 amino acids in length, (b) they contain 4 or more positively charged amino acid residues, either lysines or arginines, and (c) they are found in high abundance in the organisms from which they derive. Several of these molecules have been isolated, amino acid sequenced and described in the patent literature (*e.g.*, cecropins: WO8900199, WO 8805826, WO8604356, WO 8805826; defensins: EP 193351, EP 85250, EP 162161, US 4659692, WO 8911291). However, only limited amounts of these peptides can be isolated from the host species. For example, Sawyer, *et al.*, (*Infect. Immun.* 56:693, 1988) isolated 100-200 mg of rabbit neutrophil defensins 1 and 2 from 10^9 primed peritoneal neutrophils or lipopolysaccharide-elicited alveolar macrophages (*i.e.*, the numbers present in a whole animal).

The gene for human defensin has been cloned and sequenced, but no successful expression has been demonstrated, as yet. Furthermore, production of these peptides using peptide synthesis technology produces peptides in limited amounts and is expensive when scaled up or when many variant peptides must be produced. Also, structural analysis is difficult without specific incorporation of ^{15}N and ^{13}C tagged amino acids which is prohibitively expensive using amino acid synthesis technology.

There is a need to develop polypeptides having a broad range of potent antimicrobial activity against a plurality of microorganisms, including gram negative bacteria, gram positive bacteria, fungi, protozoa, viruses and the like.

SUMMARY OF THE INVENTION

The present invention provides a novel class of cationic peptides, referred to as bactolysins, which have antimicrobial activity. Two representative peptides are provided and include

MBI 29, NH_2-KWKSFIKKLTTAVKKVLTTGLPALIS-COOH (SEQ ID NO:1) and

MBI 26, NH_2-KWKSFIKKLTSAAKKVVTTAKPLISS-COOH (SEQ ID NO:2), analogs, derivatives and conservative variations thereof.

The invention also provides a method of inhibiting the growth of bacteria comprising contacting the bacteria with an inhibiting effective amount of a peptide having an amino acid sequence of MBI 29 (SEQ ID NO:1) or MBI 26 (SEQ ID NO:2) alone, or in combination with an antibiotic. Classes of antibiotics which can be used for synergistic therapy with the peptides of the invention include aminoglycoside, penicillin, cephalosporine, fluoroquinolone, carbepenem, tetracycline and macrolide.

In another embodiment, the invention provides a method of inhibiting an endotoxemia or sepsis associated disorder in a subject having or at risk of having such a disorder, comprising administering to the subject a therapeutically effective amount of a peptide of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a three-dimensional schematic representation of the conformational structure of CEME, MBI 29, and MBI 26.

Figure 2 shows the axial projection of the α-helical conformation of each peptide in 2D.

Figure 3 shows an inner membrane permeabilization assay for MBI 26.

Figure 4 shows an inner membrane permeabilization assay for MBI 29.

Figure 5a shows tumor necrosis factor (TNF) levels measured 6 hours after the addition of *E. coli* 0111:B4 LPS and CEME (ME), CEMA (MA), and MBI 29 to macrophage cells. The data is from two separate assays.

Figure 5b and 5c show tumor necrosis factor (TNF) levels measured 6 hours after the addition of *E. coli* Bort LPS and *E. coli* 0111:B4 LPS and MBI 29 to macrophage cells. The data is from two separate assays.

Figure 6a shows tumor necrosis factor (TNF) levels measured 6 hours after the addition of *E. coli* Bort LPS and CEME (ME), CEMA (MA) and MBI 29 to macrophage cells. The data is from two separate assays.

Figure 6b shows tumor necrosis factor (TNF) levels measured 6 hours after the addition of *P. aeruginosa* LPS and MBI 26 to RAW macrophage cells.

Figure 6c shows tumor necrosis factor (TNF) levels measured 6 hours after the addition of *E. coli* Bort LPS, *E. coli* 0111:B4 LPS and MBI 26 to RAW macrophage cells.

Figure 7a and 7b show RAW cell TNF production after addition of *E. coli* 0111:B4 LPS and the effect of the addition of MBI 29 and polymyxin B on TNF.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a novel classs of cationic peptides, called bactolysins, which have antimicrobial activity and have the ability to significantly reduce the level of lipopolysaccharide (LPS)-induced tumor necrosis factor (TNF). These peptides are useful for inhibiting microbial infection or growth, as well reducing the effects of endotoxemia and are often synergistic with conventional antibiotics and/or lysozyme. In addition, such peptides are useful as antifungal agents, antitumor agents, or antiviral agents.

The term "antimicrobial" as used herein means that the peptides of the present invention inhibit, prevent, or destroy the growth or proliferation of microbes such as bacteria, fungi, viruses or the like. The term "antiviral" as used herein means that the peptides of the present invention inhibit, prevent or destrroy the growth or proliferation of viruses or of virally-infected cells. The term "anti-tumor" as used herein means that the peptides of the present invention may be used to inhibit the growth of or destroy tumors. The term "antifungal" as used herein means that the peptides of the present inventon may be used to inhibt the growth of or destroy fungi.

In a first embodiment, the invention provides an isolated antimicrobial peptide having an amino acid sequence:

NH_2-KWK$R_2R_1R_1R_2R_2R_1R_2R_2R_1R_1R_2R_2$VLTTGLPALIS-COOH,

NH_2-KWK$R_2R_1R_1R_2R_2R_1R_2R_2R_1R_1R_2R_2$VVTTAKPLISS-COOH,

NH_2-KWK$R_2R_1R_1R_2R_2R_1R_2R_2R_1R_1R_2R_2$ILTTGLPALIS-COOH,

NH_2-KWK$R_2R_1R_1R_2R_2R_1R_2R_2R_1R_1R_2R_2$GGLLSNIVTSL-COOH,
or NH_2-KWK$R_2R_1R_1R_2R_2R_1R_2R_2R_1R_1R_2R_2$GPILANLVSIV-COOH
wherein R_1 is a hydrophobic amino acid residue and R_2 is a hydrophilic amino acid residue.

Examples of such peptides of the invention include but are not limited to:
NH_2-KWKSFIKKLTTAVKKVLTTGLPALIS-COOH (MBI 29) (SEQ ID NO:1),
NH_2-KWKSFIKKLTSAAKKVVTTAKPLISS-COOH (MBI 26) (SEQ ID NO:2),
NH_2-KWKSFIKNLTKGGSKILTTGLPALIS-COOH (MBI 201) (SEQ ID NO:3),
NH_2-KWKKFIKNLTKGGSKILTTGLPALIS-COOH (MBI 202) (SEQ ID NO:4),
NH_2-KWKSFIKNLEKVLKPGGLLSNIVTSL-COOH (MBI 490) (SEQ ID NO:5),
NH_2-KWKSFIKNLEKVLKKGPILANLVSIV-COOH (MBI 491) (SEQ ID NO:6),
NH_2-KWKEFIKKLTTAVKKVLTTGLPALIS-COOH (MBI 492) (SEQ ID NO:7),
NH_2-KWKKFIKELQKVLAPGGLLSNIVTSL-COOH (MBI 493) (SEQ ID NO:8),
NH_2-KWKSFIKKLTSVLKKVVTTALPALIS-COOH (MBI 29A1) (SEQ ID NO:9),
NH_2-KWKSFIKNLTKVLKKVVTTALPALIS-COOH (MBI 29A2) (SEQ ID NO:10),
NH_2-KWKLFKKKGTGAVLTVLTTGLPALIS-COOH (MBI 29A3) (SEQ ID NO:11),
NH_2-KWKSFIKKLTSVLKKVVTTAKPLISS-COOH (SEQ ID NO:12),
NH_2-KKKSFIKLLTSAKVSVLTTAKPLISS-COOH (SEQ ID NO:13), and
NH_2-KWKKFIKELQKVLKPGGLLSNIVTSL-COOH (SEQ ID NO:14), analogs, derivatives and conservative variations thereof, wherein the peptides have antimicrobial activity. The peptides of the invention include SEQ ID NO:1-14, as well as the broader groups of peptides having hydrophilic and hydrophobic substitutions, and conservative variations thereof.

The sequence KWKSFIKK, as found in the first 8 NH_2 terminal amino acids of peptides in SEQ ID NO:1 and 2, is important for conferring antimicrobial activity. The presence of a positively charged amino acid (lysine or arginine) at positions 11, 14, 15 and 21 are also very important in antimicrobial activity. A further enhancement of antimicrobial activity can be achieved by making conservative changes in the residues designated R_1 and R_2.

In another embodiment, the invention provides an isolated antimicrobial peptide having an amino acid sequence:

NH_2-KKWWRRR$_1$R$_1$R$_2$R$_1$R$_1$R$_2$R$_2$GPALSNV-COOH

wherein R_1 is a hydrophobic amino acid residue and R_2 is a hydrophilic amino acid residue. Examples of such peptides of the invention include but are not limited to:

NH_2-KKWWRRVLSGLKTGPALSNV-COOH (SEQ ID NO:15),

NH_2-KKWWRRVLKGLSSGPALSNV-COOH (SEQ ID NO:16), and

NH_2-KKWWRRALQALKNGPALSNV-COOH (SEQ ID NO:17), analogs, derivatives and conservative variations thereof, wherein the peptides have antimicrobial activity. The peptides of the invention include SEQ ID NO:15-17, as well as the broader groups of peptides having hydrophilic and hydrophobic substitutions, and conservative variations thereof.

In another embodiment, the invention provides an isolated antimicrobial peptide having an amino terminal amino acid sequence:

NH_2-KKWWRRX. Preferably, the peptide is from about 20 to about 30 amino acids in length and therefore X is from about 14 to about 24 amino acids. Most preferably the peptide has a sequence of

NH_2-KKWWRR $R_1R_1R_2$GLKTAGPAIQSVLNK-COOH

wherein R_1 is a hydrophobic amino acid residue and R_2 is a hydrophilic amino acid residue. Examples of such peptides of the invention include but are not limited to:

NH_2-KKWWRRVLSGLKTAGPAIQSVLNK-COOH (MBI 21A1) (SEQ ID NO:18), and

NH_2-KKWWRRALQGLKTAGPAIQSVLNK-COOH (MBI 21A2) (SEQ ID NO:19), analogs, derivatives and conservative variations thereof, wherein the peptides have antimicrobial activity. The peptides of the invention include SEQ ID NO:18-19, as well as the broader groups of peptides having hydrophilic and hydrophobic substitutions, and conservative variations thereof.

In another embodiment, the invention provides an isolated antimicrobial peptide having an amino terminal amino acid sequence:
NH_2-KKWWKX. Preferably, the peptide is from about 20 to about 30 amino acids in length and therefore X is from about 14 to about 24 amino acids. Most preferably the peptide has a sequence of
NH_2-KKWWKAQKAVNSGPNALQTLAQ-COOH (SEQ ID NO:20)
NH_2-KKWWKAKKFANSGPNALQTLAQ-COOH (SEQ ID NO:21), or
NH_2-KKWWKFIKKAVNSGTTGLQTLAS-COOH (SEQ ID NO:22), analogs, derivatives and conservative variations thereof, wherein the peptides have antimicrobial activity. The peptides of the invention include SEQ ID NO:20-22, as well as the broader groups of peptides having hydrophilic and hydrophobic substitutions, and conservative variations thereof.

Other cationic peptides of the invention include:
NH_2-KKSFFKKLTSVASSVLS-COOH (MBI 21A14) (SEQ ID NO:23),
NH_2-WKVFKSFIKKASSFAQSVLD-COOH (SEQ ID NO:24), and
NH_2-KKWRKSFFKQVGSFDNSV-COOH (SEQ ID NO:25),
analogs, derivatives and conservative variations thereof, wherein the peptides have antimicrobial activity.

The sequence of SEQ ID NO:1 contains helix-forming hydrophilic amino acids at residues 4, 8, 10, 11, and 14. Amino acids 6 and 9 are hydrophobic residues (see FIGURE 1). The helical nature of the first 10 amino acids is depicted in FIGURES 1 and 2. The cationic charge of the peptide of SEQ ID NO:1 was achieved by placing a lysine at positions 8 and 14, thereby increasing the total positive charge to 6. The carboxy terminus of SEQ ID NO:1 was converted to the methyl ester and the antibacterial activity of this derivative was the same as that of the unmodified peptide. The peptide of SEQ ID NO:1 also has antifungal activity. Amino acid residues 3, 4, 7, 8, 10, 11, 14, and 15 are preferably hydrophilic residues, while amino acid residues 2, 5, 6, 9, 12, and 13 are preferably hydrophobic residues.

SEQ ID NO:1 was modified to increase the alpha helical nature by changing amino acids in the C-terminal tail, thereby resulting in SEQ ID NO:2. Amino acids 11 (neutral hydrophilic) and 13 (hydrophobic) of SEQ ID NO:1 were changed to another neutral hydrophilic and hydrophobic amino acid, respectively, in order to increase

alpha helicity in this region. The C-terminal tail (residue 20-26) was modified to include a positive charge and increase alpha helicity. Therefore residues 20, 21, 23, 24 and 25 were changed (See FIGURES 1 and 2). SEQ ID NO:3 was obtained by changing amino acids 8, 11, 12, 13, 14, and 16 of SEQ ID NO:1. The first 15 amino acid residues contain lysine at every fourth residue of the alpha helix. Thus, the lysines at positions 8 and 14 in SEQ ID NO:1 were replaced by asparagine and serine, respectively, and the serine at position 11 was changed to lysine. Further changes were made at positions 12, 13 and 16 to conserve the helical nature. The C-terminal tail was unchanged. SEQ ID NO:4 is identical to SEQ ID NO:3, except for the amino acid at position 4, which incorporates an additional positive charge. SEQ ID NO:5 and SEQ ID NO:6 represent peptides having the same amino acid sequence as SEQ ID NO:3 for the first 9 amino acids. The amino acid at position 10 is glutamic acid. Residues 10, 11, 12, and 13 in both SEQ ID NO:5 and SEQ ID NO:6 are different than SEQ ID NO:1, but remain either hydrophilic or hydrophobic as described above.

Residue 14 in SEQ ID NO:5 is the same as in SEQ ID NO:1. All residues thereafter, 15-26, are completely changed to construct a hydrophobic helical tail, and bears no resemblance to the tail of SEQ ID NO:1. SEQ ID NO:6 contains the same residues as SEQ ID NO:1 at positions 14 and 15. Residues 16-26 have been changed to form a helical tail.

The term "isolated" as used herein refers to a peptide substantially free of proteins, lipids, nucleic acids, for example, with which it is naturally associated. Those of skill in the art can make similar substitutions to achieve peptides with greater antibacterial activity and a broader host range. For example, the invention includes the bactolysin peptides depicted in SEQ ID NO:1-25, as well as analogues or derivatives thereof, as long as the bioactivity of the peptide remains. Minor modifications of the primary amino acid sequence of the peptides of the invention may result in peptides which have substantially equivalent activity as compared to the specific peptides described herein. Such modifications may be deliberate, as by site-directed mutagenesis, or may be spontaneous. All of the peptides produced by these modifications are included herein as long as the biological activity of the original peptide still exists.

Further, deletion of one or more amino acids can also result in a modification of the structure of the resultant molecule without significantly altering its biological activity. This can lead to the development of a smaller active molecule which would also have utility. For example, amino or carboxy terminal amino acids which may not be required for biological activity of the particular peptide can be removed. Peptides of the invention include any analog, homolog, mutant, isomer or derivative of the peptides disclosed in the present invention, so long as the bioactivity as described herein is remains. All peptides were synthesized using L amino acids, however, all D forms of the peptides (*e.g.*, see Table 1b, CEMA) can be synthetically produced. In addition, C-terminal derivatives can be produced, such as C-terminal methyl esters, in order to increase the antimicrobial activity of a peptide of the invention.

The peptide of the invention include peptides which are conservative variations of those peptides specifically exemplified herein. The term "conservative variation" as used herein denotes the replacement of an amino acid residue by another, biologically similar residue. Examples of conservative variations include the substitution of one hydrophobic residue such as isoleucine, valine, leucine, alanine, cysteine, glycine, phenylalanine, proline, tryptophan, tyrosine, norleucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acids, or glutamine for asparagine, and the like. Neutral hydrophilic amino acids which can be substitued for one another include asparagine, glutamine, serine and threonine. The term "conservative variation" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid provided that antibodies raised to the substituted polypeptide also immunoreact with the unsubstituted polypeptide. Such conservative substitutions are within the definition of the classes of the peptides of the invention with respect to R_1 and R_2. For the peptides of the invention, a preferred conservative variation is substitution of lysine by arginine.

The biological activity of the peptides can be determined by standard methods known to those of skill in the art, such as "minimal inhibitory concentration (MIC)" assay described in the present examples, whereby the lowest concentration at which no change in OD is observed for a given period of time is recorded as MIC. Alternatively, "fractional inhibitory concentration (FIC)" is also useful for determination of synergy between the peptides of the invention, or the peptides in combination with

known antibiotics. FICs are performed by checkerboard titrations of peptides in one dimension of a microtiter plate, and of antibiotics in the other dimension, for example. The FIC is calculated by looking at the impact of one antibiotic on the MIC of the other and vice versa. An FIC of one indicates that the influence of the compounds is additive and an FIC of less than one indicates synergy. Preferably, an FIC of less than 0.5 is obtained for synergism. As used herein, FIC can be determined as follows:

$$FIC = \frac{\text{MIC (peptide in combination)}}{\text{MIC (peptide alone)}} + \frac{\text{MIC (antibiotic in combination)}}{\text{MIC (antibiotic alone)}}$$

Peptides of the invention can be synthesized by such commonly used methods as t-BOC or FMOC protection of alpha-amino groups. Both methods involve stepwise syntheses whereby a single amino acid is added at each step starting from the C terminus of the peptide (See, Coligan, *et al., Current Protocols in Immunology,* Wiley Interscience, 1991, Unit 9). Peptides of the invention can also be synthesized by the well known solid phase peptide synthesis methods described Merrifield, *J. Am. Chem. Soc.*, 85:2149, 1962), and Stewart and Young, *Solid Phase Peptides Synthesis*, (Freeman, San Francisco, 1969, pp.27-62), using a copoly(styrene-divinylbenzene) containing 0.1-1.0 mMol amines/g polymer. On completion of chemical synthesis, the peptides can be deprotected and cleaved from the polymer by treatment with liquid HF-10% anisole for about 1/4-1 hours at 0°C. After evaporation of the reagents, the peptides are extracted from the polymer with 1% acetic acid solution which is then lyophilized to yield the crude material. This can normally be purified by such techniques as gel filtration on Sephadex G-15 using 5% acetic acid as a solvent. Lyophilization of appropriate fractions of the column will yield the homogeneous peptide or peptide derivatives, which can then be characterized by such standard techniques as amino acid analysis, thin layer chromatography, high performance liquid chromatography, ultraviolet absorption spectroscopy, molar rotation, solubility, and quantitated by the solid phase Edman degradation.

The invention includes polynucleotides encoding:
NH_2-KWK$R_2R_1R_1R_2R_2R_1R_2R_2R_1R_1R_2R_2$VLTTGLPALIS-COOH,
NH_2-KWK$R_2R_1R_1R_2R_2R_1R_2R_2R_1R_1R_2R_2$VVTTAKPLISS-COOH,
NH_2-KWK$R_2R_1R_1R_2R_2R_1R_2R_2R_1R_1R_2R_2$ILTTGLPALIS-COOH,
NH_2-KWK$R_2R_1R_1R_2R_2R_1R_2R_2R_1R_1R_2R_2$GGLLSNIVTSL-COOH, and
NH_2-KWK$R_2R_1R_1R_2R_2R_1R_2R_2R_1R_1R_2R_2$GPILANLVSIV-COOH wherein R_1 is a hydrophobic amino acid residue and R_2 is a hydrophilic amino acid residue.

More specifically, the invention also includes an isolated polynucleotide which encodes the MBI 29 peptide of SEQ ID NO:1, an isolated polynucleotide which encodes the MBI 26 peptide of SEQ ID NO:2, and isolated polynucleotides which encode SEQ ID NO: 3-25. In addition, the invention includes polynucleotides which encode analogs, mutants and variants of the peptides of the invention. The term "isolated" as used herein refers to a polynucleotide substantially free of proteins, lipids, nucleic acids, for example, with which it is naturally associated. As used herein, "polynucleotide" refers to a polymer of deoxyribonucleotides or ribonucleotides, in the form of a separate fragment or as a component of a larger construct. DNA encoding a peptide of the invention can be assembled from cDNA fragments or from oligonucleotides which provide a synthetic gene which is capable of being expressed in a recombinant transcriptional unit. Polynucleotide sequences of the invention include DNA, RNA and cDNA sequences. A polynucleotide sequence can be deduced from the genetic code, however, the degeneracy of the code must be taken into account. Polynucleotides of the invention include sequences which are degenerate as a result of the genetic code. Such polynucleotides are useful for the recombinant production of large quantities of a peptide of interest, such as the peptide of SEQ ID NO:1-25.

In the present invention, the polynucleotides encoding the cationic peptides of the invention may be inserted into a recombinant "expression vector". The term "expression vector" refers to a plasmid, virus or other vehicle known in the art that has been manipulated by insertion or incorporation of cationic genetic sequences. Such expression vectors of the invention are preferably plasmids which contain a promoter sequence which facilitates the efficient transcription of the inserted genetic sequence in the host. The expression vector typically contains an origin of replication, a promoter, as well as specific genes which allow phenotypic selection of the transformed cells. For example, the expression of the peptides of the

invention can be placed under control of *E. coli* chromosomal DNA comprising a lactose or lac operon which mediates lactose utilization by elaborating the enzyme beta-galactosidase. The lac control system can be induced by IPTG. A plasmid can be constructed to contain the *lac Iq* repressor gene, permitting repression of the *lac* promoter until IPTG is added. Other promoter systems known in the art include beta lactamase, lambda promoters, the protein A promoter, and the tryptophan promoter systems. While these are the most commonly used, other microbial promoters, both inducible and constitutive, can be utilized as well. The vector contains a replicon site and control sequences which are derived from species compatible with the host cell. In addition, the vector may carry specific gene(s) which are capable of providing phenotypic selection in transformed cells. For example, the beta-lactamase gene confers ampicillin resistance to those transformed cells containing the vector with the beta-lactamase gene.

Transformation of a host cell with the polynucleotide may be carried out by conventional techniques well known to those skilled in the art. For example, where the host is prokaryotic, such as *E. coli*, competent cells which are capable of DNA uptake can be prepared from cells harvested after exponential growth and subsequently treated by the $CaCl_2$ method using procedures well known in the art. Alternatively, $MgCl_2$ or RbCl could be used.

In addition to conventional chemical methods of transformation, the plasmid vectors of the invention may be introduced into a host cell by physical means, such as by electroporation or microinjection. Electroporation allows transfer of the vector by high voltage electric impulse, which creates pores in the plasma membrane of the host and is performed according to methods well known in the art. Additionally, cloned DNA can be introduced into host cells by protoplast fusion, using methods well known in the art.

DNA sequences encoding the cationic peptides can be expressed *in vivo* by DNA transfer into a suitable host cell. "Host cells" of the invention are those in which a vector can be propagated and its DNA expressed. The term also includes any progeny of the subject host cell. It is understood that not all progeny are identical to the parental cell, since there may be mutations that occur during replication. However, such progeny are included when the terms above are used. Preferred host

cells of the invention include *E. coli, S. aureus* and *P. aeruginosa*, although other Gram-negative and Gram-positive organisms known in the art can be utilized as long as the expression vectors contain an origin of replication to permit expression in the host.

The cationic peptide polynucleotide sequence used according to the method of the invention can be isolated from an organism or synthesized in the laboratory. Specific DNA sequences encoding the cationic peptide of interest can be obtained by: 1) isolation of a double-stranded DNA sequence from the genomic DNA; 2) chemical manufacture of a DNA sequence to provide the necessary codons for the cationic peptide of interest; and 3) *in vitro* synthesis of a double-stranded DNA sequence by reverse transcription of mRNA isolated from a donor cell. In the latter case, a double-stranded DNA complement of mRNA is eventually formed which is generally referred to as cDNA.

The synthesis of DNA sequences is frequently the method of choice when the entire sequence of amino acid residues of the desired peptide product is known. In the present invention, the synthesis of a DNA sequence has the advantage of allowing the incorporation of codons which are more likely to be recognized by a bacterial host, thereby permitting high level expression without difficulties in translation. In addition, virtually any peptide can be synthesized, including those encoding natural cationic peptides, variants of the same, or synthetic peptides.

When the entire sequence of the desired peptide is not known, the direct synthesis of DNA sequences is not possible and the method of choice is the formation of cDNA sequences. Among the standard procedures for isolating cDNA sequences of interest is the formation of plasmid or phage containing cDNA libraries which are derived from reverse transcription of mRNA which is abundant in donor cells that have a high level of genetic expression. When used in combination with polymerase chain reaction technology, even rare expression products can be cloned. In those cases where significant portions of the amino acid sequence of the cationic peptide are known, the production of labeled single or double-stranded DNA or RNA probe sequences duplicating a sequence putatively present in the target cDNA may be employed in DNA/DNA hybridization procedures which are carried out on cloned

copies of the cDNA which have been denatured into a single stranded form (Jay, *et al.*, *Nuc. Acid Res.*, 11:2325, 1983).

The invention also provides a method of inhibiting the growth of bacteria comprising contacting the bacteria with an inhibiting effective amount of a peptide of the invention, including:

NH_2-KWK$R_2R_1R_1R_2R_2R_1R_2R_2R_1R_1R_2R_2$VLTTGLPALIS-COOH,

NH_2-KWK$R_2R_1R_1R_2R_2R_1R_2R_2R_1R_1R_2R_2$VVTTAKPLISS-COOH,

NH_2-KWK$R_2R_1R_1R_2R_2R_1R_2R_2R_1R_1R_2R_2$ILTTGLPALIS-COOH,

NH_2-KWK$R_2R_1R_1R_2R_2R_1R_2R_2R_1R_1R_2R_2$GGLLSNIVTSL-COOH,

NH_2-KWK$R_2R_1R_1R_2R_2R_1R_2R_2R_1R_1R_2R_2$GPILANLVSIV-COOH,

NH_2-KKWWRR$R_1R_1R_2R_1R_1R_2R_2$GPALSNV-COOH, or

NH_2-KKWWRR $R_1R_1R_2$GLKTAGPAIQSVLNK-COOH

wherein R_1 is a hydrophobic amino acid residue and R_2 is a hydrophilic amino acid residue. More specifically, the invention provides a method of inhibiting the growth of bacteria comprising contacting the bacteria with an inhibiting effective amount of a peptide of the invention, such as MBI 26 or MBI 29,

NH_2-KWKSFIKKLTTAVKKVLTTGLPALIS-COOH (SEQ ID NO:1),

NH_2-KWKSFIKKLTSAAKKVVTTAKPLISS-COOH (SEQ ID NO:2), or

NH_2-KWKSFIKNLTKGGSKILTTGLPALIS-COOH (SEQ ID NO:3),

NH_2-KWKKFIKNLTKGGSKILTTGLPALIS-COOH (SEQ ID NO:4),

NH_2-KWKSFIKNLEKVLKPGGLLSNIVTSL-COOH (MBI 490) (SEQ ID NO:5),

NH_2-KWKSFIKNLEKVLKKGPILANLVSIV-COOH (MBI 491) (SEQ ID NO:6),

NH_2-KWKEFIKKLTTAVKKVLTTGLPALIS-COOH (MBI 492) (SEQ ID NO:7),

NH_2-KWKKFIKELQKVLAPGGLLSNIVTSL-COOH (MBI 493) (SEQ ID NO:8),

NH_2-KWKSFIKKLTSVLKKVVTTALPALIS-COOH (MBI 29A1) (SEQ ID NO:9),

NH_2-KWKSFIKNLTKVLKKVVTTALPALIS-COOH (MBI 29A2) (SEQ ID NO:10),

NH_2-KWKLFKKKGTGAVLTVLTTGLPALIS-COOH (MBI 29A3) (SEQ ID NO:11),

NH_2-KWKSFIKKLTSVLKKVVTTAKPLISS-COOH (SEQ ID NO:12),

NH_2-KKKSFIKLLTSAKVSVLTTAKPLISS-COOH (SEQ ID NO:13),

NH_2-KWKKFIKELQKVLKPGGLLSNIVTSL-COOH (SEQ ID NO:14),

NH_2-KKWWRRVLSGLKTGPALSNV-COOH (SEQ ID NO:15),

NH_2-KKWWRRVLKGLSSGPALSNV-COOH (SEQ ID NO:16),

NH_2-KKWWRRALQALKNGPALSNV-COOH (SEQ ID NO:17),

NH_2-KKWWRRVLSGLKTAGPAIQSVLNK-COOH (MBI 21A1) (SEQ ID NO:18),

NH_2-KKWWRRALQGLKTAGPAIQSVLNK-COOH (MBI 21A2) (SEQ ID NO:19),
NH_2-KKWWKAQKAVNSGPNALQTLAQ-COOH (SEQ ID NO:20)
NH_2-KKWWKAKKFANSGPNALQTLAQ-COOH (SEQ ID NO:21),
NH_2-KKWWKFIKKAVNSGTTGLQTLAS-COOH (SEQ ID NO:22),
NH_2-KKSFFKKLTSVASSVLS-COOH (MBI 21A14) (SEQ ID NO:23),
NH_2-WKVFKSFIKKASSFAQSVLD-COOH (SEQ ID NO:24), and
NH_2-KKWRKSFFKQVGSFDNSV-COOH (SEQ ID NO:25).
and analogs, derivatives, or conservative variations thereof.

The term "contacting" refers to exposing the bacteria to the peptide so that the peptide can effectively inhibit, kill, or lyse bacteria, bind endotoxin (LPS), or p-ermeabilize gram- negative bacterial outer membranes. Contacting may be *in vitro*, for example by adding the peptide to a bacterial culture to test for susceptibility of the bacteria to the peptide. Contacting may be *in vivo*, for example administering the peptide to a subject with a bacterial disorder, such as septic shock. "Inhibiting" or "inhibiting effective amount" refers to the amount of peptide which is required to cause a bacteriostatic or bactericidal effect. Examples of bacteria which may be inhibited include *E. coli, P. aeruginosa, E. cloacae, S. typhimurium,* and *S. aureus.*

The method of inhibiting the growth of bacteria may further include the addition of antibiotics for combination or synergistic therapy. The appropriate antibiotic administered will typically depend on the susceptibility of the bacteria such as whether the bacteria is gram negative or gram positive, and will be easily discernable by one of skill in the art. Examples of particular classes of antibiotics useful for synergistic therapy with the peptides of the invention include aminoglycosides (*e.g.*, tobramycin), penicillins (*e.g.*, piperacillin), cephalosporins (*e.g.*, ceftazidime), fluoroquinolones (*e.g.*, ciprofloxacin), carbepenems (*e.g.*, imipenem), tetracyclines and macrolides (*e.g.*, erythromycin and clarithromycin). The peptides and/or analogues or derivatives thereof may be administered to any host, including a human or non-human animal, in an amount effective to inhibit not only growth of a bacterium, but also a virus or fungus. These peptides are useful as antimicrobial agents, antiviral agents, and antifungal agents.

The peptide of the invention can be administered parenterally by injection or by gradual infusion over time. The peptide can be administered intravenously,

intraperitoneally, intramuscularly, subcutaneously, intracavity, or transdermally. Preferred methods for delivery of the peptide include orally, by encapsulation in microspheres or proteinoids, by aerosol delivery to the lungs, or transdermally by iontophoresis or transdermal electroporation. Other methods of administration will be known to those skilled in the art.

Preparations for parenteral administration of a peptide of the invention include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

The invention provides a method of treating or ameliorating an endotoxemia or septic shock (sepsis) associated disorder, or one or more of the symptoms of sepsis comprising administering to a subject displaying symptoms of sepsis or at risk for developing sepsis, a therapeutically effective amount of a cationic peptide of the invention, for example, SEQ ID NO:1 or SEQ ID NO:2, or analogs, derivatives, or conservative variations thereof. The term "ameliorate" refers to a decrease or lessening of the symptoms of the disorder being treated. Such symptoms which may be ameliorated include those associated with a transient increase in the blood level of TNF, such as fever, hypotension, neutropenia, leukopenia, thrombocytopenia, disseminated intravascular coagulation, adult respiratory distress syndrome, shock and multiple organ failure. Patients who require such treatment include those at risk for or those suffering from toxemia, such as endotoxemia resulting from a gram-negative bacterial infection, venom poisoning, or hepatic failure, for example. In addition, patients having a gram-positive bacterial, viral or fungal infection may display symptoms of sepsis and may benefit from such a therapeutic method as described herein. Those patients who are more particularly able to benefit from the method of the invention are those suffering from infection by *E. coli, Haemophilus*

influenza B, Neisseria meningitides, staphylococci, or pneumococci. Patients at risk for sepsis include those suffering from gunshot wounds, renal or hepatic failure, trauma, burns, immunocompromised (HIV), hematopoietic neoplasias, multiple myeloma, Castleman's disease or cardiac myxoma.

The term "therapeutically effective amount" as used herein for treatment of endotoxemia refers to the amount of cationic peptide used is of sufficient quantity to decrease the subject's response to LPS and decrease the symptoms of sepsis. The term "therapeutically effective" therefore includes that the amount of cationic peptide sufficient to prevent, and preferably reduce by at least 50%, and more preferably sufficient to reduce by 90%, a clinically significant increase in the plasma level of TNF. The dosage ranges for the administration of cationic peptide are those large enough to produce the desired effect. Generally, the dosage will vary with the age, condition, sex, and extent of the infection with bacteria or other agent as described above, in the patient and can be determined by one skilled in the art. The dosage can be adjusted by the individual physician in the event of any contraindications. In any event, the effectiveness of treatment can be determined by monitoring the level of LPS and TNF in a patient. A decrease in serum LPS and TNF levels should correlate with recovery of the patient.

In addition, patients at risk for or exhibiting the symptoms of sepsis can be treated by the method as described above, further comprising administering, substantially simultaneously with the therapeutic administration of cationic peptide, an inhibitor of TNF, an antibiotic, or both. For example, intervention in the role of TNF in sepsis, either directly or indirectly, such as by use of an anti-TNF antibody and/or a TNF antagonist, can prevent or ameliorate the symptoms of sepsis. Particularly preferred is the use of an anti-TNF antibody as an active ingredient, such as a monoclonal antibody with TNF specificity as described by Tracey, *et al. (Nature*, 330:662, 1987).

A patient who exhibits the symptoms of sepsis may be treated with an antibiotic in addition to the treatment with cationic peptide. Typical antibiotics include an amino-glycoside, such as gentamicin or a beta-lactam such as penicillin, or cephalosporin. Therefore, a preferred therapeutic method of the invention includes administering a therapeutically effective amount of cationic peptide substantially simultaneously with administration of a bactericidal amount of an antibiotic. Preferably, administration of

cationic peptide occurs within about 48 hours and preferably within about 2-8 hours, and most preferably, substantially concurrently with administration of the antibiotic.

The term "bactericidal amount" as used herein refers to an amount sufficient to achieve a bacteria-killing blood concentration in the patient receiving the treatment. The bactericidal amount of antibiotic generally recognized as safe for administration to a human is well known in the art, and as is known in the art, varies with the specific antibiotic and the type of bacterial infection being treated.

Because of the antibiotic, antimicrobial, and antiviral properties of the peptides, they may also be used as preservatives or sterillants of materials susceptible to microbial or viral contamination. The peptides of the invention can be utilized as broad spectrum antimicrobial agents directed toward various specific applications. Such applications include use of the peptides as preservatives in processed foods (organisms including Salmonella, Yersinia, Shigella), either alone or in combination with antibacterial food additives such as lysozymes; as a topical agent (Pseudomonas, Streptococcus) and to kill odor producing microbes (Micrococci). The relative effectiveness of the cationic peptides of the invention for the applications described can be readily determined by one of skill in the art by determining the sensitivity of any organism to one of the peptides.

The following examples are intended to illustrate but not limit the invention. While they are typical of those that might be used, other procedures known to those skilled in the art may alternatively be used.

EXAMPLE 1
MIC VALUES FOR CATIONIC PEPTIDES

The minimum inhibitory concentrations of (MIC) of CEME, CEMA, the first 8 amino acid residues of CEME (NH_2-KWKLFKKIGIGAVLKVLTTGLPALIS-COOH; SEQ ID NO:1 with changes at residues 4, 6, 8-11, 14) and CEMA (NH_2-K-WKLFKKIGIGAVLKVLTTGLPALKLTK-COOH; SEQ NO:1 with changes at residues 4, 6, 8-11, 14, and 25-28), (Piers, K. and Hancock, R., *Molec. Microbiology,* 12(6), 1994) 20 carboxy terminal amino acids of melittin (MA), 8 amino terminal amino acids from cecropin, and the peptides shown in SEQ ID NO:1, MBI 29 and SEQ ID

NO:2, MBI 26, were determined for a number of different bacteria (Table 1a and 1b). Briefly, cells were grown overnight at 37°C in LB-S (Luria broth without any salt supplement) and diluted one in 10,000 in the same medium to give concentrations of about 10^4 to 10^5 CFU/ml. The broth dilutions were set up in a 96 well microtiter plate by putting 200μl of LB-S containing the initial concentration of antibiotic or compound in column 1 and 100 μl of the same medium in columns 2-12. The compounds were diluted by taking 100 μl of broth from column 1 and mixing it with column 2, resulting in a one in two dilution. This was continued to column 10. Finally, 10 μl of bacteria were pipetted into columns 1-11, and the plates incubated overnight at 37°C. The next day the plates were scored for growth in the wells, and the MIC determined.

TABLE 1a

MINIMUM INHIBITORY CONCENTRATION (MIC) VALUES FOR CATIONIC PEPTIDES

STRAIN	CEME	CEMA	MBI 29	MBI 26	CE-8	MA-20
S. aureus (K147)	32	≥64	20	≥64	≥64	≥64
S. aureus (SAP0017)	24	≥64	18	≥64	≥64	≥64
S. epidermis	16	≥64	10	≥64	≥64	≥64
E. coli (UB1005)	5	10	3	3	≥64	≥64
P. aeruginosa (H187)	8	26	5	11	≥64	≥64
Candida albicans (CAN105)	≥64	≥64	40	≥64	≥64	≥64

Additional MICs were determined for MBI-26 and MBI-29 and MBI 29 and MBI21 derivatives as noted for the following strains:

Strain	MBI 26	MBI 29
P. aeruginosa (H 103)	4	4
E. coli. Bort	2	6
E. coli 0111:B4	2	2
X maltophilia	2	3
A. calcoaceticus	2	4
E. cloacae	2	2
K. pneumoniae	8	16

Peptide	*E.coli* 0111:B4 µg/ml	*E. faecalis*	*B. Subtilis*	H103	S.epi	SAP 0017	K147	C610
MBI29A1	4	64	16	16	16	16	16	8
MBI29A2	8	64	8	16	16	8	16	8
MBI29A3	8	>128	16	16	16	32	32	8
MBI21A1	16	>128	>128	64	128	>128	>128	16
MBI21A2	32	>128	>128	128	>128	>128	>128	16
MBI21A14	>128	>128	>128	>128	>128	>128	>128	128

In a separate set of experiments, the following MIC values were obtained:

TABLE 1b
MINIMUM INHIBITORY CONCENTRATION (MIC) VALUES FOR CATIONIC PEPTIDES

STRAIN	CEMA	CEMA^{me}	MBI 29	MBI 29^{me}	MBI 26D	MBI 26L
S. aureus (K147)	≥64	≥64	32	16	≥64	≥64
S. aureus (SAP0017)	≥64	≥64	32	16	≥64	≥64
S. epidermis	43	43	16	8	8-16	32
P. aeruginosa (H187)	32	53	16	8	16	32
E. coli (UB1005)	13	32	4	4	4-8	4
E. faecalis	≥64	≥64	32	≥64	≥64	≥64
Candida albicans (CAN105)	≥64	≥64	32	32	≥64	≥64

The results show that both MBI 29 (SEQ ID NO:1) and MBI 26 (SEQ ID NO:2) are effective antimicrobial agents for a variety of gram positive and gram negative bacteria. In addition, all peptides were effective against *Candida albicans.* Modification of the peptides, such as methyl ester modification, or L to D amino acids provided a broader class of antimicrobial agents.

Agarose Dilution Susceptibility Testing

The following is a novel agarose-based solid phase assay system to measure antimicrobial activity of the cationic peptides of the invention, for example, either alone or in combination with a conventional antibiotic. Standard MIC methods, as described above or this method can be used to determine MICs of the peptides described herein.

A) Preparing plates

1) Unsupplemented Mueller-Hinton agarose (*i.e.* no Ca^{2+} or Mg^{2+} added) was used. (Unsupplemented broth is used with agar because agar contains divalent cations).

Prepared as follows:

22 g/L Mueller-Hinton powder (BBI, #11443)

15 g/L agarose (Sigma, A-0576)

QS to 1 L with sdH_2O

Heat to boiling and boil 1 min

Adjust pH to 7.3 with NaOH or KOH (adjust the meter to the correct temperature

-note agarose solidifies at <40°C)

Autoclave for 10 min at 121°C

After the agarose has been autoclaved, it is allowed to cool to 40-45°C in a water bath before aseptically adding antimicrobial solutions, and pouring the plates.

2) Appropriate dilutions of antimicrobial solutions are added to molten test agarose that has been allowed to equilibrate in a water bath to 40-45°C.

In general, a scheme in which 1 part of antimicrobial solution is added to 9 parts of liquid agarose is used (*i.e.* 2 mL of drug to 18 mL of agar for each plate; 1 mL of each drug in the combination if testing synergy).

3) The agarose and antimicrobial solution are mixed throughly by gentle inversion about 10X to avoid bubbles and the mixture poured into petri-dishes on a level surface to result in an agar depth of 3-4 mm.

4) The plates are poured as quickly as possible after mixing to prevent cooling and partial solidification.

5) The agarose is allowed to solidify at room temperature and the plates are either used immediately or stored in sealed plastic bags at 4°C. Storage time can vary but plates should be used within a week.

6) Plates stored at 4°C should be allowed to equilibrate at room temperature before use. The surface should be dry before inoculating the plates. If necessary, plates can be placed in the fume hood for 30 min with lids ajar to hasten drying of agarose surface.

B) Inoculum Preparation

1) To standardize the inoculum density, a 0.5 McFarland standard can be prepared as follows:

A 0.5 mL aliquot of 0.48 m $BaCl_2$ is added to 99.5 mL of 0.36 N H_2SO_4 (1% v/v) with constant stirring to maintain a suspension. The correct density should be verified using a spectrophotometer with a 1 cm light path and matched cuvettes to determine the absorbance. The absorbance at 630 nm should be 0.08 to 0.10. The suspension should be transferred to 10 mL aliquot into screwcap tubes of the same size as those used in growing or diluting the bacterial inoculum. These tubes should be tightly sealed and stored in the dark at room temperature. The turbidity standard should be rigorously agitated on a vortex mixer prior to use. If large particles appear, the standard should be replaced. Replacement should occur every month (use a new standard tubes each time, and when ten are used up, make a new batch).

2) 3 to 5 morphologically identical colonies are selected from a TSA plate (the top of each is touched with a sterile loop) and transferred to a tube of 5 mL TSB.

3) The broth culture is incubated at 37°C with shaking until it achieved or exceeds the turbidity of the standard (2 to 6 hours) -approx. 1 to 2 x 10^8 CFU/mL.

4) The turbidity can be adjusted with broth to obtain a turbidity equal to the 0.5 standard. Use adequate lighting and hold up a white card with black lines behind the cultures to assist in the comparison.

5) Cultures adjusted to the standard are diluted 1/10 in sterile broth to obtain a density of approximately 10^7 CFU/mL. Most inoculum replicators deposit approximately 1 to 2 μL on the agarose surface. The final inoculum on the agarose will then be approximately 10^4 CFU in a spot of 5 to 8 mm in diameter on the agarose. The adjusted suspensions should be used for final inoculation within 15 min of preparation.

The tubes containing the adjusted and diluted bacterial suspensions should be arranged in order on a rack. An aliquot of each well-mixed suspension is placed into the corresponding well in the replicator inoculum block. The agarose plates are marked for orientation of the inoculum spots. A growth control plate (no antimicrobial) is inoculated first and then, starting with the lowest concentration, the plates continuing the different antimicrobial concentration are inoculated (*Optional-* A second growth control plate is inoculated last to ensure that there was no contamination or significant antimicrobial carryover during the inoculation).

Optional- a sample of each inoculum is streaked on a suitable agar plate and incubated

6) The inoculated plates are allowed to stand at room temperature until the moisture in the inoculum spots have been absorbed into the agarose. The plates are inverted and incubated at 35°C for 24 hours, 48 hours.

7) The plates should be placed on a dark, nonreflecting surface to determine the end points. The MIC is recorded as the lowest concentration of antimicrobial agent that completely inhibits growth, disregarding a single-colony or a faint haze caused by the inoculum.

8) If two or more colonies persist in concentrations of the agent beyond an obvious end point, or if there is no growth at lower concentrations, the culture purity is checked and test possibly repeated.

9) After 48 hours a replica plating tool can be used to transfer cells onto a fresh TSA or BHI plate and growth is read after 24 hrs.

EXAMPLE 2

EFFECT OF FORMULATION ON BACTERICIDAL ACTIVITY

Effect of formulation on bactericidal activity and MIC was determined using the test solutions listed below. The results show that citrate and related formulations reduced the MIC (Table 2). Similar effects were seen with acetate formulated peptide (Tables 2 and 3).

TABLE 2

EFFECT OF CITRATE, ASCORBIC ACID AND DEXTROSE ON MBI 26 (10MG/ML) KILLING OF *P. AERUGINOSA*

			Survivors at Time (min.)			
Test Solution	Peptide	0	15	30	60
Citrate (0.129M)	Control	$1.1x10^7$	$1.1x10^7$	$1.2x10^7$	$1.4x10^7$
	+MBI 26	$1.1x10^7$	0	0	0
Citrate (0.129M) +Ascorbic Acid (100mM)	Control	$1.3x10^7$	$1.2x10^7$	$1.7x10^7$	$1.7x10^7$
	+MBI 26	$1.3x10^7$	0	0	0
Citrate-Dextrose	Control	$1.2x10^7$	$5.8x10^6$		$6.9x10^6$
	+MBI26	$9x10^6$	20	0	0
Citrate-Phosphate Dextrose	Control	$7.8x10^6$	$6.5x10^7$	$1.5x10^7$	$1.1x10^7$
	+MBI 26	0		0	0
Dextran	Control	$2.3x10^7$	$2.2x10^7$	$1.4x10^7$	$2x10^7$
	+MBI 26 (20µg)	$1.9x10^7$	$1.2x10^6$	$1x10^6$	$8.5x10^5$

TABLE 3

EFFECT OF CITRATE ADDITION TO CATION ADJUSTED MEDIA ON MINIMAL INHIBITORY CONCENTRATIONS

Strain	MIC(μg/mL)	
	CAMHB	CAMHB + Citrate
E. coli (UB 1005)	4	2
E. coli (111:B4)	8	2
E. coli (Bort)	16	8
S. typhimurium (C610)	8	2
E. facaelis (C625)	16	>32
A. calcoacet. (C402)	8	4
A. calcoacet (C403)	1	1
E. cloacae (C601)	8	4
E. cloacae (C602)	8	4
E. cloacae (C603)	4	2
P. aeruginosa (H103)	16	2

The media used in this experiment was Cation Adjusted Muller Hinton (CAMHB), and CAMHB +8mMcitrate. MBI 26 was prepared in sterile distilled water.

EXAMPLE 3

INNER MEMBRANE PERMEABILIZATION

Inner membrane permeability was determined by measurement in *E. coli* ML 35 of β-galactosidase activity using ONPG as a substrate. Normally ONPG cannot pass across the cytoplasmic membrane to permit hydrolysis by the cytoplasmic enzyme β-galactosidase. However, cationic peptides, by permeabilizing the inner membrane, unmasks this enzyme leading to color development as ONP is released. MBI 26 and MBI 29 were tested with the same conditions as shown in Figure 3 and 4 (4 ug MBI 26 or MBI 29 alone, ▬; + 100mM NaCl, □; + 100 uM CCCP, ▬▬; +5 mM Mg, ▬▪▪▬). While 100mM NaCl had little effect on MBI 26 permeabilization, 5mM Mg++ had a large inhibitory effect. MBI 29 permeabilization of the inner membrane was the least affected by any of the conditions especially Mg^{++} as seen in Figure 4. Addition of citrate increased the lag time before the

peptides were able to permeabilize the membranes. When the experiments were executed in cation adjusted media (CAMHB), the lag time was increased up to 30 minutes. The data in Table 4 shows the rate and lag time for inner membrane permeabilization of MBI 26, MBI 29 and peptides 490, 491, 492 and 493 (SEQ ID NO:2, 1, and 5-8, respectively).

TABLE 4

INNER MEMBRANE PERMEABILIZATION

Peptide	Lag Time (Sec.)	Rate of Hydrolysis ($\Delta AU\ min^{-1}$)
MBI 26 (SEQ ID NO:2)	210	0.081
MBI 29 (SEQ ID NO:1)	66	0.21
490 (SEQ ID NO:5)	156	0.081
491 (SEQ ID NO:6)	152	0.14
492 (SEQ ID NO:7)	68	0.23
493 (SEQ ID NO:8)	136	0.14

EXAMPLE 4

INHIBITION OF LPS-MEDIATED TNF INDUCTION IN MACROPHAGES BY CATIONIC PEPTIDES

The effect of cationic peptides, CEME (KWKLFKKIGIGAVLKVLTTGLPALIS) and CEMA (KWKLFKKIGIGAVLKVLTTGLPALKLTK) and the peptides of the invention, MBI 29, NH_2-KWKSFIKKLTTAVKKVLTTGLPALIS-COOH (SEQ ID NO:1) and MBI 26, NH_2-KWKSFIKKLTSAAKKVVTTAKPLISS-COOH (SEQ ID NO:2), on LPS-induced TNF in macrophages was examined. RAW 264.7 macrophage cells were grown by seeding 10^6 cells into a $162cm^2$ cell culture flask and incubated at 37°C, 5% CO_2 for 1 week. RAW cell media [(Dulbecco's Modified Eagle Medium with Hepes buffer 450 ml; 2.4mM L-glutamine 3ml (400mM); Pen/Strep 3ml (10^4U/ml of Pen, 1 mg/ml strep); and 10% heat inactivated fetal bovine serum (FBS) 50ml)] was then completely removed from flasks. 10 mls of cell dissociation solution (Sigma) was added to each flask and incubated at 37°C for 10 minutes. Cells were removed from flasks, diluted in RAW cell media and centrifuged for 6 minutes. The cell pellet was resuspended in 5ml of media / flask used. 100µl cell suspension was removed and added to

400µl of trypan blue and cells were counted using a hemocytometer. The cell suspension was diluted to 1 x 10^6 cells / ml and 1 ml of suspension was added per well of a 24 well plate. The 24 well plates were incubated at 37°C, 5% CO_2 overnight for use in the assay.

After an overnight incubation, the media was aspirated from all the wells. 100µl of Lipopolysaccharide (LPS) was added at 100ng/100µl. CEME, CEMA or MBI 29 was added at the desired concentration/100µl to specified wells. RAW cell media was added to all the wells so they all had a final volume of 1 ml. The plates were then incubated for six hours at 37°C, 5% CO_2. The supernatant was then removed from the wells and stored overnight at 4°C. For those wells in which whole bacteria were added directly to the wells, the supernatant was centrifuged in 0.2µm filter eppendorf tubes for 5 minutes.

The supernatants were then used in cell cytotoxic L929 assay. The samples were transferred to 96 well plates. 50µl of TNF media was added to all the wells in all the plates except to those wells in the first row. 10µl of murine TNF standard (20ng/ml) and 90µl of TNF media was added in duplicate to the plate and diluted 1:2 down the plate to the second to last row. Test samples (75µl), comprising the supernatants from the RAW cell assays, were added to separate rows in duplicate and diluted 1:3 to the second to last rows.

TNF-sensitive L929 mouse fibroblast cells were grown by seeding 10^6 cells into a 162cm^2 cell culture flask and left to grow for 1 week. L929 cells were removed from the flask with 10mls of trypsin-EDTA/flask and incubated 3-5 minutes. Cell suspension was diluted and centrifuged for 6 minutes. The pellet was resuspended in 5 mls of fresh L929 media/flask and counted (same as RAW cells). Cell suspension was diluted to 10^6 cells/ml. 100µl was used to inoculate each well of the 96 well plates with the supernatants. (L929 Growth Media was the same as RAW cell media except instead of FBS, 50 mls of 10% heat inactivated horse serum was utilized; TNF Assay Media was the same as RAW cell media except 4µg/ml Actinomycin D was added.)

The plates were incubated at 37°C at 5% CO_2 for 2 days. The media was then aspirated and replaced with 100µl of the dye MTT (0.5mg/ml) in modified Eagle

Medium without phenol red. The plates were then incubated at 37°C at 5% CO_2 for 3 hours. The dye was then removed and replaced with 100µl of absolute ethanol. The plates were left at room temperature for 10 - 15 minutes to dissolve the formazan dye crystals.

The plates were read at 570nm in a ELISA plate reader with 690nm reference filter. One unit of TNF activity is defined as the amount required to kill 50% of the L929 cells. The TNF level in Units per ml therefore was the reciprocal of the dilution which led to a 50% killing of L929 cells.

FIGURES 5and 6 show levels of TNF (U/ml) after a 6 hour treatment with increasing amounts (0, 5, 10, 20, 30, 40 or 50 µg) of either CEME (ME) , CEMA (MA), MBI 26 (SEQ ID NO:2) or MBI 29 (SEQ ID NO:1) peptide and 100 ng of LPS. TNF levels were measured six hours after the addition of *E. coli* 0111:B4 or *E. coli* Bort LPS (FIGURE 5a, 5b, 5c and FIGURE 6a and 6c as labeled). *P. aeruginosa* LPS was also added in Figure 6b. The data shows the results of several separate experiments and indicate that both peptides efficiently reduce the level of LPS-induced TNF in the culture with three distinct LPS samples at c-oncentrations of peptides as low as 5 µg/ml.

In addition, the peptides of SEQ ID NO: 5-8 also bound effectively to LPS with high affinity based on Dansyl polymyxin displacement assays (Table 5).

TABLE 5

Peptide	I_{50} (mM)	I_{max} (%)
MBI 26 (SEQ ID NO:2)	1.8	38
MBI 29 (SEQ ID NO:1)	2.8	40
490 (SEQ ID NO:5)	4.9	40
491 (SEQ ID NO:6)	4.5	24
492 (SEQ ID NO:7)	5.4	48
493 (SEQ ID NO:8)	3.8	56

EXAMPLE 5

CATIONIC PEPTIDE REDUCTION OF LPS-INDUCED TNF

In order to determine how rapidly the cationic peptides reduced LPS-induced TNF production, *E. coli* 0111:B4 was added at time 0 to RAW macrophages. MBI 29 or Polymyxin B was added at time 0, 30 and 60 minutes. Levels of TNF were measured after 6 hours. The results are shown in FIGURE 7a and 7b(PMB, Polymyxin B; 29, MBI 29 (SEQ ID NO:1)). The results show that MBI 29 inhibited TNF induction by LPS in a similar manner to polymyxin B. Furthermore, MBI 29 was effective at reducing the ability of LPS to induce TNF in RAW cell lines even when added 60 minutes after the addition of LPS. MBI 29 demonstrated a distinct and reproducible advantage over polymyxin B when added 60 minutes after the addition of LPS. To confirm that MBI 29 was acting on LPS rather than directly upon macrophage cell lines, 20 µg of MBI 29 was added to RAW cells and incubated for 60 minutes prior to aspiration of the medium and washing the cells 3 times with HBSS (Hanks Buffered Salt Solution). Addition of 10 ng or 100 ng of LPS to the washed RAW cells resulted in a high level of TNF induction (14,000-20,000 Units of TNF per ml), suggesting that the MBI 29 had not permanently depressed the ability of RAW cells to induce TNF in response to LPS addition. In contrast, the aspirated medium containing MBI 29 could depress the ability of fresh RAW cells to induce TNF in response to 10 ng or 100 ng of *E. coli* LPS by 98.5% and 75% respectively. Up to 50 µg of MBI 29 caused no apparent decrease in RAW cell viability as judged by Trypan blue exclusion.

EXAMPLE 6

PROTECTION FROM LETHAL LPS ENDOTOXICITY IN A MOUSE ENDOTOXIC SHOCK MODEL

The ability of MBI 29 and MBI 26 to protect against LPS-induced endotoxemia is assessed *in vivo*. Mice (8-10 weeks old) are injected intraperitoneally with 20 µg D-galactosamine (Dgal) to sensitize them to LPS according to the method of Galanos (Galanos, *et al., Proc. Natl. Acad. Sci., USA,* 76:5939-5943, 1979), followed by 0, 50, 100, or 200 µg MBI 29 or MBI 26 in 100 µl. Immediately afterwards LPS (10 or 20 µg) in 100 µl is injected. The mice are observed at 24

and 48 hours after injections and survivors noted. MBI 26 was shown to be non-toxic up to 30.6 mg/kg in mice.

To demonstrate that survival is associated with a reduction in TNF levels, 10 µg of LPS and 20 mg of Dgal are injected at time 0. Thirty minutes later, the mice are sacrificed and the blood is taken and centrifuged to separate the serum which was used in the cell cytotoxic L929 assay. The results suggest that the bactolysins have potential in therapy against endotoxin-associated disorders.

EXAMPLE 7

CHARACTERIZATION OF DERIVATIVE PEPTIDES

Peptides 490-493 (SEQ ID NO:5-8) were found to possess comparable anti microbial activity to MBI 26 and MBI 29 (Table 6). These peptides do not lyse Red Blood Cells at concentration below 55 µg/mL.

TABLE 6

Peptide	MIC (µg/mL)		RBC Lysis
	E. coli (UB 1005)	*P. aeruginosa* (H187)	(µg/mL)
MBI 26	1.1	4.5	>55
MBI 29	0.5	1.0	>55
490	1	8	>55
491	2	8	>55
492	2.4	4.8	>55
493	5	10	>55

α Helical Content of Cationic Peptides by CD Spectra

The α helical content of the peptides were measured in buffer, liposomes and 20%

Hexafluoroisopropanol (HFPIP). The liposomes constituted of POPC alone or POPC and POPC and 30% POPG (Table 7).

Table 7

Peptide	% α helicity			
	Buffer	POPC Liposomes	POPC/POPG Liposomes	20% HFIP
MBI 26	0	0	8	70
MBI 29	0	16	8	50
490	0	29	27	41
491	0	0	15	44
492	0	42	19	48
493	0	18	20	42

EXAMPLE 8

SYNERGISM BETWEEN CATIONIC PEPTIDES AND ANTIBIOTICS

Synergy between the peptides of the invention and conventional antibiotics from several different classes was determined by MIC and FIC values. The following Tables 8-47 show the MIC and FIC values for MBI 26 and MBI 29 with different bacteria (*A. calcoaceticus, P. aeruginosa, E. cloacae* and *X. maltophila*) and different classes of antibiotics. Synergism is identified as an FIC of about ≤0.5.

Table 8

Synergy of MBI-26 + Ciprofloxacin against *A. calcoaceticus*

Isolate Number	Strain Number	MIC (μg · mL^{-1}) MBI-26	MIC (μg · mL^{-1}) Ciprofloxacin	Lowest FIC MBI-26 and Ciprofloxacin
1	ATCC 23055	128	0.5	0.51
2	23	32	0.5	0.75
3	27	64	0.5	0.75
4	28	16	8	0.53
5	8220	64	2	0.5
6	8221	64	1	0.5
7	8222	64	1	0.5
8	8223	64	2	0.38
9	8224	64	2	0.5
10	8225	64	2	0.38
11	8226	64	2	0.38

Table 9

Synergy of MBI-29 + Ciprofloxacin against *A. calcoaceticus*

Isolate Number	Strain Number	MIC (μg · mL^{-1}) MBI-29	MIC (μg · mL^{-1}) Ciprofloxacin	Lowest FIC MBI-29 and Ciprofloxacin
1	ATCC 23055	32	0.5	0.51
2	23	32	0.5	0.75
3	27	32	8	0.63
4	28	32	8	0.63
5	8220	16	2	0.56
6	8221	16	0.5	1
7	8222	16	2	0.75
8	8223	16	2	0.75
9	8224	16	1	1
10	8225	16	2	0.75
11	8226	16	2	0.75

Table 10

Synergy of MBI-26 + Imipenem against *A. calcoaceticus*

Isolate Number	Strain Number	MIC (μg · mL^{-1}) MBI-26	MIC (μg · mL^{-1}) Imipenem	Lowest FIC MBI-26 and Imipenem
1	ATCC 23055	128	2	1.0
2	23	64	512	0.13
3	27	32	512	0.14
4	28	32	16	0.25
5	8220	32	2	0.53
6	8221	64	1	1.01
7	8222	64	4	0.31
8	8223	32	2	0.75
9	8224	64	2	0.63
10	8225	64	2	0.63
11	8226	64	4	0.5

Table 11

Synergy of MBI-29 + Imipenem against *A. calcoaceticus*

Isolate Number	Strain Number	MIC (μg · mL^{-1}) MBI-29	MIC (μg · mL^{-1}) Imipenem	Lowest FIC MBI-29 and Imipenem
1	ATCC 23055	64	2	1
2	23	32	512	0.13
3	27	8	512	0.5
4	28	32	8	0.5
5	8220	8	2	1
6	8221	8	1	1.03
7	8222	32	2	0.75
8	8223	16	2	1
9	8224	32	2	0.51
10	8225	32	2	0.51
11	8226	32	2	1

TABLE 12

Synergy of MBI-26 + Tobramycin against *A. calcoaceticus*

Isolate Number	Strain Number	MIC (μg · mL^{-1}) MBI-26	MIC (μg · mL^{-1}) Tobramycin	Lowest FIC MBI-26 and Tobramycin
1	ATCC 23055	64	4	0.31
2	23	32	16	ND
3	27	32	1	1.01
4	28	32	128	0.26
5	8220	64	4	0.5
6	8221	64	4	0.5
7	8222	64	4	0.5
8	8223	64	4	0.5
9	8224	64	4	0.5
10	8225	64	4	0.5
11	8226	64	4	0.5

Table 13

Synergy of MBI-29 + Tobramycin against *A. calcoaceticus*

Isolate Number	Strain Number	MIC (μg · mL^{-1}) MBI-29	MIC (μg · mL^{-1}) Tobramycin	Lowest FIC MBI-29 and Tobramycin
1	ATCC 23055	32	2	0.75
2	23	4	4	1.25
3	27	8	1	1.03
4	28	32	128	0.26
5	8220	32	4	0.5
6	8221	32	4	0.5
7	8222	32	4	0.5
8	8223	32	4	0.5
9	8224	32	4	0.5
10	8225	32	4	0.5
11	8226	32	4	0.5

Table 14

Synergy of MBI-26 + Piperacillin against *A. calcoaceticus*

Isolate Number	Strain Number	MIC (μg · mL^{-1}) MBI-26	MIC (μg · mL^{-1}) Piperacillin	Lowest FIC MBI-26 and Piperacillin
1	ATCC 23055	64	8	0.75
2	23	32	1024	0.26
3	27	32	16	0.38
4	28	16	4	1.03
5	8220	64	16	0.38
6	8221	64	16	0.38
7	8222	64	32	0.25
8	8223	64	128	0.13
9	8224	64	256	0.16
10	8225	64	32	0.25
11	8226	64	32	0.25

Table 15

Synergy of MBI-29 + Piperacillin against *A. calcoaceticus*

Isolate Number	Strain Number	MIC ($\mu g \cdot mL^{-1}$) MBI-29	MIC ($\mu g \cdot mL^{-1}$) Piperacillin	Lowest FIC MBI-29 and Piperacillin
1	ATCC 23055	32	8	1
2	23	16	1024	0.28
3	27	16	16	0.5
4	28	8	4	1.03
5	8220	32	16	0.5
6	8221	32	16	0.38
7	8222	32	32	0.25
8	8223	32	32	0.25
9	8224	32	128	0.19
10	8225	32	32	0.25
11	8226	32	32	0.25

Table 16

Synergy of MBI-26 + Ceftazidime against *A. calcoaceticus*

Isolate Number	Strain Number	MIC ($\mu g \cdot mL^{-1}$) MBI-26	MIC ($\mu g \cdot mL^{-1}$) Ceftazidime	Lowest FIC MBI-26 and Ceftazidime
1	ATCC 23055	128	2	1.01
2	23	64	128	0.25
3	27	32	32	0.38
4	28	32	16	0.38
5	8220	32	16	0.38
6	8221	64	8	0.25
7	8222	64	16	0.5
8	8223	32	16	0.25
9	8224	64	64	0.25
10	8225	64	32	0.31
11	8226	64	64	0.38

Table 17

Synergy of MBI-29 + Ceftazidime against *A. calcoaceticus*

Isolate Number	Strain Number	MIC (μg · mL^{-1}) MBI-29	MIC (μg · mL^{-1}) Ceftazidime	Lowest FIC MBI-29 and Ceftazidime
1	ATCC 23055	64	4	0.63
2	23	32	128	0.26
3	27	8	4	1.5
4	28	32	16	0.63
5	8220	8	8	1.03
6	8221	16	8	1
7	8222	32	16	0.38
8	8223	16	16	0.75
9	8224	32	64	0.25
10	8225	32	8	0.5
11	8226	32	16	0.38

Table 18

Synergy of MBI-26 + Ciprofloxacin against *E. cloacae*

Isolate Number	Strain Number	MIC (μg · mL^{-1}) MBI-26	MIC (μg · mL^{-1}) Ciprofloxacin	Lowest FIC MBI-26 and Ciprofloxacin
12	ATCC 13047	64	0.25	1.01
13	37-1	256	1	0.26
14	938-2	64	1	0.31
15	B5546	64	1	0.38
16	R4148	64	2	0.38
17	R4148-2	64	2	0.38
18	3077	64	0.5	0.75
19	3356	128	0.25	1
20	14269	256	1	0.28
21	14661	512	1	0.28
22	18801	32	1	0.38

Table 19

Synergy of MBI-29 + Ciprofloxacin against *E. cloacae*

Isolate Number	Strain Number	MIC ($\mu g \cdot mL^{-1}$) MBI-29	MIC ($\mu g \cdot mL^{-1}$) Ciprofloxacin	Lowest FIC MBI-29 and Ciprofloxacin
12	ATCC 13047	32	0.25	1.01
13	37-1	32	1	1
14	938-2	32	1	0.5
15	B5546	32	1	0.5
16	R4148	32	2	0.63
17	R4148-2	16	2	0.75
18	3077	32	1	0.75
19	3356	32	0.25	1.01
20	14269	32	1	0.75
21	14661	32	1	0.75
22	18801	32	1	0.75

Table 20

Synergy of MBI-26 + Imipenem against *E. cloacae*

Isolate Number	Strain Number	MIC ($\mu g \cdot mL^{-1}$) MBI-26	MIC ($\mu g \cdot mL^{-1}$) Imipenem	Lowest FIC MBI-26 and Imipenem
12	ATCC 13047	16	1	1
13	37-1	64	2	0.75
14	938-2	64	4	0.75
15	B5546	64	2	1
16	R4148	64	4	0.75
17	R4148-2	32	2	1
18	3077	64	4	0.75
19	3356	64	4	0.75
20	14269	64	4	0.51
21	14661	64	2	1.01
22	18801	64	2	1.01

Table 21

Synergy of MBI-29 + Imipenem against *E. cloacae*

Isolate Number	Strain Number	MIC (μg · mL^{-1}) MBI-29	MIC (μg · mL^{-1}) Imipenem	Lowest FIC MBI-29 and Imipenem
12	ATCC 13047	64	2	1
13	37-1	128	2	1
14	938-2	32	2	1.5
15	B5546	32	2	0.75
16	R4148	64	4	0.56
17	R4148-2	32	2	0.75
18	3077	32	2	1
19	3356	32	2	1.5
20	14269	128	2	1
21	14661	64	2	1
22	18801	64	2	1

Table 22

Synergy of MBI-26 + Tobramycin against *E. cloacae*

Isolate Number	Strain Number	MIC (μg · mL^{-1}) MBI-26	MIC (μg · mL^{-1}) Tobramycin	Lowest FIC MBI-26 and Tobramycin
12	ATCC 13047	64	1	1.01
13	37-1	64	1	1.01
14	938-2	64	2	1.01
15	B5546	32	2	0.51
16	R4148	32	1	1.06
17	R4148-2	64	1	ND
18	3077	64	1	1.06
19	3356	64	1	1.01
20	14269	64	1	1.01
21	14661	64	1	1.01
22	18801	32	1	1.01

Table 23

Synergy of MBI-29 + Tobramycin against *E. cloacae*

Isolate Number	Strain Number	MIC ($\mu g \cdot mL^{-1}$) MBI-29	MIC ($\mu g \cdot mL^{-1}$) Tobramycin	Lowest FIC MBI-29 and Tobramycin
12	ATCC 13047	32	1	1
13	37-1	32	1	1
14	938-2	16	1	1.01
15	B5546	32	1	1.01
16	R4148	16	1	1.03
17	R4148-2	16	1	1.01
18	3077	32	1	1.01
19	3356	32	1	1.01
20	14269	16	1	1.01
21	14661	32	1	1.01
22	18801	16	1	1.01

Table 24

Synergy of MBI-26 + Piperacillin against *E. cloacae*

Isolate Number	Strain Number	MIC ($\mu g \cdot mL^{-1}$) MBI-26	MIC ($\mu g \cdot mL^{-1}$) Piperacillin	Lowest FIC MBI-26 and Piperacillin
12	ATCC 13047	64	8	0.75
13	37-1	64	4	1.01
14	938-2	64	8	0.51
15	B5546	64	8	0.56
16	R4148	64	1024	0.13
17	R4148-2	64	1024	0.25
18	3077	64	8	0.56
19	3356	64	64	0.25
20	14269	512	1024	0.03
21	14661	64	16	0.5
22	18801	32	4	1.01

Table 25

Synergy of MBI-29 + Piperacillin against *E. cloacae*

Isolate Number	Strain Number	MIC (μg · mL^{-1}) MBI-29	MIC (μg · mL^{-1}) Piperacillin	Lowest FIC MBI-29 and Piperacillin
12	ATCC 13047	32	8	0.75
13	37-1	32	4	1.01
14	938-2	32	4	1.01
15	B5546	32	8	0.63
16	R4148	32	1024	0.28
17	R4148-2	32	1024	0.28
18	3077	32	4	1.01
19	3356	32	64	0.31
20	14269	128	1024	0.07
21	14661	32	8	1.01
22	18801	16	4	1.01

Table 26

Synergy of MBI-26 + Ceftazidime against *E. cloacae*

Isolate Number	Strain Number	MIC (μg · mL^{-1}) MBI-26	MIC (μg · mL^{-1}) Ceftazidime	Lowest FIC MBI-26 and Ceftazidime
12	ATCC 13047	16	4	0.75
13	37-1	256	2	1
14	938-2	64	2	1.01
15	B5546	64	2	1.01
16	R4148	64	512	0.25
17	R4148-2	32	512	31
18	3077	64	2	1.01
19	3356	128	128	0.19
20	14269	512	512	0.03
21	14661	512	512	0.03
22	18801	256	2	1

Table 27

Synergy of MBI-29 + Ceftazidime against *E. cloacae*

Isolate Number	Strain Number	MIC (μg · mL^{-1}) MBI-29	MIC (μg · mL^{-1}) Ceftazidime	Lowest FIC MBI-29 and Ceftazidime
12	ATCC 13047	64	4	0.53
13	37-1	128	2	1
14	938-2	32	2	1.01
15	B5546	32	2	1.01
16	R4148	64	512	0.16
17	R4148-2	32	512	0.28
18	3077	32	2	1.01
19	3356	32	128	0.3
20	14269	128	512	0.09
21	14661	128	512	0.09
22	18801	128	2	1

Table 28

Synergy of MBI-26 + Ciprofloxacin against *P. aeruginosa*

Isolate Number	Strain Number	MIC (μg · mL^{-1}) MBI-26	MIC (μg · mL^{-1}) Ciprofloxacin	Lowest FIC MBI-26 and Ciprofloxacin
23	ATCC 27853	64	1	0.56
24	34	64	1	0.53
25	923-1	64	0.25	1.01
26	3101	64	0.25	1.01
27	14644	256	32	ND
28	15036	256	16	1
29	15545	64	0.25	1.01
30	B3999-1	64	0.25	1.01
31	U7688-1	64	0.25	1.01
32	W2897-1	64	0.25	1.01
33	W5483-2	64	0.25	1.01

Table 29

Synergy of MBI-29 + Ciprofloxacin against *P. aeruginosa*

Isolate Number	Strain Number	MIC ($\mu g \cdot mL^{-1}$) MBI-29	MIC ($\mu g \cdot mL^{-1}$) Ciprofloxacin	Lowest FIC MBI-29 and Ciprofloxacin
23	ATCC 27853	32	1	0.75
24	34	32	1	0.75
25	923-1	32	0.25	1.01
26	3101	32	0.25	1.01
27	14644	128	32	ND
28	15036	128	32	ND
29	15545	32	0.25	0.63
30	B3999-1	32	0.25	1.01
31	U7688-1	32	0.25	1.01
32	W2897-1	32	0.25	1.01
33	W5483-2	32	0.25	1.01

Table 30

Synergy of MBI-26 + Imipenem against *P. aeruginosa*

Isolate Number	Strain Number	MIC ($\mu g \cdot mL^{-1}$) MBI-26	MIC ($\mu g \cdot mL^{-1}$) Imipenem	Lowest FIC MBI-26 and Imipenem
23	ATCC 27853	64	8	0.5
24	34	128	512	0.38
25	923-1	64	4	1.01
26	3101	64	8	0.75
27	14644	64	16	0.51
28	15036	64	16	1
29	15545	64	128	ND
30	B3999-1	64	128	ND
31	U7688-1	64	16	1
32	W2897-1	64	128	0.25
33	W5483-2	64	64	ND

Table 31

Synergy of MBI-29 + Imipenem against *P. aeruginosa*

Isolate Number	Strain Number	MIC (μg · mL^{-1}) MBI-29	MIC (μg · mL^{-1}) Imipenem	Lowest FIC MBI-29 and Imipenem
23	ATCC 27853	64	4	0.75
24	34	32	512	0.16
25	923-1	64	2	1.13
26	3101	32	4	0.75
27	14644	64	8	1
28	15036	64	8	1
29	15545	32	256	0.63
30	B3999-1	64	256	1
31	U7688-1	64	256	0.75
32	W2897-1	32	256	0.75
33	W5483-2	64	256	0.5

Table 32

Synergy of MBI-26 + Tobramycin against *P. aeruginosa*

Isolate Number	Strain Number	MIC (μg · mL^{-1}) MBI-26	MIC (μg · mL^{-1}) Tobramycin	Lowest FIC MBI-26 and Tobramycin
23	ATCC 27853	64	1	1.01
24	34	128	128	0.5
25	923-1	64	1	1.01
26	3101	64	1	1.01
27	14644	64	2	0.53
28	15036	64	1	1.01
29	15545	64	2	1
30	B3999-1	64	2	0.51
31	U7688-1	64	1	1.01
32	W2897-1	64	1	1.01
33	W5483-2	64	1	1.01

Table 33

Synergy of MBI-29 + Tobramycin against *P. aeruginosa*

Isolate Number	Strain Number	MIC ($\mu g \cdot mL^{-1}$) MBI-29	MIC ($\mu g \cdot mL^{-1}$) Tobramycin	Lowest FIC MBI-29 and Tobramycin
23	ATCC 27853	32	1	1.01
24	34	16	64	0.5
25	923-1	32	1	1.01
26	3101	32	1	1.01
27	14644	32	1	1.01
28	15036	32	1	1.01
29	15545	32	2	0.75
30	B3999-1	32	1	1.01
31	U7688-1	32	2	0.56
32	W2897-1	32	1	1.01
33	W5483-2	32	2	0.51

Table 34

Synergy of MBI-26 + Piperacillin against *P. aeruginosa*

Isolate Number	Strain Number	MIC ($\mu g \cdot mL^{-1}$) MBI-26	MIC ($\mu g \cdot mL^{-1}$) Piperacillin	Lowest FIC MBI-26 and Piperacillin
23	ATCC 27853	128	256	0.19
24	34	128	1024	0.5
25	923-1	64	64	0.5
26	3101	64	64	0.63
27	14644	64	512	ND
28	15036	256	1024	0.09
29	15545	256	512	0.14
30	B3999-1	256	512	0.25
31	U7688-1	256	512	ND
32	W2897-1	64	64	1
33	W5483-2	256	128	1

Table 35

Synergy of MBI-29 + Piperacillin against *P. aeruginosa*

Isolate Number	Strain Number	MIC ($\mu g \cdot mL^{-1}$) MBI-29	MIC ($\mu g \cdot mL^{-1}$) Piperacillin	Lowest FIC MBI-29 and Piperacillin
23	ATCC 27853	64	128	ND
24	34	32	1024	0.28
25	923-1	32	32	0.5
26	3101	32	128	0.31
27	14644	128	1024	ND
28	15036	128	1024	0.09
29	15545	64	512	0.16
30	B3999-1	128	256	0.12
31	U7688-1	32	256	0.5
32	W2897-1	32	32	0.75
33	W5483-2	128	512	0.19

Table 36

Synergy of MBI-26 + Ceftazidime against *P. aeruginosa*

Isolate Number	Strain Number	MIC ($\mu g \cdot mL^{-1}$) MBI-26	MIC ($\mu g \cdot mL^{-1}$) Ceftazidime	Lowest FIC MBI-26 and Ceftazidime
23	ATCC 27853	128	32	0.31
24	34	128	32	0.75
25	923-1	128	4	1.01
26	3101	128	8	0.56
27	14644	256	128	1
28	15036	256	2	2
29	15545	256	8	0.53
30	B3999-1	256	16	1.01
31	U7688-1	256	32	0.25
32	W2897-1	256	32	0.26
33	W5483-2	256	8	1.01

Table 37

Synergy of MBI-29 + Ceftazidime against *P. aeruginosa*

Isolate Number	Strain Number	MIC ($\mu g \cdot mL^{-1}$) MBI-29	MIC ($\mu g \cdot mL^{-1}$) Ceftazidime	Lowest FIC MBI-29 and Ceftazidime
23	ATCC 27853	64	32	0.38
24	34	32	64	0.31
25	923-1	64	4	0.56
26	3101	64	8	0.56
27	14644	128	256	1
28	15036	128	4	0.51
29	15545	64	8	0.5
30	B3999-1	128	8	0.31
31	U7688-1	128	8	0.63
32	W2897-1	64	4	0.51
33	W5483-2	128	8	0.56

Table 38

Synergy of MBI-26 + Ciprofloxacin against *X. maltophila*

Isolate Number	Strain Number	MIC ($\mu g \cdot mL^{-1}$) MBI-26	MIC ($\mu g \cdot mL^{-1}$) Ciprofloxacin	Lowest FIC MBI-26 and Ciprofloxacin
34	ATCC 13637	64	0.5	0.63
35	6	64	4	0.38
36	16	64	8	1
37	3095	64	16	1
38	7901	256	32	ND
39	B3110.1	64	2	2.01
40	R1302-1	128	4	1
41	R4230	64	2	1
42	TF29-1	128	8	ND
43	TF29-2	128	8	1
44	W6896-2	256	4	0.13
45	W11291	64	2	2.01

Table 39

Synergy of MBI-29 + Ciprofloxacin against *X. maltophila*

Isolate Number	Strain Number	MIC (μg · mL^{-1}) MBI-29	MIC (μg · mL^{-1}) Ciprofloxacin	Lowest FIC MBI-29 and Ciprofloxacin
34	ATCC 13637	32	0.5	1.01
35	6	32	2	0.38
36	16	32	8	ND
37	3095	64	16	0.5
38	7901	64	32	0.75
39	B3110.1	32	2	1.01
40	R1302-1	32	4	0.56
41	R4230	16	4	0.5
42	TF29-1	16	8	1
43	TF29-2	16	8	0.53
44	W6896-2	16	8	1
45	W11291	32	4	0.75

Table 40

Synergy of MBI-26 + Imipenem against *X. maltophila*

Isolate Number	Strain Number	MIC (μg · mL^{-1}) MBI-26	MIC (μg · mL^{-1}) Imipenem	Lowest FIC MBI-26 and Imipenem
34	ATCC 13637	64	512	0.13
35	6	128	512	0.16
36	16	256	512	ND
37	3095	512	512	ND
38	7901	256	512	ND
39	B3110.1	256	512	ND
40	R1302-1	128	512	ND
41	R4230	64	512	ND
42	TF29-1	128	512	ND
43	TF29-2	128	512	ND
44	W6896-2	256	512	0.25
45	W11291	512	512	ND

Table 41

Synergy of MBI-29 + Imipenem against *X. maltophila*

Isolate Number	Strain Number	MIC ($\mu g \cdot mL^{-1}$) MBI-29	MIC ($\mu g \cdot mL^{-1}$) Imipenem	Lowest FIC MBI-29 and Imipenem
34	ATCC 13637	32	512	0.19
35	6	32	512	0.25
36	16	32	512	0.3
37	3095	64	512	ND
38	7901	64	512	0.38
39	B3110.1	32	512	ND
40	R1302-1	64	512	0.25
41	R4230	32	512	0.5
42	TF29-1	16	512	0.56
43	TF29-2	16	512	0.56
44	W6896-2	64	512	0.38
45	W11291	64	512	0.38

Table 42

Synergy of MBI-26 + Tobramycin against *X. maltophila*

Isolate Number	Strain Number	MIC ($\mu g \cdot mL^{-1}$) MBI-26	MIC ($\mu g \cdot mL^{-1}$) Tobramycin	Lowest FIC MBI-26 and Tobramycin
34	ATCC 13637	64	32	0.16
35	6	64	4	0.25
36	16	256	128	0.63
37	3095	32	32	0.5
38	7901	32	32	1.01
39	B3110.1	256	32	0.63
40	R1302-1	128	32	0.63
41	R4230	64	16	0.38
42	TF29-1	32	2	1.01
43	TF29-2	32	2	1.01
44	W6896-2	32	16	0.5
45	W11291	512	128	ND

Table 43

Synergy of MBI-29 + Tobramycin against *X. maltophila*

Isolate Number	Strain Number	MIC ($\mu g \cdot mL^{-1}$) MBI-29	MIC ($\mu g \cdot mL^{-1}$) Tobramycin	Lowest FIC MBI-29 and Tobramycin
34	ATCC 13637	8	8	0.63
35	6	16	4	0.75
36	16	32	128	0.31
37	3095	64	32	0.16
38	7901	64	32	0.28
39	B3110.1	32	16	0.56
40	R1302-1	64	32	0.53
41	R4230	16	16	0.56
42	TF29-1	8	1	1.03
43	TF29-2	16	1	1.5
44	W6896-2	16	8	0.63
45	W11291	64	128	ND

Table 44

Synergy of MBI-26 + Piperacillin against *X. maltophila*

Isolate Number	Strain Number	MIC ($\mu g \cdot mL^{-1}$) MBI-26	MIC ($\mu g \cdot mL^{-1}$) Piperacillin	Lowest FIC MBI-26 and Piperacillin
34	ATCC 13637	64	1024	0.14
35	6	64	512	ND
36	16	256	1024	ND
37	3095	64	512	ND
38	7901	64	512	ND
39	B3110.1	64	512	ND
40	R1302-1	64	512	ND
41	R4230	64	1024	0.5
42	TF29-1	128	1024	0.2
43	TF29-2	128	1024	0.5
44	W6896-2	32	1024	0.6
45	W11291	64	512	ND

Table 45

Synergy of MBI-29 + Piperacillin against *X. maltophila*

Isolate Number	Strain Number	MIC (μg · mL^{-1}) MBI-29	MIC (μg · mL^{-1}) Piperacillin	Lowest FIC MBI-29 and Piperacillin
34	ATCC 13637	32	1024	0.19
35	6	32	64	0.19
36	16	32	1024	0.38
37	3095	64	1024	0.38
38	7901	64	1024	0.28
39	B3110.1	32	1024	0.63
40	R1302-1	64	1024	0.25
41	R4230	32	1024	0.3
42	TF29-1	32	1024	0.2
43	TF29-2	32	1024	0.28
44	W6896-2	64	1024	0.13
45	W11291	64	1024	0.31

Table 46

Synergy of MBI-26 + Ceftazidime against *X. maltophila*

Isolate Number	Strain Number	MIC (μg · mL^{-1}) MBI-26	MIC (μg · mL^{-1}) Ceftazidime	Lowest FIC MBI-26 and Ceftazidime
34	ATCC 13637	64	64	0.28
35	6	128	64	0.25
36	16	256	32	0.51
37	3095	512	512	ND
38	7901	256	128	1
39	B3110.1	256	64	1.13
40	R1302-1	128	16	1
41	R4230	64	4	1
42	TF29-1	128	4	1
43	TF29-2	128	4	1.01
44	W6896-2	256	64	1
45	W11291	512	128	1

Table 47

Synergy of MBI-29 + Ceftazidime against *X. maltophila*

Isolate Number	Strain Number	MIC ($\mu g \cdot mL^{-1}$) MBI-29	MIC ($\mu g \cdot mL^{-1}$) Ceftazidime	Lowest FIC MBI-29 and Ceftazidime
34	ATCC 13637	32	64	0.28
35	6	32	32	0.31
36	16	32	16	0.63
37	3095	64	512	ND
38	7901	64	256	0.31
39	B3110.1	32	128	0.75
40	R1302-1	64	32	0.25
41	R4230	32	8	0.5
42	TF29-1	16	4	1
43	TF29-2	16	2	1.01
44	W6896-2	64	64	0.5
45	W11291	64	256	0.28

Note: MBI 26 (1.25µg/mL) also showed synergy with Lysozyme (>64x reduction in MIC), Novobiocin (>4x reduction in MIC), Nalidixic acid (2x reduction in MIC) and carbenicillin (2x reduction in MIC).

Although the invention has been described with reference to the presently preferred embodiment, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

WE CLAIM:

1. An isolated antimicrobial peptide having an amino acid sequence selected from the group consisting of:

NH_2-KWK$R_2R_1R_1R_2R_2R_1R_2R_2R_1R_1R_2R_2$VLTTGLPALIS-COOH,

NH_2-KWK$R_2R_1R_1R_2R_2R_1R_2R_2R_1R_1R_2R_2$VVTTAKPLISS-COOH,

NH_2-KWK$R_2R_1R_1R_2R_2R_1R_2R_2R_1R_1R_2R_2$ILTTGLPALIS-COOH,

NH_2-KWK$R_2R_1R_1R_2R_2R_1R_2R_2R_1R_1R_2R_2$GGLLSNIVTSL-COOH,

NH_2-KWK$R_2R_1R_1R_2R_2R_1R_2R_2R_1R_1R_2R_2$GPILANLVSIV-COOH,

NH_2-KKWWRR$R_1R_1R_2R_1R_1R_2R_2$GPALSNV-COOH,

NH_2-KKWWRRX, and

NH_2-KKWWKX

wherein R_1 is a hydrophobic amino acid residue and R_2 is a hydrophilic amino acid residue, and X is from about 14 to 24 amino acid residues.

2. The peptide of claim 1, wherein the peptide has an amino acid sequence selected from the group consisting of:

NH_2-KWKSFIKKLTTAVKKVLTTGLPALIS-COOH (SEQ ID NO:1),

NH_2-KWKSFIKKLTSAAKKVVTTAKPLISS-COOH (SEQ ID NO:2),

NH_2-KWKSFIKNLTKGGSKILTTGLPALIS-COOH (SEQ ID NO:3),

NH_2-KWKKFIKNLTKGGSKILTTGLPALIS-COOH (SEQ ID NO:4),

NH_2-KWKSFIKNLEKVLKPGGLLSNIVTSL-COOH (SEQ ID NO:5),

NH_2-KWKSFIKNLEKVLKKGPILANLVSIV-COOH (SEQ ID NO:6),

NH_2-KWKEFIKKLTTAVKKVLTTGLPALIS-COOH (SEQ ID NO:7),

NH_2-KWKKFIKELQKVLAPGGLLSNIVTSL-COOH (SEQ ID NO:8),

NH_2-KWKSFIKKLTSVLKKVVTTALPALIS-COOH (SEQ ID NO:9),
NH_2-KWKSFIKNLTKVLKKVVTTALPALIS-COOH (SEQ ID NO:10),
NH_2-KWKLFKKKGTGAVLTVLTTGLPALIS-COOH (SEQ ID NO:11),
NH_2-KWKSFIKKLTSVLKKVVTTAKPLISS-COOH (SEQ ID NO:12),
NH_2-KKKSFIKLLTSAKVSVLTTAKPLISS-COOH (SEQ ID NO:13),
NH_2-KWKKFIKELQKVLKPGGLLSNIVTSL-COOH (SEQ ID NO:14),
NH_2-KKWWRRVLSGLKTGPALSNV-COOH (SEQ ID NO:15),
NH_2-KKWWRRVLKGLSSGPALSNV-COOH (SEQ ID NO:16),
NH_2-KKWWRRALQALKNGPALSNV-COOH (SEQ ID NO:17),
NH_2-KKWWRRVLSGLKTAGPAIQSVLNK-COOH (SEQ ID NO:18),
NH_2-KKWWRRALQGLKTAGPAIQSVLNK-COOH (SEQ ID NO:19),
NH_2-KKWWKAQKAVNSGPNALQTLAQ-COOH (SEQ ID NO:20)
NH_2-KKWWKAKKFANSGPNALQTLAQ-COOH (SEQ ID NO:21), and
NH_2-KKWWKFIKKAVNSGTTGLQTLAS-COOH (SEQ ID NO:22).

3. An isolated polynucleotide which encodes the peptide of claim 1.

4. An isolated polynucleotide which encodes a peptide selected from the group consisting of:

NH_2-KWKSFIKKLTTAVKKVLTTGLPALIS-COOH (SEQ ID NO:1),

NH_2-KWKSFIKKLTSAAKKVVTTAKPLISS-COOH (SEQ ID NO:2),

NH_2-KWKSFIKNLTKGGSKILTTGLPALIS-COOH (SEQ ID NO:3),

NH_2-KWKKFIKNLTKGGSKILTTGLPALIS-COOH (SEQ ID NO:4),

NH_2-KWKSFIKNLEKVLKPGGLLSNIVTSL-COOH (SEQ ID NO:5),

NH_2-KWKSFIKNLEKVLKKGPILANLVSIV-COOH (SEQ ID NO:6),

NH_2-KWKEFIKKLTTAVKKVLTTGLPALIS-COOH (SEQ ID NO:7),

NH_2-KWKKFIKELQKVLAPGGLLSNIVTSL-COOH (SEQ ID NO:8),

NH_2-KWKSFIKKLTSVLKKVVTTALPALIS-COOH (SEQ NO:9),

NH_2-KWKSFIKNLTKVLKKVVTTALPALIS-COOH (SEQ ID NO:10),

NH_2-KWKLFKKKGTGAVLTVLTTGLPALIS-COOH (SEQ ID NO:11),

NH_2-KWKSFIKKLTSVLKKVVTTAKPLISS-COOH (SEQ ID NO:12),

NH_2-KKKSFIKLLTSAKVSVLTTAKPLISS-COOH (SEQ ID NO:13),

NH_2-KWKKFIKELQKVLKPGGLLSNIVTSL-COOH (SEQ ID NO:14),

NH_2-KKWWRRVLSGLKTGPALSNV-COOH (SEQ ID NO:15),

NH_2-KKWWRRVLKGLSSGPALSNV-COOH (SEQ ID NO:16),

NH_2-KKWWRRALQALKNGPALSNV-COOH (SEQ ID NO:17),

NH_2-KKWWRRVLSGLKTAGPAIQSVLNK-COOH (SEQ ID NO:18),

NH_2-KKWWRRALQGLKTAGPAIQSVLNK-COOH (SEQ ID NO:19),
NH_2-KKWWKAQKAVNSGPNALQTLAQ-COOH (SEQ ID NO:20)
NH_2-KKWWKAKKFANSGPNALQTLAQ-COOH (SEQ ID NO:21), and
NH_2-KKWWKFIKKAVNSGTTGLQTLAS-COOH (SEQ ID NO:22)..

5. A method of inhibiting the growth of bacteria comprising contacting the bacteria with an inhibiting effective amount of a peptide having an amino acid sequence selected from the group consisting of:

NH_2-KWK$R_2R_1R_1R_2R_2R_1R_2R_2R_1R_1R_2R_2$VLTTGLPALIS-COOH,
NH_2-KWK$R_2R_1R_1R_2R_2R_1R_2R_2R_1R_1R_2R_2$VVTTAKPLISS-COOH,
NH_2-KWK$R_2R_1R_1R_2R_2R_1R_2R_2R_1R_1R_2R_2$ILTTGLPALIS-COOH,
NH_2-KWK$R_2R_1R_1R_2R_2R_1R_2R_2R_1R_1R_2R_2$GGLLSNIVTSL-COOH,
NH_2-KWK$R_2R_1R_1R_2R_2R_1R_2R_2R_1R_1R_2R_2$GPILANLVSIV-COOH,
NH_2-KKWWRR$R_1R_1R_2R_1R_1R_2R_2$GPALSNV-COOH,
NH_2-KKWWRRX, and
NH_2-KKWWKX

wherein R_1 is a hydrophobic amino acid residue and R_2 is a hydrophilic amino acid residue, and X is from about 14 to 24 amino acid residues.

6. The method of claim 5, wherein the peptide is selected from the group consisting of:

NH_2-KWKSFIKKLTTAVKKVLTTGLPALIS-COOH (SEQ ID NO:1),

NH_2-KWKSFIKKLTSAAKKVVTTAKPLISS-COOH (SEQ ID NO:2),

NH_2-KWKSFIKNLTKGGSKILTTGLPALIS-COOH (SEQ ID NO:3),

NH_2-KWKKFIKNLTKGGSKILTTGLPALIS-COOH (SEQ ID NO:4),

NH_2-KWKSFIKNLEKVLKPGGLLSNIVTSL-COOH (SEQ ID NO:5),

NH_2-KWKSFIKNLEKVLKKGPILANLVSIV-COOH (SEQ ID NO:6),

NH_2-KWKEFIKKLTTAVKKVLTTGLPALIS-COOH (SEQ ID NO:7),

NH_2-KWKKFIKELQKVLAPGGLLSNIVTSL-COOH (SEQ ID NO:8),

NH_2-KWKSFIKKLTSVLKKVVTTALPALIS-COOH (SEQ NO:9),

NH_2-KWKSFIKNLTKVLKKVVTTALPALIS-COOH (SEQ ID NO:10),

NH_2-KWKLFKKKGTGAVLTVLTTGLPALIS-COOH (SEQ ID NO:11),

NH_2-KWKSFIKKLTSVLKKVVTTAKPLISS-COOH (SEQ ID NO:12),

NH_2-KKKSFIKLLTSAKVSVLTTAKPLISS-COOH (SEQ ID NO:13),

NH_2-KWKKFIKELQKVLKPGGLLSNIVTSL-COOH (SEQ ID NO:14)

NH_2-KKWWRRVLSGLKTGPALSNV-COOH (SEQ ID NO:15),

NH_2-KKWWRRVLKGLSSGPALSNV-COOH (SEQ ID NO:16),

NH_2-KKWWRRALQALKNGPALSNV-COOH (SEQ ID NO:17),

NH_2-KKWWRRVLSGLKTAGPAIQSVLNK-COOH (SEQ ID NO:18),

NH_2-KKWWRRALQGLKTAGPAIQSVLNK-COOH (SEQ ID NO:19),
NH_2-KKWWKAQKAVNSGPNALQTLAQ-COOH (SEQ ID NO:20)
NH_2-KKWWKAKKFANSGPNALQTLAQ-COOH (SEQ ID NO:21),
and
NH_2-KKWWKFIKKAVNSGTTGLQTLAS-COOH (SEQ ID NO:22).

7. The method of claim 5, wherein the bacteria is gram positive.

8. The method of claim 7, wherein the bacteria is *Staphylococcuus aureus* or *S. epidermidis*.

9. The method of claim 5, wherein the bacteria is gram negative.

10. The method of claim 9, wherein the bacteria is selected from the group consisting of *E. coli, P. aeruginosa*, and *S. typhimurium*.

11. The method of claim 5, wherein the peptide is administered in combination with at least one antibiotic.

12. The method of claim 11, wherein the antibiotic is selected from the group consisting of the class aminoglycoside, penicillin, cephalosporine, fluoroquinolone, carbepenem, tetracycline and macrolide.

13. A method of inhibiting an endotoxemia or sepsis associated disorder in a subject having or at risk of having such a disorder, comprising administering to the subject a therapeutically effective amount of a peptide having an amino acid sequence selected from the group consisting of:

NH_2-KWK$R_2R_1R_1R_2R_2R_1R_2R_2R_1R_1R_2R_2$VLTTGLPALIS-COOH,
NH_2-KWK$R_2R_1R_1R_2R_2R_1R_2R_2R_1R_1R_2R_2$VVTTAKPLISS-COOH,
NH_2-KWK$R_2R_1R_1R_2R_2R_1R_2R_2R_1R_1R_2R_2$ILTTGLPALIS-COOH,
NH_2-KWK$R_2R_1R_1R_2R_2R_1R_2R_2R_1R_1R_2R_2$GGLLSNIVTSL-COOH,
NH_2-KWK$R_2R_1R_1R_2R_2R_1R_2R_2R_1R_1R_2R_2$GPILANLVSIV-COOH
NH_2-KKWWRR$R_1R_1R_2R_1R_1R_2R_2$GPALSNV-COOH,
NH_2-KKWWRRX, and
NH_2-KKWWKX

wherein R_1 is a hydrophobic amino acid residue and R_2 is a hydrophilic amino acid residue, and X is from about 14 to 24 amino acid residues.

14. The method of claim 13, wherein the peptide is selected from the group consisting of:

NH_2-KWKSFIKKLTTAVKKVLTTGLPALIS-COOH
(SEQ ID NO:1),
NH_2-KWKSFIKKLTSAAKKVVTTAKPLISS-COOH
(SEQ ID NO:2),
NH_2-KWKSFIKNLTKGGSKILTTGLPALIS-COOH
(SEQ ID NO:3),
NH_2-KWKKFIKNLTKGGSKILTTGLPALIS-COOH
(SEQ ID NO:4),
NH_2-KWKSFIKNLEKVLKPGGLLSNIVTSL-COOH
(SEQ ID NO:5),
NH_2-KWKSFIKNLEKVLKKGPILANLVSIV-COOH
(SEQ ID NO:6),
NH_2-KWKEFIKKLTTAVKKVLTTGLPALIS-COOH (SEQ ID NO:7),
NH_2-KWKKFIKELQKVLAPGGLLSNIVTSL-COOH (SEQ ID NO:8),

NH_2-KWKSFIKKLTSVLKKVVTTALPALIS-COOH (SEQ ID NO:9),
NH_2-KWKSFIKNLTKVLKKVVTTALPALIS-COOH (SEQ ID NO:10),
NH_2-KWKLFKKKGTGAVLTVLTTGLPALIS-COOH (SEQ ID NO:11),
NH_2-KWKSFIKKLTSVLKKVVTTAKPLISS-COOH (SEQ ID NO:12),
NH_2-KKKSFIKLLTSAKVSVLTTAKPLISS-COOH (SEQ ID NO:13),
NH_2-KWKKFIKELQKVLKPGGLLSNIVTSL-COOH (SEQ ID NO:14),
NH_2-KKWWRRVLSGLKTGPALSNV-COOH (SEQ ID NO:15),
NH_2-KKWWRRVLKGLSSGPALSNV-COOH (SEQ ID NO:16),
NH_2-KKWWRRALQALKNGPALSNV-COOH (SEQ ID NO:17),
NH_2-KKWWRRVLSGLKTAGPAIQSVLNK-COOH (SEQ ID NO:18),
NH_2-KKWWRRALQGLKTAGPAIQSVLNK-COOH (SEQ ID NO:19),
NH_2-KKWWKAQKAVNSGPNALQTLAQ-COOH (SEQ ID NO:20)
NH_2-KKWWKAKKFANSGPNALQTLAQ-COOH (SEQ ID NO:21), and
NH_2-KKWWKFIKKAVNSGTTGLQTLAS-COOH (SEQ ID NO:22).

15. The method of claim 12, wherein the disorder is septic shock.

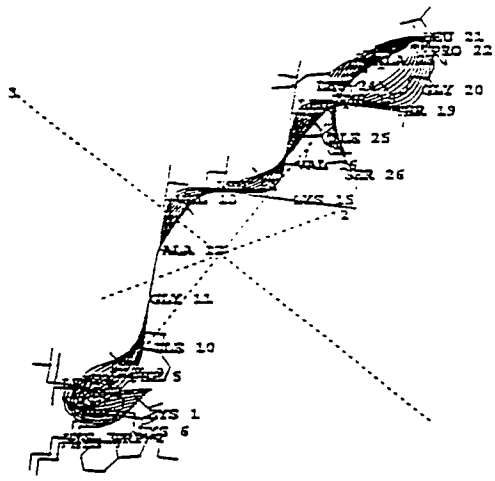
16. An isolated antimicrobial peptide having an amino acid sequence selected from the group consisting of:
NH_2-KKSFFKKLTSVASSVLS-COOH (SEQ ID NO:23),
NH_2-WKVFKSFIKKASSFAQSVLD-COOH (SEQ ID NO:24), and
NH_2-KKWRKSFFKQVGSFDNSV-COOH (SEQ ID NO:25).

17. An isolated polynucleotide which encodes a peptide selected from the group consisting of:
NH_2-KKSFFKKLTSVASSVLS-COOH (SEQ ID NO:23),
NH_2-WKVFKSFIKKASSFAQSVLD-COOH (SEQ ID NO:24), and
NH_2-KKWRKSFFKQVGSFDNSV-COOH (SEQ ID NO:25).

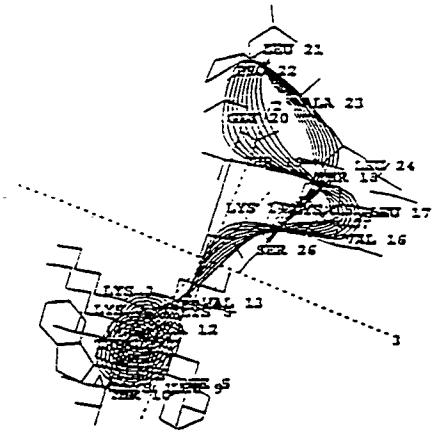
18. A method of inhibiting the growth of bacteria comprising contacting the bacteria with an inhibiting effective amount of a peptide having an amino acid sequence selected from the group consisting of:
NH_2-KKSFFKKLTSVASSVLS-COOH (SEQ ID NO:23),
NH_2-WKVFKSFIKKASSFAQSVLD-COOH (SEQ ID NO:24), and
NH_2-KKWRKSFFKQVGSFDNSV-COOH (SEQ ID NO:25).

19. A method of inhibiting an endotoxemia or sepsis associated disorder in a subject having or at risk of having such a disorder, comprising administering to the subject a therapeutically effective amount of a peptide having an amino acid sequence selected from the group consisting of:
NH_2-KKSFFKKLTSVASSVLS-COOH (SEQ ID NO:23),
NH_2-WKVFKSFIKKASSFAQSVLD-COOH (SEQ ID NO:24), and
NH_2-KKWRKSFFKQVGSFDNSV-COOH (SEQ ID NO:25).

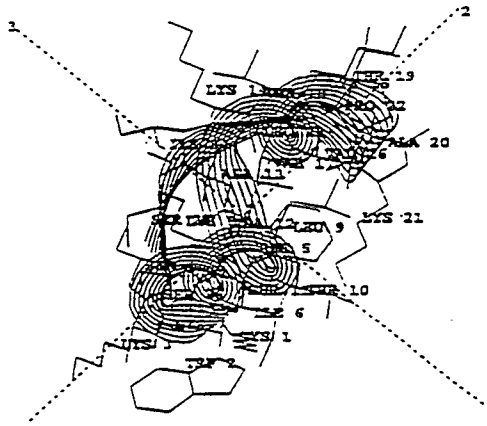
FIGURE 1



CEME

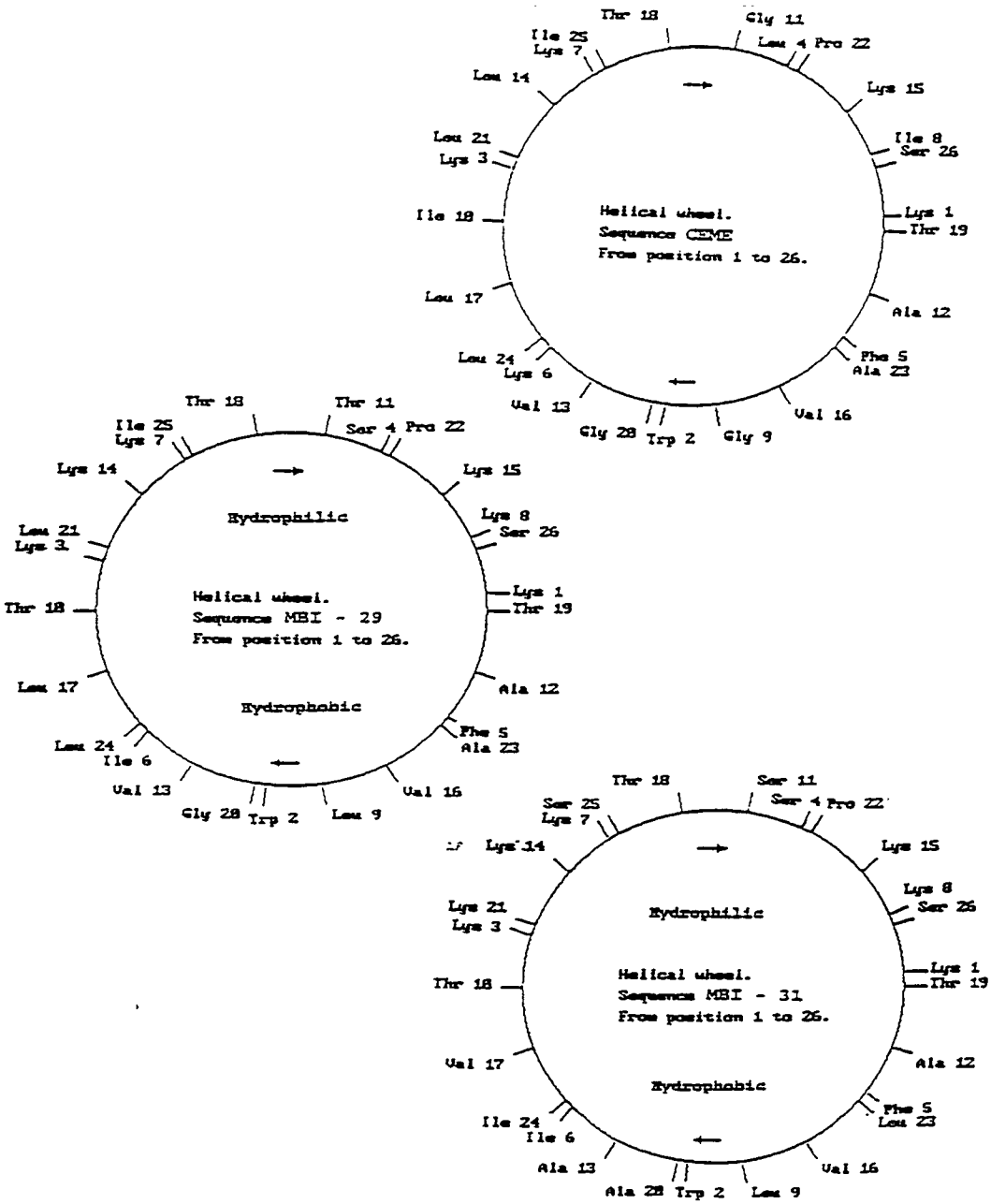


MBI - 29



MBI - 31

FIGURE 2


Helical wheel.
Sequence CEME
From position 1 to 26.
Hydrophilic
Helical wheel.
Sequence MBI - 29
From position 1 to 26.
Hydrophobic
Hydrophilic
Helical wheel.
Sequence MBI - 31
From position 1 to 26.
Hydrophobic

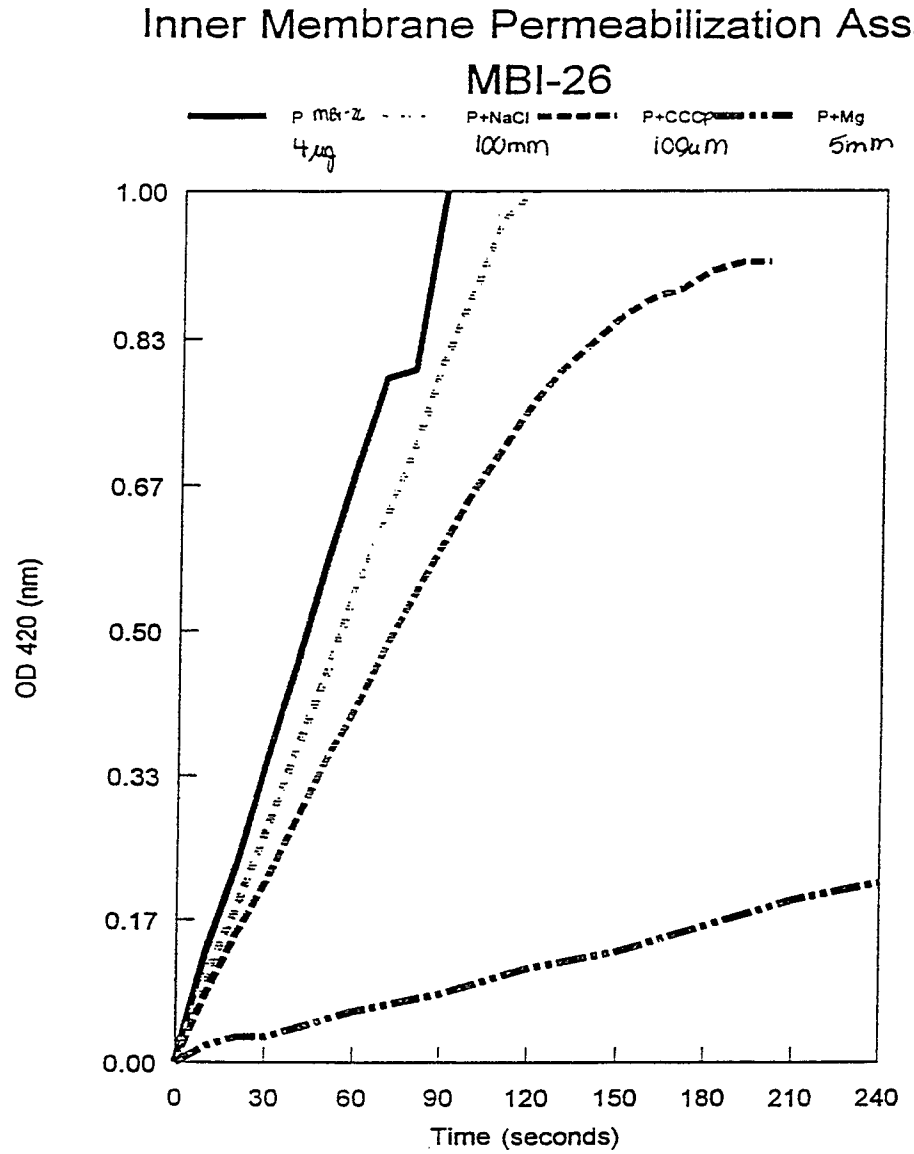

Inner Membrane Permeabilization Assay
MBI-26
P MBI-2
4µg
P+NaCl
100mm
P+CCCP
100µm
P+Mg
5mm
OD 420 (nm)
1.00
0.83
0.67
0.50
0.33
0.17
0.00
0
30
60
90
120
150
180
210
240
Time (seconds)

Fig. 3

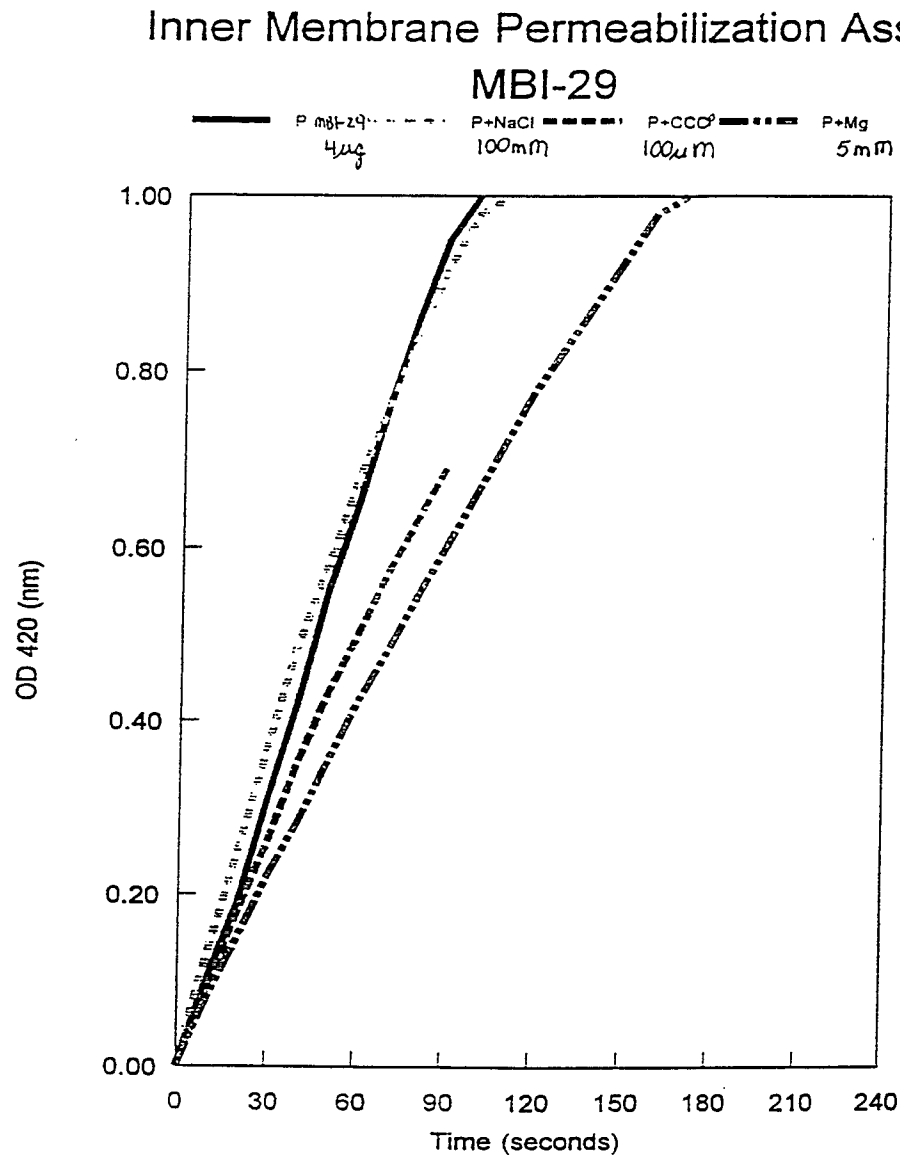

Inner Membrane Permeabilization Assay
MBI-29
P MBI-29
4μg
P+NaCl
100mM
P+CCCP
100μM
P+Mg
5mM
1.00
0.80
0.60
0.40
0.20
0.00
OD 420 (nm)
0
30
60
90
120
150
180
210
240
Time (seconds)

Fig. 4

FIGURE 5a

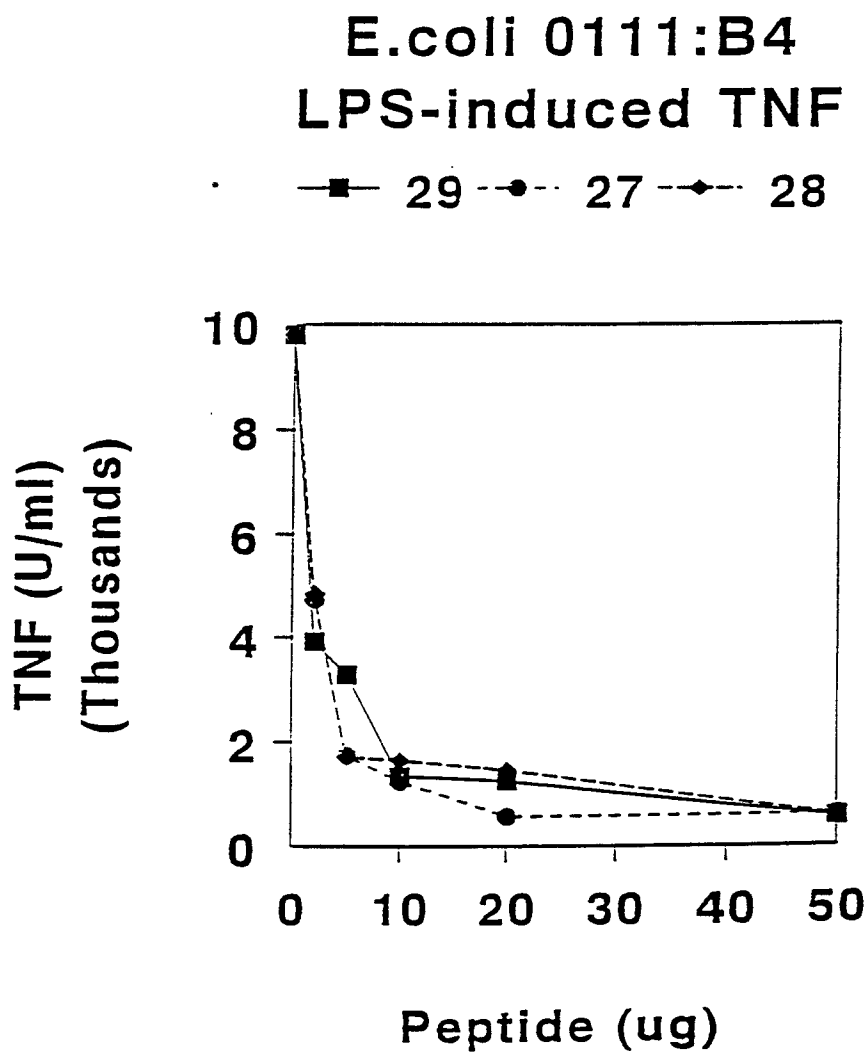

E.coli 0111:B4
LPS-induced TNF
29
27
28
TNF (U/ml)
(Thousands)
10
8
6
4
2
0
0
10
20
30
40
50
Peptide (ug)

FIGURE 5b

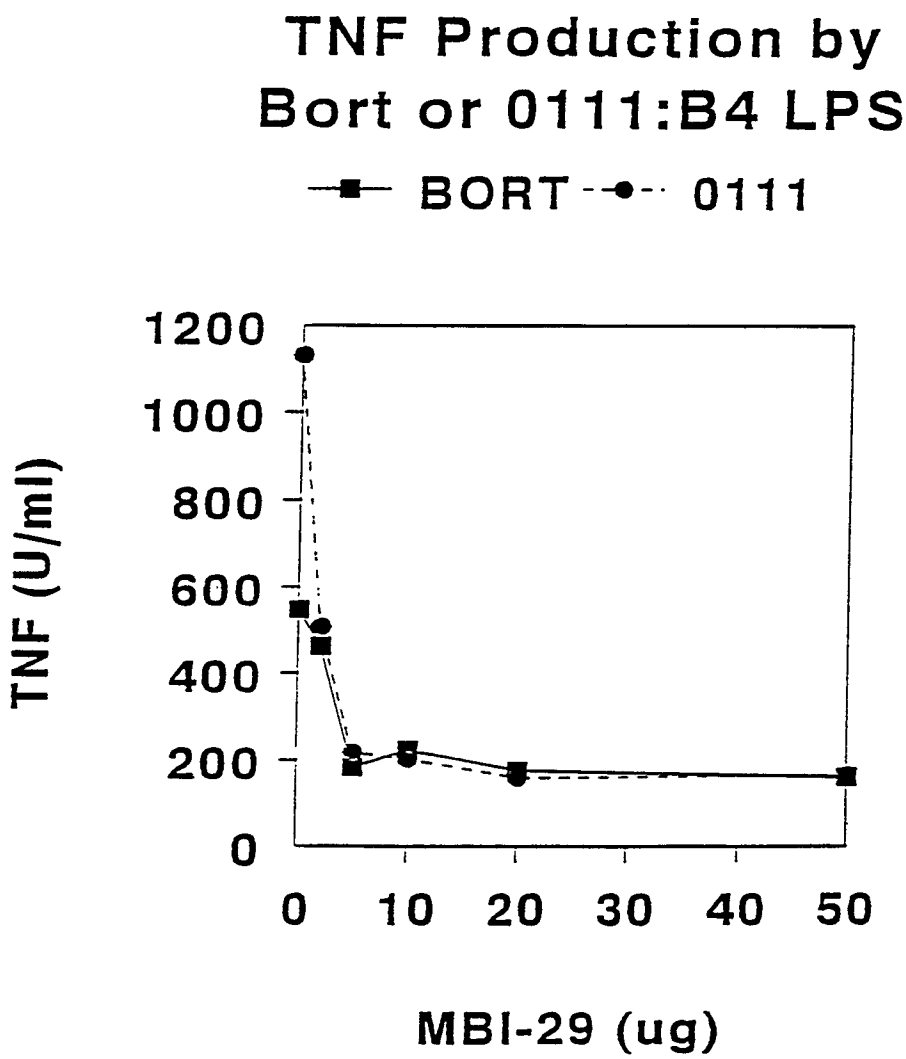

TNF Production by
Bort or 0111:B4 LPS
BORT
0111
TNF (U/ml)
1200
1000
800
600
400
200
0
0
10
20
30
40
50
MBI-29 (ug)

FIGURE 5c

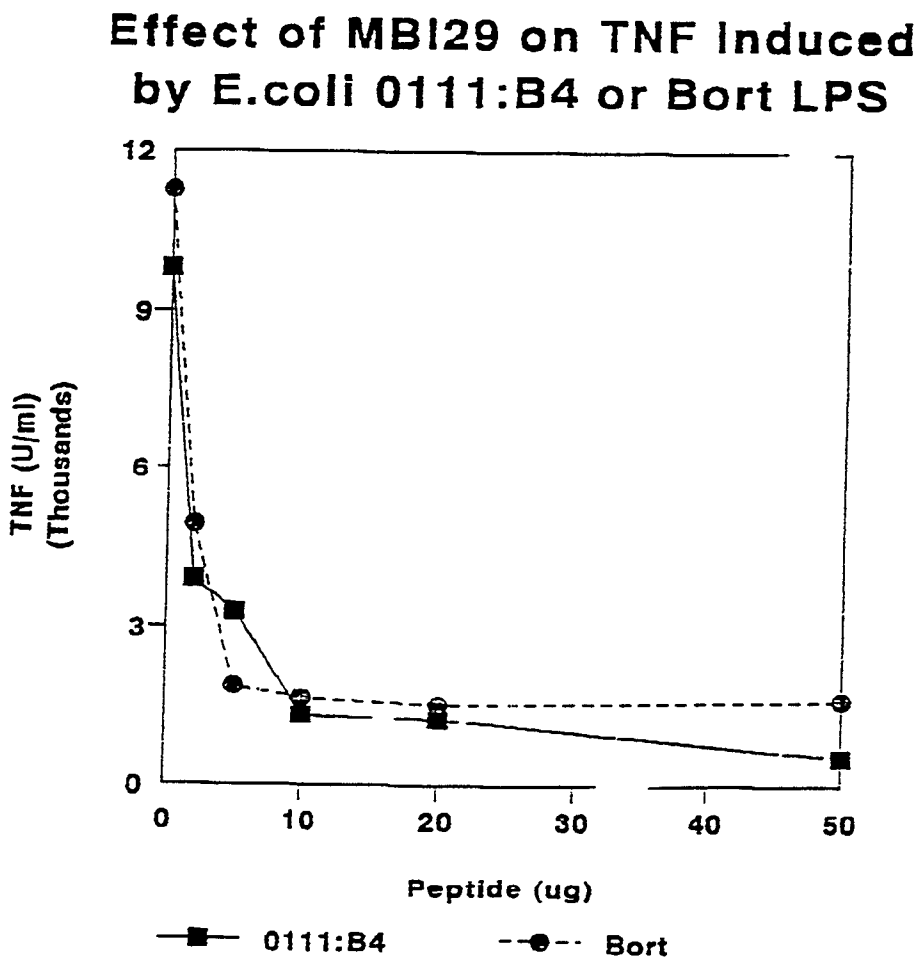
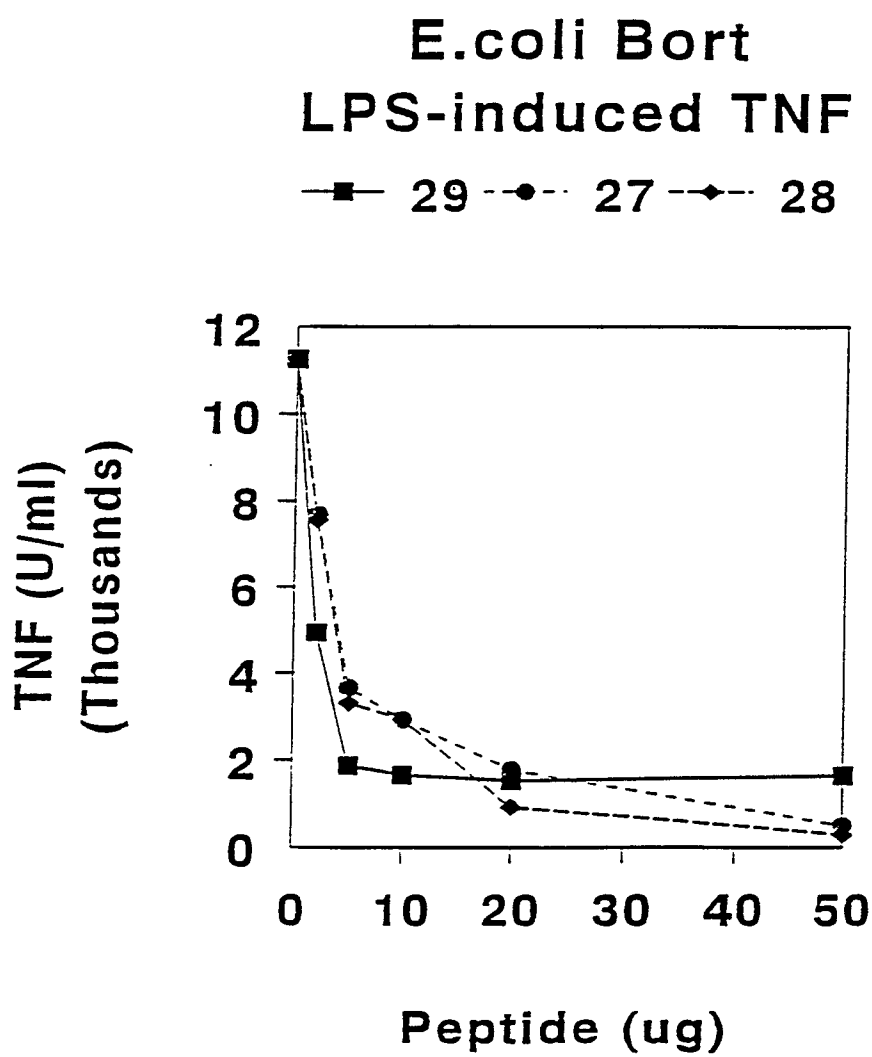

Effect of MBI29 on TNF Induced by E.coli 0111:B4 or Bort LPS
TNF (U/ml) (Thousands)
12
9
6
3
0
0
10
20
30
40
50
Peptide (ug)
0111:B4
Bort

FIGURE 6a


E.coli Bort
LPS-induced TNF
29
27
28
TNF (U/ml)
(Thousands)
12
10
8
6
4
2
0
0
10
20
30
40
50
Peptide (ug)

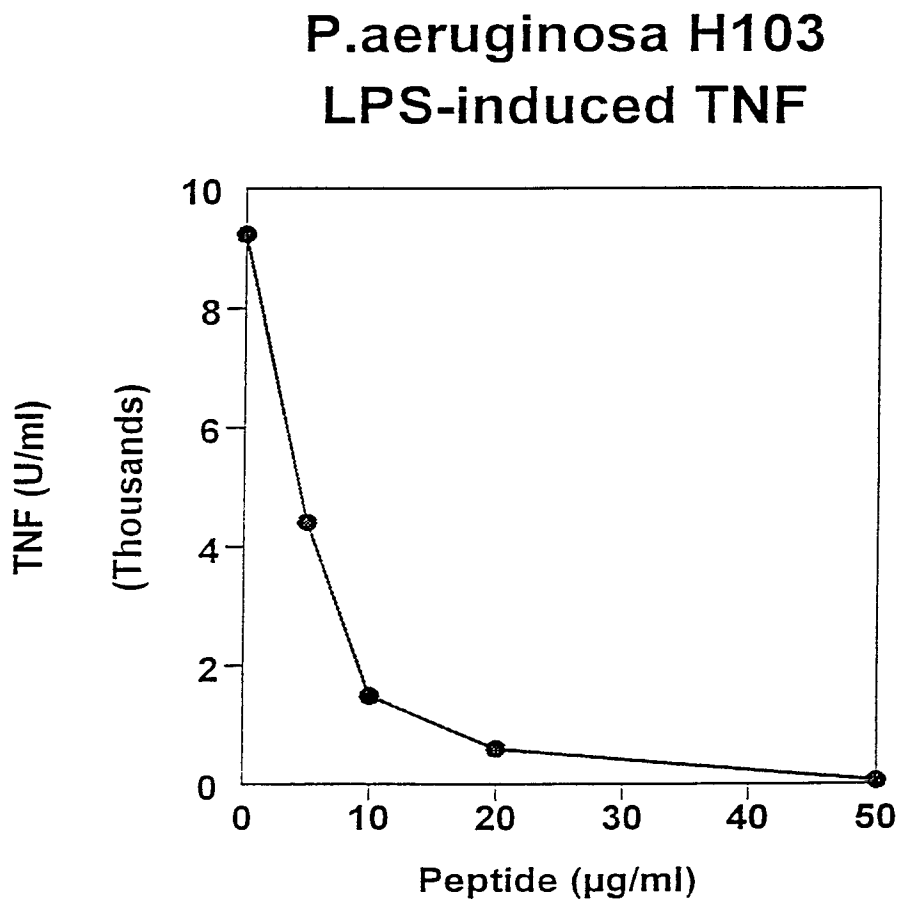

P.aeruginosa H103
LPS-induced TNF
TNF (U/ml)
(Thousands)
10
8
6
4
2
0
0
10
20
30
40
50
Peptide (µg/ml)

Fig 6b

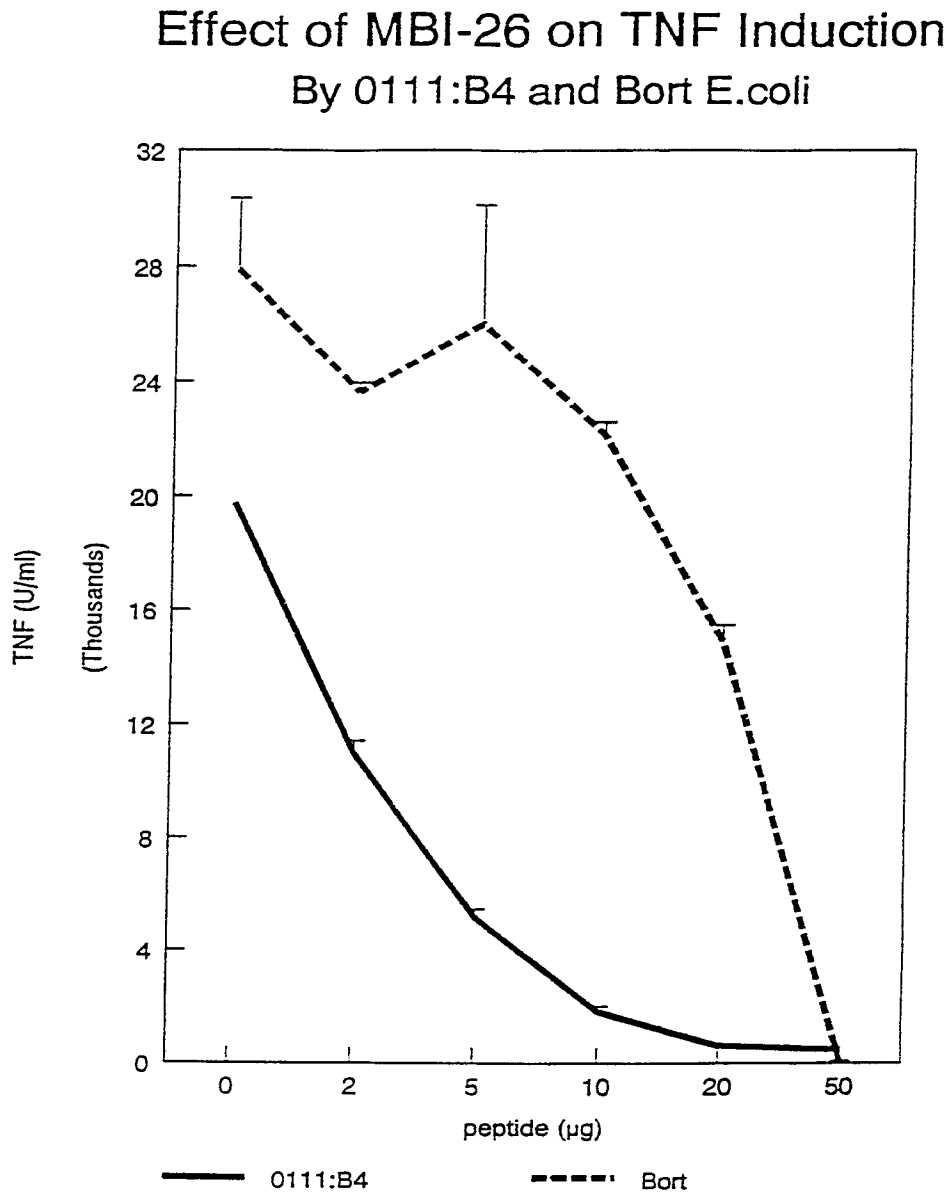

Effect of MBI-26 on TNF Induction
By 0111:B4 and Bort E.coli
TNF (U/ml)
(Thousands)
32
28
24
20
16
12
8
4
0
0
2
5
10
20
50
peptide (µg)
0111:B4
Bort

Fig. 6c

FIGURE 7a

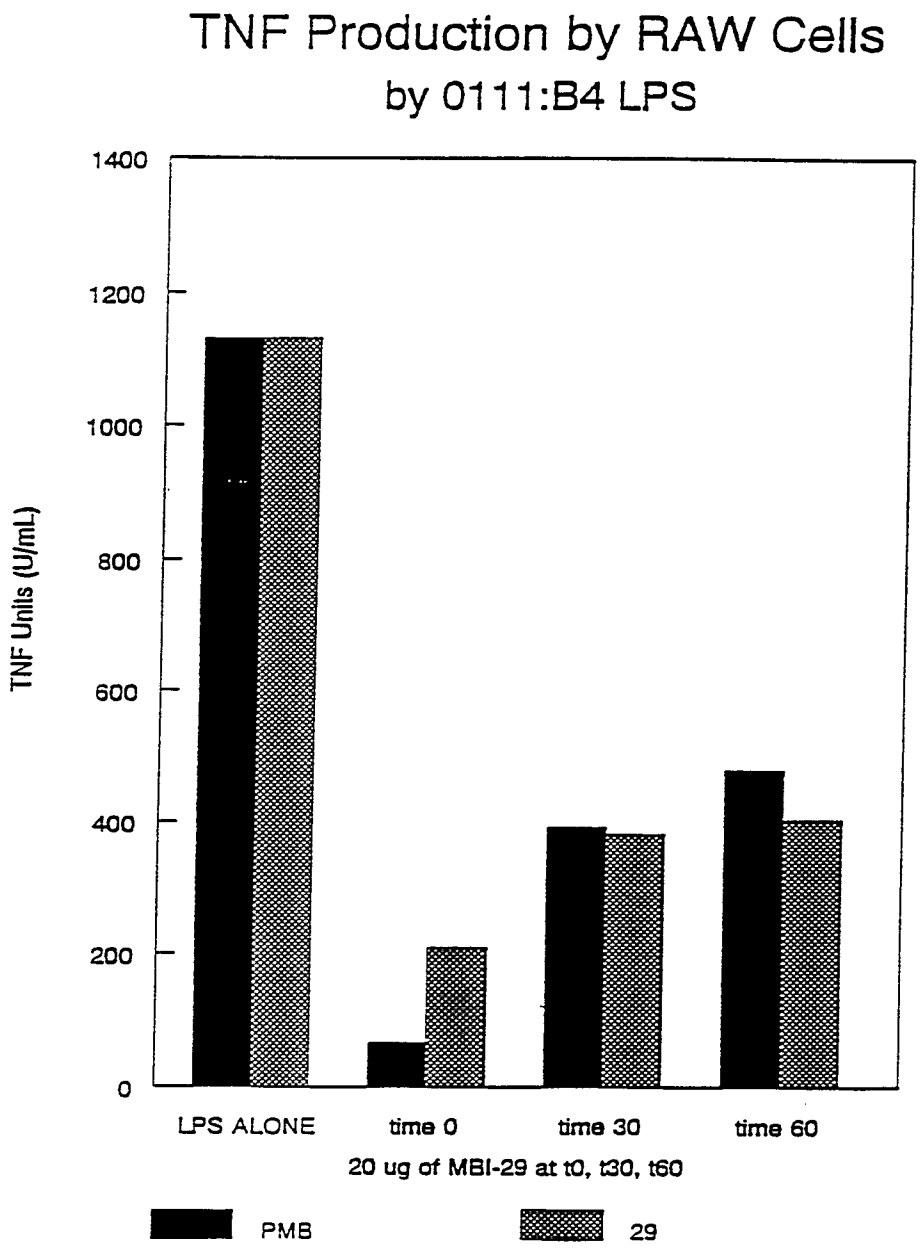
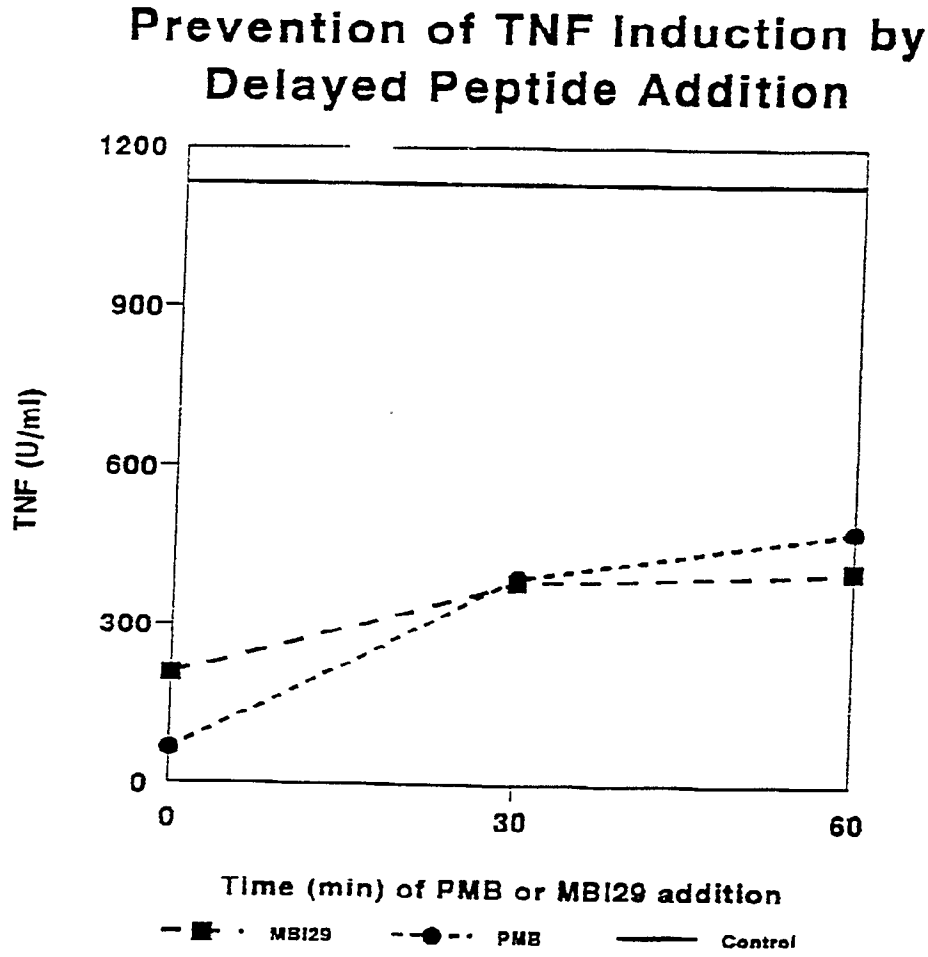

TNF Production by RAW Cells
by 0111:B4 LPS
TNF Units (U/mL)
1400
1200
1000
800
600
400
200
0
LPS ALONE
time 0
time 30
time 60
20 ug of MBI-29 at t0, t30, t60
PMB
29

FIGURE 7b


Prevention of TNF Induction by Delayed Peptide Addition
1200
900
600
300
0
TNF (U/ml)
0
30
60
Time (min) of PMB or MBI29 addition
MBI29
PMB
Control