EFFECTORS OF INNATE IMMUNITY

RELATED APPLICATION DATA

This application claims priority under 35 USC 119(e) to US Patent Application Serial No. 60/336,632, filed December 3, 2001, herein incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0001] The present invention relates generally to peptides and specifically to peptides effective as therapeutics and for drug discovery related to pathologies resulting from microbial infections and for modulating innate immunity or anti-inflammatory activity.

BACKGROUND OF THE INVENTION

[0002] Infectious diseases are the leading cause of death worldwide. According to a 1999 World Health Organization study, over 13 million people die from infectious diseases each year. Infectious diseases are the third leading cause of death in North America, accounting for 20% of deaths annually and increasing by 50% since 1980. The success of many medical and surgical treatments also hinges on the control of infectious diseases. The discovery and use of antibiotics has been one of the great achievements of modern medicine. Without antibiotics, physicians would be unable to perform complex surgery, chemotherapy or most medical interventions such as catheterization.

[0003] Current sales of antibiotics are US$26 billion worldwide. However, the overuse and sometimes unwarranted use of antibiotics have resulted in the evolution of new antibiotic-resistant strains of bacteria. Antibiotic resistance has become part of the medical landscape. Bacteria such as vancomycin-resistant Enterococcus, VRE, and methicillin-resistant Staphylococcus aureus and MRSA, strains cannot be treated with antibiotics and often, patients suffering from infections with such bacteria die.

Antibiotic discovery has proven to be one of the most difficult areas for new drug development and many large pharmaceutical companies have cut back or completely halted their antibiotic development programs. However, with the dramatic rise of antibiotic resistance, including the emergence of untreatable infections, there is a clear unmet medical need for novel types of anti-microbial therapies, and agents that impact on innate immunity would be one such class of agents.

[0004] The innate immune system is a highly effective and evolved general defense system. Elements of innate immunity are always present at low levels and are activated very rapidly when stimulated. Stimulation can include interaction of bacterial signaling molecules with pattern recognition receptors on the surface of the body's cells or other mechanisms of disease. Every day, humans are exposed to tens of thousands of potential pathogenic microorganisms through the food and water we ingest, the air we breathe and the surfaces, pets and people that we touch. The innate immune system acts to prevent these pathogens from causing disease. The innate immune system differs from so-called adaptive immunity (which includes antibodies and antigen-specific B- and T-lymphocytes) because it is always present, effective immediately, and relatively non-specific for any given pathogen. The adaptive immune system requires amplification of specific recognition elements and thus takes days to weeks to respond. Even when adaptive immunity is pre-stimulated by vaccination, it may take three days or more to respond to a pathogen whereas innate immunity is immediately or rapidly (hours) available. Innate immunity involves a variety of effector functions including phagocytic cells, complement, etc, but is generally incompletely understood. Generally speaking many innate immune responses are "triggered" by the binding of microbial signaling molecules with pattern recognition receptors termed Toll-like receptors on the surface of host cells. Many of these effector functions are grouped together in the inflammatory response. However too severe an inflammatory response can result in responses that are harmful to the body, and in an extreme case sepsis and potentially death can occur.

[0005] The release of structural components from infectious agents during infection causes an inflammatory response, which when unchecked can lead to the potentially lethal condition, sepsis. Sepsis occurs in approximately 780,000 patients in North

America annually. Sepsis may develop as a result of infections acquired in the community such as pneumonia, or it may be a complication of the treatment of trauma, cancer or major surgery. Severe sepsis occurs when the body is overwhelmed by the inflammatory response and body organs begin to fail. Up to 120,000 deaths occur annually in the United Stated due to sepsis. Sepsis may also involve pathogenic microorganisms or toxins in the blood (e.g., septicemia), which is a leading cause of death among humans. Gram-negative bacteria are the organisms most commonly associated with such diseases. However, gram-positive bacteria are an increasing cause of infections. Gram-negative and Gram-positive bacteria and their components can all cause sepsis.

[0006] The presence of microbial components induce the release of pro-inflammatory cytokines of which tumor necrosis factor-α (TNF-α) is of extreme importance. TNF-α and other pro-inflammatory cytokines can then cause the release of other pro-inflammatory mediators and lead to an inflammatory cascade. Gram-negative sepsis is usually caused by the release of the bacterial outer membrane component, lipopolysaccharide (LPS; also referred to as endotoxin). Endotoxin in the blood, called endotoxemia comes primarily from a bacterial infection, and may be released during treatment with antibiotics. Gram-positive sepsis can be caused by the release of bacterial cell wall components such as lipoteichoic acid (LTA), peptidoglycan (PG), rhamnose-glucose polymers made by *Streptococci*, or capsular polysaccharides made by *Staphylococci*. Bacterial or other non-mammalian DNA that, unlike mammalian DNA, frequently contains unmethylated cytosine-guanosine dimers (CpG DNA) has also been shown to induce septic conditions including the production of TNF-α. Mammalian DNA contains CpG dinucleotides at a much lower frequency, often in a methylated form. In addition to their natural release during bacterial infections, antibiotic treatment can also cause release of the bacterial cell wall components LPS and LTA and probably also bacterial DNA. This can then hinder recovery from infection or even cause sepsis.

[0007] Cationic peptides are being increasingly recognized as a form of defense against infection, although the major effects recognized in the scientific and patent literature are the antimicrobial effects (Hancock, R.E.W., and R. Lehrer. 1998.

Cationic peptides: a new source of antibiotics. Trends in Biotechnology 16: 82-88.). Cationic peptides having antimicrobial activity have been isolated from a wide variety of organisms. In nature, such peptides provide a defense mechanism against microorganisms such as bacteria and yeast. Generally, these cationic peptides are thought to exert their antimicrobial activity on bacteria by interacting with the cytoplasmic membrane, and in most cases, forming channels or lesions. In gram-negative bacteria, they interact with LPS to permeabilize the outer membrane, leading to self promoted uptake across the outer membrane and access to the cytoplasmic membrane. Examples of cationic antimicrobial peptides include indolicidin, defensins, cecropins, and magainins.

[0008] Recently it has been increasingly recognized that such peptides are effectors in other aspects of innate immunity (Hancock, R.E.W. and G. Diamond. 2000. The role of cationic peptides in innate host defenses. Trends in Microbiology 8:402-410.; Hancock, R.E.W. 2001. Cationic peptides: effectors in innate immunity and novel antimicrobials. Lancet Infectious Diseases 1:156-164) although it was not known if the antimicrobial and effector functions are independent.

[0009] Some cationic peptides have an affinity for binding bacterial products such as LPS and LTA. Such cationic peptides can suppress cytokine production in response to LPS, and to varying extents can prevent lethal shock. However it has not been proven as to whether such effects are due to binding of the peptides to LPS and LTA, or due to a direct interaction of the peptides with host cells. Cationic peptides are induced, in response to challenge by microbes or microbial signaling molecules like LPS, by a regulatory pathway similar to that used by the mammalian immune system (involving Toll like receptors and the transcription factor; NFκB). Cationic peptides therefore appear to have a key role in innate immunity. Mutations that affect the induction of antibacterial peptides can reduce survival in response to bacterial challenge. As well, mutations of the Toll pathway of *Drosophila* that lead to decreased antifungal peptide expression result in increased susceptibility to lethal fungal infections. In humans, patients with specific granule deficiency syndrome, completely lacking in α-defensins, suffer from frequent and severe bacterial infections. Other evidence includes the inducibility of some peptides by infectious

agents, and the very high concentrations that have been recorded at sites of inflammation. Cationic peptides may also regulate cell migration, to promote the ability of leukocytes to combat bacterial infections. For example, two human α-defensin peptides, HNP-1 and HNP-2, have been indicated to have direct chemotactic activity for murine and human T cells and monocytes, and human β-defensins appear to act as chemoattractants for immature dendritic cells and memory T cells through interaction with CCR6. Similarly, the porcine cationic peptide, PR-39 was found to be chemotactic for neutrophils. It is unclear however as to whether peptides of different structures and compositions share these properties.

[00010] The single known cathelicidin from humans, LL-37, is produced by myeloid precursors, testis, human keratinocytes during inflammatory disorders and airway epithelium. The characteristic feature of cathelicidin peptides is a high level of sequence identity at the N-terminus prepro regions termed the cathelin domain. Cathelicidin peptides are stored as inactive propeptide precursors that, upon stimulation, are processed into active peptides.

SUMMARY OF THE INVENTION

[00011] The present invention is based on the seminal discovery that based on patterns of polynucleotide expression regulated by endotoxic lipopolysaccharide, lipoteichoic acid, CpG DNA, or other cellular components (e.g., microbes or their cellular components), and affected by cationic peptides, one can screen for novel compounds that block or reduce sepsis and/or inflammation in a subject. Further, based on the use of cationic peptides as a tool, one can identify selective enhancers of innate immunity that do not trigger the sepsis reaction and that can block/dampen inflammatory and/or septic responses.

[00012] Thus, in one embodiment, a method of identifying a polynucleotide or pattern of polynucleotides regulated by one or more sepsis or inflammatory inducing agents and inhibited by a cationic peptide is provided. The method of the invention includes contacting the polynucleotide or polynucleotides with one or more sepsis or inflammatory inducing agents and contacting the polynucleotide or polynucleotides

with a cationic peptide either simultaneously or immediately thereafter. Differences in expression are detected in the presence and absence of the cationic peptide, and a change in expression, either up- or down-regulation, is indicative of a polynucleotide or pattern of polynucleotides that is regulated by a sepsis or inflammatory inducing agent and inhibited by a cationic peptide. In another aspect the invention provides a polynucleotide or polynucleotides identified by the above method. Examples of sepsis or inflammatory regulatory agents include LPS, LTA or CpG DNA or microbial components (or any combination thereof), or related agents.

[0010] In another embodiment, the invention provides a method of identifying an agent that blocks sepsis or inflammation including combining a polynucleotide identified by the method set forth above with an agent wherein expression of the polynucleotide in the presence of the agent is modulated as compared with expression in the absence of the agent and wherein the modulation in expression affects an inflammatory or septic response.

[0011] In another embodiment, the invention provides a method of identifying a pattern of polynucleotide expression for inhibition of an inflammatory or septic response by 1) contacting cells with LPS, LTA and/or CpG DNA in the presence or absence of a cationic peptide and 2) detecting a pattern of polynucleotide expression for the cells in the presence and absence of the peptide. The pattern obtained in the presence of the peptide represents inhibition of an inflammatory or septic response. In another aspect the pattern obtained in the presence of the peptide is compared to the pattern of a test compound to identify a compound that provides a similar pattern. In another aspect the invention provides a compound identified by the foregoing method.

[0012] In another embodiment, the invention provides a method of identifying an agent that enhances innate immunity by contacting a polynucleotide or polynucleotides that encode a polypeptide involved in innate immunity, with an agent of interest, wherein expression of the polynucleotide in the presence of the agent is modulated as compared with expression of the polynucleotide in the absence of the agent and wherein the modulated expression results in enhancement of innate

immunity. Preferably, the agent does not stimulate a sepsis reaction in a subject. In one aspect, the agent increases the expression of an anti-inflammatory polynucleotide. Exemplary, but non-limiting anti-inflammatory polynucleotides encode proteins such as IL-1 R antagonist homolog 1 (AI167887), IL-10 R beta (AA486393), IL-10 R alpha (U00672) TNF Receptor member 1B (AA150416), TNF receptor member 5 (H98636), TNF receptor member 11b (AA194983), IK cytokine down-regulator of HLA II (R39227), TGF-B inducible early growth response 2 (AI473938), CD2 (AA927710), IL-19 (NM_013371) or IL-10 (M57627). In one aspect, the agent decreases the expression of polynucleotides encoding proteasome subunits involved in NF-κB activation such as proteasome subunit 26S (NM_013371). In one aspect, the agent may act as an antagonist of protein kinases. In one aspect, the agent is a peptide selected from SEQ ID NO:4-54.

[0013] In another embodiment, the invention provides a method of identifying a pattern of polynucleotide expression for identification of a compound that selectively enhances innate immunity. The invention includes detecting a pattern of polynucleotide expression for cells contacted in the presence and absence of a cationic peptide, wherein the pattern in the presence of the peptide represents stimulation of innate immunity; detecting a pattern of polynucleotide expression for cells contacted in the presence of a test compound, wherein a pattern with the test compound that is similar to the pattern observed in the presence of the cationic peptide, is indicative of a compound that enhances innate immunity. It is preferred that the compound does not stimulate a septic reaction in a subject.

[0014] In another embodiment, the invention provides a method for inferring a state of infection in a mammalian subject from a nucleic acid sample of the subject by identifying in the nucleic acid sample a polynucleotide expression pattern exemplified by an increase in polynucleotide expression of at least 2 polynucleotides in Table 50, 51 and or 52, as compared to a non-infected subject. Also included is a polynucleotide expression pattern obtained by any of the methods described above.

[00013] In another aspect a cationic peptide that is an antagonist of CXCR-4 is provided. In still another aspect, a method of identifying a cationic peptide that is an

antagonist of CXCR-4 by contacting T cells with SDF-1 in the presence of absence of a test peptide and measuring chemotaxis is provided. A decrease in chemotaxis in the presence of the test peptide is indicative of a peptide that is an antagonist of CXCR-4. Cationic peptide also acts to reduce the expression of the SDF-1 receptor polynucleotide (NM_013371).

[0015] In all of the above described methods, the compounds or agents of the invention include but are not limited to peptides, cationic peptides, peptidomimetics, chemical compounds, polypeptides, nucleic acid molecules and the like.

[0016] In still another aspect the invention provides an isolated cationic peptide. An isolated cationic peptide of the invention is represented by one of the following general formulas and the single letter amino acid code:

$X_1X_2X_3IX_4PX_4IPX_5X_2X_1$ (SEQ ID NO: 4), where X_1 is one or two of R, L or K, X_2 is one of C, S or A, X_3 is one of R or P, X_4 is one of A or V and X_5 is one of V or W;

$X_1LX_2X_3KX_4X_2X_5X_3PX_3X_1$ (SEQ ID NO: 11), where X_1 is one or two of D, E, S, T or N, X2 is one or two of P, G or D, X_3 is one of G, A, V, L, I or Y, X_4 is one of R, K or H and X_5 is one of S, T, C, M or R;

$X_1X_2X_3X_4WX_4WX_4X_5K$ (SEQ ID NO: 18), where X_1 is one to four chosen from A, P or R, X_2 is one or two aromatic amino acids (F, Y and W), X_3 is one of P or K, X_4 is one, two or none chosen from A, P, Y or W and X_5 is one to three chosen from R or P;

$X_1X_2X_3X_4X_1VX_3X_4RGX_4X_3X_4X_1X_3X_1$ (SEQ ID NO: 25) where X_1 is one or two of R or K, X_2 is a polar or charged amino acid (S, T, M, N, Q, D, E, K, R and H), X_3 is C, S, M, D or A and X_4 is F, I, V, M or R;

$X_1X_2X_3X_4X_1VX_5X_4RGX_4X_5X_4X_1X_3X_1$ (SEQ ID NO: 32), where X_1 is one or two of R or K, X_2 is a polar or charged amino acid (S, T, M, N, Q, D, E, K, R and H), X_3 is one of C, S, M, D or A, X_4 is one of F, I, V, M or R and X_5 is one of A, I, S, M, D or R; and

$KX_1KX_2FX_2KMLMX_2ALKKX_3$ (SEQ ID NO: 39), where X_1 is a polar amino acid (C, S, T, M, N and Q); X_2 is one of A, L, S or K and X_3 is 1-17 amino acids

chosen from G, A, V, L, I, P, F, S, T, K and H;

$KWKX_2X_1X_1X_2X_2X_1X_2X_2X_1X_1X_2X_2$IFHTALKPISS (SEQ ID NO: 46), where X_1 is a hydrophobic amino acid and X_2 is a hydrophilic amino acid.

[0017] Additionally, in another aspect the invention provides isolated cationic peptides KWKSFLRTFKSPVRTVFHTALKPISS (SEQ ID NO: 53) and KWKSYAHTIMSPVRLVFHTALKPISS (SEQ ID NO: 54).

[0018] Also provided are nucleic acid sequences encoding the cationic peptides of the invention, vectors including such polynucleotides and host cells containing the vectors.

DETAILED DESCRIPTION OF THE INVENTION

[0019] The present invention provides novel cationic peptides, characterized by a group of generic formulas, which have ability to modulate (e.g., up- and/or down regulate) polynucleotide expression, thereby regulating sepsis and inflammatory responses and/or innate immunity.

[0020] "Innate immunity" as used herein refers to the natural ability of an organism to defend itself against invasions by pathogens. Pathogens or microbes as used herein may include, but are not limited to bacteria, fungi, parasites and viruses. Innate immunity is contrasted with acquired/adaptive immunity in which the organism develops a defensive mechanism based substantially on antibodies and/or immune lymphocytes that is characterized by specificity, amplifiability and self vs. non-self dsicrimination. With innate immunity, broad, nonspecific immunity is provided and there is no immunologic memory of prior exposure. The hallmarks of innate immunity are effectiveness against a broad variety of potential pathogens, independence of prior exposure to a pathogen, and immediate effectiveness (in contrast to the specific immune response which takes days to weeks to be elicited). In addition, innate immunity includes immune responses that affect other diseases, such as cancer, inflammatory diseases, multiple sclerosis, various viral infections, and the like.

[0021] As used herein, the term "cationic peptide" refers to a sequence of amino acids from about 5 to about 50 amino acids in length. In one aspect, the cationic peptide of the invention is from about 10 to about 35 amino acids in length. A peptide is "cationic" if it possesses sufficient positively charged amino acids to have a pKa greater than 9.0. Typically, at least two of the amino acid residues of the cationic peptide will be positively charged, for example, lysine or arginine. "Positively charged" refers to the side chains of the amino acid residues which have a net positive charge at pH 7.0. Examples of naturally occurring cationic antimicrobial peptides which can be recombinantly produced according to the invention include defensins, cathelicidins, magainins, melittin, and cecropins, bactenecins, indolicidins, polyphemusins, tachyplesins, and analogs thereof. A variety of organisms make cationic peptides, molecules used as part of a non-specific defense mechanism against microorganisms. When isolated, these peptides are toxic to a wide variety of microorganisms, including bacteria, fungi, and certain enveloped viruses. While cationic peptides act against many pathogens, notable exceptions and varying degrees of toxicity exist. However this patent reveals additional cationic peptides with no toxicity towards microorganisms but an ability to protect against infections through stimulation of innate immunity, and this invention is not limited to cationic peptides with antimicrobial activity. In fact, many peptides useful in the present invention do not have antimicrobial activity.

[0022] Cationic peptides known in the art include for example, the human cathelicidin LL-37, and the bovine neutrophil peptide indolicidin and the bovine variant of bactenecin, Bac2A.

LL-37 LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES (SEQ ID NO: 1)
Indolicidin ILPWKWPWWPWRR-NH_2 (SEQ ID NO: 2)
Bac2A RLARIVVIRVAR-NH_2 (SEQ ID NO: 3)

[0023] In innate immunity, the immune response is not dependent upon antigens. The innate immunity process may include the production of secretory molecules and cellular components as set forth above. In innate immunity, the pathogens are recognized by receptors encoded in the germline. These Toll-like receptors have

broad specificity and are capable of recognizing many pathogens. When cationic peptides are present in the immune response, they aid in the host response to pathogens. This change in the immune response induces the release of chemokines, which promote the recruitment of immune cells to the site of infection.

[0024] Chemokines, or chemoattractant cytokines, are a subgroup of immune factors that mediate chemotactic and other pro-inflammatory phenomena (See, Schall, 1991, *Cytokine* 3:165-183). Chemokines are small molecules of approximately 70-80 residues in length and can generally be divided into two subgroups, α which have two N-terminal cysteines separated by a single amino acid (CxC) and β which have two adjacent cysteines at the N terminus (CC). RANTES, MIP-1α and MIP-1β are members of the β subgroup (reviewed by Horuk, R., 1994, *Trends Pharmacol. Sci*, 15:159-165; Murphy, P. M., 1994, *Annu. Rev. Immunol.*, 12:593-633). The amino terminus of the β chemokines RANTES, MCP-1, and MCP-3 have been implicated in the mediation of cell migration and inflammation induced by these chemokines. This involvement is suggested by the observation that the deletion of the amino terminal 8 residues of MCP-1, amino terminal 9 residues of MCP-3, and amino terminal 8 residues of RANTES and the addition of a methionine to the amino terminus of RANTES, antagonize the chemotaxis, calcium mobilization and/or enzyme release stimulated by their native counterparts (Gong et al., 1996 *J. Biol. Chem.* 271:10521-10527; Proudfoot et al., 1996 *J. Biol. Chem.* 271:2599-2603). Additionally, α chemokine-like chemotactic activity has been introduced into MCP-1 via a double mutation of Tyr 28 and Arg 30 to leucine and valine, respectively, indicating that internal regions of this protein also play a role in regulating chemotactic activity (Beall et al., 1992, *J. Biol. Chem.* 267:3455-3459).

[0025] The monomeric forms of all chemokines characterized thus far share significant structural homology, although the quaternary structures of α and β groups are distinct. While the monomeric structures of the β and α chemokines are very similar, the dimeric structures of the two groups are completely different. An additional chemokine, lymphotactin, which has only one N terminal cysteine has also been identified and may represent an additional subgroup (γ) of chemokines (Yoshida

et al., 1995, *FEBS Lett.* 360:155-159; and Kelner et al., 1994, *Science* 266:1395-1399).

[0026] Receptors for chemokines belong to the large family of G-protein coupled, 7 transmembrane domain receptors (GCR's) (See, reviews by Horuk, R., 1994, *Trends Pharmacol. Sci.* 15:159-165; and Murphy, P. M., 1994, *Annu. Rev. Immunol.* 12:593-633). Competition binding and cross-desensitization studies have shown that chemokine receptors exhibit considerable promiscuity in ligand binding. Examples demonstrating the promiscuity among β chemokine receptors include: CC CKR-1, which binds RANTES and MIP-1α (Neote et al., 1993, *Cell* 72: 415-425), CC CKR-4, which binds RANTES, MIP-1α, and MCP-1 (Power et al., 1995, *J. Biol. Chem.* 270:19495-19500), and CC CKR-5, which binds RANTES, MIP-1α, and MIP-1β (Alkhatib et al., 1996, *Science*, in press and Dragic et al., 1996, *Nature* 381:667-674). Erythrocytes possess a receptor (known as the Duffy antigen) which binds both α and β chemokines (Horuk et al., 1994, *J. Biol. Chem.* 269:17730-17733; Neote et al., 1994, *Blood* 84:44-52; and Neote et al., 1993, *J. Biol. Chem.* 268:12247-12249). Thus the sequence and structural homologies evident among chemokines and their receptors allows some overlap in receptor-ligand interactions.

[0027] In one aspect, the present invention provides the use of compounds including cationic peptides of the invention to reduce sepsis and inflammatory responses by acting directly on host cells. In this aspect, a method of identification of a polynucleotide or polynucleotides that are regulated by one or more sepsis or inflammatory inducing agents is provided, where the regulation is altered by a cationic peptide. Such sepsis or inflammatory inducing agents include, but are not limited to endotoxic lipopolysaccharide (LPS), lipoteichoic acid (LTA) and/or CpG DNA or intact bacteria or other bacterial components. The identification is performed by contacting the polynucleotide or polynucleotides with the sepsis or inflammatory inducing agents and further contacting with a cationic peptide either simultaneously or immediately after. The expression of the polynucleotide in the presence and absence of the cationic peptide is observed and a change in expression is indicative of a polynucleotide or pattern of polynucleotides that is regulated by a sepsis or

inflammatory inducing agent and inhibited by a cationic peptide. In another aspect, the invention provides a polynucleotide identified by the method.

[0028] Once identified, such polynucleotides will be useful in methods of screening for compounds that can block sepsis or inflammation by affecting the expression of the polynucleotide. Such an effect on expression may be either up regulation or down regulation of expression. By identifying compounds that do not trigger the sepsis reaction and that can block or dampen inflammatory or septic responses, the present invention also presents a method of identifying enhancers of innate immunity. Additionally, the present invention provides compounds that are used or identified in the above methods.

[0029] Candidate compounds are obtained from a wide variety of sources including libraries of synthetic or natural compounds. For example, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides and oligopeptides. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. Additionally, natural or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical means, and may be used to produce combinatorial libraries. Known pharmacological agents may be subjected to directed or random chemical modifications, such as acylation, alkylation, esterification, amidification, and the like to produce structural analogs. Candidate agents are also found among biomolecules including, but not limited to: peptides, peptidiomimetics, saccharides, fatty acids, steroids, purines, pyrimidines, polypeptides, polynucleotides, chemical compounds, derivatives, structural analogs or combinations thereof.

[0030] Incubating components of a screening assay includes conditions which allow contact between the test compound and the polynucleotides of interest. Contacting includes in solution and in solid phase, or in a cell. The test compound may optionally be a combinatorial library for screening a plurality of compounds. Compounds identified in the method of the invention can be further evaluated,

detected, cloned, sequenced, and the like, either in solution or after binding to a solid support, by any method usually applied to the detection of a compound.

[0031] Generally, in the methods of the invention, a cationic peptide is utilized to detect and locate a polynucleotide that is essential in the process of sepsis or inflammation. Once identified, a pattern of polynucleotide expression may be obtained by observing the expression in the presence and absence of the cationic peptide. The pattern obtained in the presence of the cationic peptide is then useful in identifying additional compounds that can inhibit expression of the polynucleotide and therefore block sepsis or inflammation. It is well known to one of skill in the art that non-peptidic chemicals and peptidomimetics can mimic the ability of peptides to bind to receptors and enzyme binding sites and thus can be used to block or stimulate biological reactions. Where an additional compound of interest provides a pattern of polynucleotide expression similar to that of the expression in the presence of a cationic peptide, that compound is also useful in the modulation of sepsis or an innate immune response. In this manner, the cationic peptides of the invention, which are known inhibitors of sepsis and inflammation and enhancers of innate immunity are useful as tools in the identification of additional compounds that inhibit sepsis and inflammation and enhance innate immunity.

[0032] As can be seen in the Examples below, peptides of the invention have a widespread ability to reduce the expression of polynucleotides regulated by LPS. High levels of endotoxin in the blood are responsible for many of the symptoms seen during a serious infection or inflammation such as fever and an elevated white blood cell count. Endotoxin is a component of the cell wall of Gram-negative bacteria and is a potent trigger of the pathophysiology of sepsis. The basic mechanisms of inflammation and sepsis are related. In Example 1, polynucleotide arrays were utilized to determine the effect of cationic peptides on the transcriptional response of epithelial cells. Specifically, the effects on over 14,000 different specific polynucleotide probes induced by LPS were observed. The tables show the changes seen with cells treated with peptide compared to control cells. The resulting data indicated that the peptides have the ability to reduce the expression of polynucleotides induced by LPS.

[0033] Example 2, similarly, shows that peptides of the invention are capable of neutralizing the stimulation of immune cells by Gram positive and Gram negative bacterial products. Additionally, it is noted that certain pro-inflammatory polynucleotides are down-regulated by cationic peptides, as set forth in table 24 such as TLR1 (AI339155), TLR2 (T57791), TLR5 (N41021), TNF receptor-associated factor 2 (T55353), TNF receptor-associated factor 3 (AA504259), TNF receptor superfamily, member 12 (W71984), TNF receptor superfamily, member 17 (AA987627), small inducible cytokine subfamily B, member 6 (AI889554), IL-12R beta 2 (AA977194), IL-18 receptor 1 (AA482489), while anti-inflammatory polynucleotides are up-regulated by cationic peptides, as seen in table 25 such as IL-1 R antagonist homolog 1 (AI167887), IL-10 R beta (AA486393), TNF Receptor member 1B (AA150416), TNF receptor member 5 (H98636), TNF receptor member 11b (AA194983), IK cytokine down-regulator of HLA II (R39227), TGF-B inducible early growth response 2 (AI473938), or CD2 (AA927710). The relevance and application of these results are confirmed by an *in vivo* application to mice. Example 3 demonstrates that such peptides do not generally demponstrate toxicity towards the host cells they contact.

[0034] In Example 4 it can be seen that the cationic peptides of the invention alter polynucleotide expression in macrophage and epithelial cells. The results of this example show that pro-inflammatory polynucleotides are down-regulated by cationic peptides (Table 24) whereas anti-inflammatory polynucleotides are up-regulated by cationic peptides (Table 25).

[0035] In another aspect, the invention provides a method of identifying an agent that enhances innate immunity. In the method, a host cell polynucleotide or polynucleotides that encode a polypeptide involved in innate immunity is contacted with an agent of interest. Expression of the polynucleotide is determined, both in the presence and absence of the agent. The expression is compared and of the specific modulation of expression was indicative of an enhancement of innate immunity. In another aspect, the agent does not stimulate a septic reaction as revealed by the lack of upregulation of the pro-inflammatory cytokine TNF-α. In still another aspect the agent reduces or blocks the inflammatory or septic response. In yet another aspect,

the agent reduces the expression of TNF-α and/or interleukins including, but not limited to, IL-1β, IL-6, IL-12 p40, IL-12 p70, and IL-8.

[0036] In another aspect, the invention provides methods of direct polynucleotide regulation by cationic peptides and the use of compounds including cationic peptides to stimulate elements of innate immunity. In this aspect, the invention provides a method of identification of a pattern of polynucleotide expression for identification of a compound that enhances innate immunity. In the method of the invention, an initial detection of a pattern of polynucleotide expression for cells contacted in the presence and absence of a cationic peptide is made. The pattern resulting from polynucleotide expression in the presence of the peptide represents stimulation of innate immunity. A pattern of polynucleotide expression is then detected in the presence of a test compound, where a resulting pattern with the test compound that is similar to the pattern observed in the presence of the cationic peptide is indicative of a compound that enhances innate immunity. In another aspect, the invention provides compounds that are identified in the above methods. In another aspect, the compound of the invention stimulates chemokine or chemokine receptor expression. Chemokine or chemokine receptors may include, but are not limited to CXCR4, CXCR1, CXCR2, CCR2, CCR4, CCR5, CCR6, MIP-1 alpha, MDC, MIP-3 alpha, MCP-1, MCP-2, MCP-3, MCP-4, MCP-5, and RANTES. In still another aspect, the compound is a peptide, peptidomimetic, chemical compound, or a nucleic acid molecule.

[0037] In still another aspect the polynucleotide expression pattern includes expression of pro-inflammatory polynucleotides. Such pro-inflammatory polynucleotides may include, but are not limited to, ring finger protein 10 (D87451), serine/threonine protein kinase MASK (AB040057), KIAA0912 protein (AB020719), KIAA0239 protein (D87076), RAP1, GTPase activating protein 1 (M64788), FEM-1-like death receptor binding protein (AB007856), cathepsin S (M90696), hypothetical protein FLJ20308 (AK000315), pim-1 oncogene (M54915), proteasome subunit beta type 5 (D29011), KIAA0239 protein (D87076), mucin 5 subtype B tracheobronchial (AJ001403), cAMP response element-binding protein CREBPa, integrin alpha M (J03925), Rho-associated kinase 2 (NM_004850), PTD017 protein (AL050361) unknown genes (AK001143, AK034348, AL049250, AL16199, AL031983) and any

combination thereof. In still another aspect the polynucleotide expression pattern includes expression of cell surface receptors that may include but is not limited to retinoic acid receptor (X06614), G protein-coupled receptors (Z94155, X81892, U52219, U22491, AF015257, U66579) chemokine (C-C motif) receptor 7 (L31584), tumor necrosis factor receptor superfamily member 17 (Z29575), interferon gamma receptor 2 (U05875), cytokine receptor-like factor 1 (AF059293), class I cytokine receptor (AF053004), coagulation factor II (thrombin) receptor-like 2 (U92971), leukemia inhibitory factor receptor (NM_002310), interferon gamma receptor 1 (AL050337).

[0038] It is shown below, for example, in tables 1-15, that cationic peptides can neutralize the host response to the signaling molecules of infectious agents as well as modify the transcriptional responses of host cells, mainly by down-regulating the pro-inflammatory response and/or up-regulating the anti-inflammatory response. Example 5 shows that the cationic peptides can aid in the host response to pathogens by inducing the release of chemokines, which promote the recruitment of immune cells to the site of infection. The results are confirmed by an *in vivo* application to mice.

[0039] It is seen from the examples below that cationic peptides have a substantial influence on the host response to pathogens in that they assist in regulation of the host immune response by inducing selective pro-inflammatory responses that for example promote the recruitment of immune cells to the site of infection but not inducing potentially harmful pro-inflammatory cytokines. Sepsis appears to be caused in part by an overwhelming pro-inflammatory response to infectious agents. Cationic peptides aid the host in a "balanced" response to pathogens by inducing an anti-inflammatory response and suppressing certain potentially harmful pro-inflammatory responses.

[0040] In Example 7, the activation of selected MAP kinases was examined, to study the basic mechanisms behind the effects of interaction of cationic peptides with cells. Macrophages activate MEK/ERK kinases in response to bacterial infection. MEK is a MAP kinase kinase that when activated, phosphorylates the downstream kinase ERK

(extracellular regulated kinase), which then dimerizes and translocates to the nucleus where it activates transcription factors such as Elk-1 to modify polynucleotide expression. MEK/ERK kinases have been shown to impair replication of *Salmonella* within macrophages. Signal transduction by MEK kinase and NADPH oxidase may play an important role in innate host defense against intracellular pathogens. By affecting the MAP kinases as shown below the cationic peptides have an effect on bacterial infection. The cationic peptides can directly affect kinases. Table 21 demonstrates but is not limited to MAP kinase polynucleotide expression changes in response to peptide. The kinases include MAP kinase kinase 6 (H070920), MAP kinase kinase 5 (W69649), MAP kinase 7 (H39192), MAP kinase 12 (AI936909) and MAP kinase-activated protein kinase 3 (W68281).

[0041] In another method, the methods of the invention may be used in combination, to identify an agent with multiple characteristics, i.e. a peptide with anti-inflammatory/anti-sepsis activity, and the ability to enhance innate immunity, in part by inducing chemokines *in vivo*.

[0042] In another aspect, the invention provides a method for inferring a state of infection in a mammalian subject from a nucleic acid sample of the subject by identifying in the nucleic acid sample a polynucleotide expression pattern exemplified by an increase in polynucleotide expression of at least 2 polynucleotides in Table 55 as compared to a non-infected subject. In another aspect the invention provides a method for inferring a state of infection in a mammalian subject from a nucleic acid sample of the subject by identifying in the nucleic acid sample a polynucleotide expression pattern exemplified by a polynucleotide expression of at least 2 polynucleotides in Table 56 or Table 57 as compared to a non-infected subject. In one aspect of the invention, the state of infection is due to infectious agents or signaling molecules derived therefrom, such as, but not limited to, Gram negative bacteria and Gram positive bacteria, viral, fungal or parasitic agents. In still another aspect the invention provides a polynucleotide expression pattern of a subject having a state of infection identified by the above method. Once identified, such polynucleotides will be useful in methods of diagnosis of a condition associated with the activity or presence of such infectious agents or signaling molecules.

[0043] Example 10 below demonstrates this aspect of the invention. Specifically, table 61 demonstrates that both MEK and the NADPH oxidase inhibitors can limit bacterial replication (infection of IFN-γ-primed macrophages by S. typhimurium triggers a MEK kinase). This is an example of how bacterial survival can be impacted by changing host cell signaling molecules.

[0044] In still another aspect of the invention, compounds are presented that inhibit stromal derived factor-1 (SDF-1) induced chemotaxis of T cells. . Compounds are also presented which decrease expression of SDF-1 receptor. Such compounds also may act as an antagonist or inhibitor of CXCR-4. In one aspect the invention provides a cationic peptide that is an antagonist of CXCR-4. In another aspect the invention provides a method of identifying a cationic peptide that is an antagonist of CXCR-4. The method includes contacting T cells with SDF-1 in the presence of absence of a test peptide and measuring chemotaxis. A decrease in chemotaxis in the presence of the test peptide is then indicative of a peptide that is an antagonist of CXCR-4. Such compounds and methods are useful in therapeutic applications in HIV patients. These types of compounds and the utility thereof is demonstrated, for example, in Example 11 (see also Tables 62, 63). In that example, cationic peptides are shown to inhibit cell migration and therefore antiviral activity.

[0045] In one embodiment, the invention provides an isolated cationic peptides having an amino acid sequence of the general formula (Formula A): $X_1X_2X_3IX_4PX_4IPX_5X_2X_1$ (SEQ ID NO: 4), wherein X_1 is one or two of R, L or K, X_2 is one of C, S or A, X_3 is one of R or P, X_4 is one of A or V and X_5 is one of V or W. Examples of the peptides of the invention include, but are not limited to: LLCRIVPVIPWCK (SEQ ID NO: 5), LRCPIAPVIPVCKK (SEQ ID NO: 6), KSRIVPAIPVSLL (SEQ ID NO: 7), KKSPIAPAIPWSR (SEQ ID NO: 8), RRARIVPAIPVARR (SEQ ID NO: 9) and LSRIAPAIPWAKL (SEQ ID NO: 10).

[0046] In another embodiment, the invention provides an isolated linear cationic peptide having an amino acid sequence of the general formula (Formula B): $X_1LX_2X_3KX_4X_2X_5X_3PX_3X_1$ (SEQ ID NO: 11), wherein X_1 is one or two of D, E, S, T or N, X2 is one or two of P, G or D, X_3 is one of G, A, V, L, I or Y, X_4 is one of R, K

or H and X_5 is one of S, T, C, M or R. Examples of the peptides of the invention include, but are not limited to: DLPAKRGSAPGST (SEQ ID NO: 12), SELPGLKHPCVPGS (SEQ ID NO: 13), TTLGPVKRDSIPGE (SEQ ID NO: 14), SLPIKHDRLPATS (SEQ ID NO: 15), ELPLKRGRVPVE (SEQ ID NO: 16) and NLPDLKKPRVPATS (SEQ ID NO: 17).

[0047] In another embodiment, the invention provides an isolated linear cationic peptide having an amino acid sequence of the general formula (Formula C): $X_1X_2X_3X_4WX_4WX_4X_5K$ (SEQ ID NO: 18) (this formula includes CP12a and CP12d) , wherein X_1 is one to four chosen from A, P or R, X_2 is one or two aromatic amino acids (F, Y and W), X_3 is one of P or K, X_4 is one, two or none chosen from A, P, Y or W and X_5 is one to three chosen from R or P. Examples of the peptides of the invention include, but are not limited to: RPRYPWWPWWPYRPRK (SEQ ID NO: 19), RRAWWKAWWARRK (SEQ ID NO: 20), RAPYWPWAWARPRK (SEQ ID NO: 21), RPAWKYWWPWPWPRRK (SEQ ID NO: 22), RAAFKWAWAWWRRK (SEQ ID NO: 23) and RRRWKWAWPRRK (SEQ ID NO: 24).

[0048] In another embodiment, the invention provides an isolated hexadecameric cationic peptide having an amino acid sequence of the general formula (Formula D): $X_1X_2X_3X_4X_1VX_3X_4RGX_4X_3X_4X_1X_3X_1$ (SEQ ID NO: 25) wherein X_1 is one or two of R or K, X_2 is a polar or charged amino acid (S, T, M, N, Q, D, E, K, R and H), X_3 is C, S, M, D or A and X_4 is F, I, V, M or R. Examples of the peptides of the invention include, but are not limited to: RRMCIKVCVRGVCRRKCRK (SEQ ID NO: 26), KRSCFKVSMRGVSRRRCK (SEQ ID NO: 27), KKDAIKKVDIRGMDMRRAR (SEQ ID NO: 28), RKMVKVDVRGIMIRKDRR (SEQ ID NO: 29), KQCVKVAMRGMALRRCK (SEQ ID NO: 30) and RREAIRRVAMRGRDMKRMRR (SEQ ID NO: 31).

[0049] In still another embodiment, the invention provides an isolated hexadecameric cationic peptide having an amino acid sequence of the general formula (Formula E): $X_1X_2X_3X_4X_1VX_5X_4RGX_4X_5X_4X_1X_3X_1$ (SEQ ID NO: 32), wherein X_1 is one or two of R or K, X_2 is a polar or charged amino acid (S, T, M, N, Q, D, E, K, R and H), X_3 is one of C, S, M, D or A, X_4 is one of F, I, V, M or R and X_5 is one of A, I, S, M, D

or R. Examples of the peptides of the invention include, but are not limited to: RTCVKRVAMRGIIRKRCR (SEQ ID NO: 33), KKQMMKRVDVRGISVKRKR (SEQ ID NO: 34), KESIKVIIRGMMVRMKK (SEQ ID NO: 35), RRDCRRVMVRGIDIKAK (SEQ ID NO: 36), KRTAIKKVSRRGMSVKARR (SEQ ID NO: 37) and RHCIRRVSMRGIIMRRCK (SEQ ID NO: 38).

[0050] In another embodiment, the invention provides an isolated longer cationic peptide having an amino acid sequence of the general formula (Formula F): $KX_1KX_2FX_2KMLMX_2ALKKX_3$ (SEQ ID NO: 39), wherein X_1 is a polar amino acid (C, S, T, M, N and Q); X_2 is one of A, L, S or K and X_3 is 1-17 amino acids chosen from G, A, V, L, I, P, F, S, T, K and H. Examples of the peptides of the invention include, but are not limited to: KCKLFKKMLMLALKKVLTTGLPALKLTK (SEQ ID NO: 40), KSKSFLKMLMKALKKVLTTGLPALIS (SEQ ID NO: 41), KTKKFAKMLMMALKKVVSTAKPLAILS (SEQ ID NO: 42), KMKSFAKMLMLALKKVLKVLTTALTLKAGLPS (SEQ ID NO: 43), KNKAFAKMLMKALKKVTTAAKPLTG (SEQ ID NO: 44) and KQKLFAKMLMSALKKKTLVTTPLAGK (SEQ ID NO: 45).

[0051] In yet another embodiment, the invention provides an isolated longer cationic peptide having an amino acid sequence of the general formula (Formula G): $KWKX_2X_1X_1X_2X_2X_1X_2X_2X_1X_1X_2X_2IFHTALKPISS$ (SEQ ID NO: 46), wherein X_1 is a hydrophobic amino acid and X_2 is a hydrophilic amino acid. Examples of the peptides of the invention include, but are not limited to: KWKSFLRTFKSPVRTIFHTALKPISS (SEQ ID NO: 47), KWKSYAHTIMSPVRLIFHTALKPISS (SEQ ID NO: 48), KWKRGAHRFMKFLSTIFHTALKPISS (SEQ ID NO: 49), KWKKWAHSPRKVLTRIFHTALKPISS (SEQ ID NO: 50), KWKSLVMMFKKPARRIFHTALKPISS (SEQ ID NO: 51) and KWKHALMKAHMLWHMIFHTALKPISS (SEQ ID NO: 52).

[0052] In still another embodiment, the invention provides an isolated cationic peptide having an amino acid sequence of the formula:

KWKSFLRTFKSPVRTVFHTALKPISS (SEQ ID NO: 53) or
KWKSYAHTIMSPVRLVFHTALKPISS (SEQ ID NO: 54).

[0053] The term "isolated" as used herein refers to a peptide that is substantially free of other proteins, lipids, and nucleic acids (*e.g.*, cellular components with which an *in vivo*-produced peptide would naturally be associated). Preferably, the peptide is at least 70%, 80%, or most preferably 90% pure by weight.

[0054] The invention also includes analogs, derivatives, conservative variations, and cationic peptide variants of the enumerated polypeptides, provided that the analog, derivative, conservative variation, or variant has a detectable activity in which it enhances innate immunity or has anti-inflammatory activity. It is not necessary that the analog, derivative, variation, or variant have activity identical to the activity of the peptide from which the analog, derivative, conservative variation, or variant is derived.

[0055] A cationic peptide "variant" is an peptide that is an altered form of a referenced cationic peptide. For example, the term "variant" includes a cationic peptide in which at least one amino acid of a reference peptide is substituted in an expression library. The term "reference" peptide means any of the cationic peptides of the invention (*e.g.*, as defined in the above formulas), from which a variant, derivative, analog, or conservative variation is derived. Included within the term "derivative" is a hybrid peptide that includes at least a portion of each of two cationic peptides (*e.g.*, 30-80% of each of two cationic peptides). Also included are peptides in which one or more amino acids are deleted from the sequence of a peptide enumerated herein, provided that the derivative has activity in which it enhances innate immunity or has anti-inflammatory 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 enhancing innate immunity or anti-inflammatory activity of a peptide can be removed. Likewise, additional derivatives can be produced by adding one or a few (*e.g.*, less than 5) amino acids to a cationic peptide without completely inhibiting the activity of the peptide. In addition, C-terminal derivatives, *e.g.*, C-terminal methyl esters, and N-

terminal derivatives can be produced and are encompassed by the invention. 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 remains. Also included is the reverse sequence of a peptide encompassed by the general formulas set forth above. Additionally, an amino acid of "D" configuration may be substituted with an amino acid of "L" configuration and vice versa. Alternatively the peptide may be cyclized chemically or by the addition of two or more cysteine residues within the sequence and oxidation to form disulphide bonds.

[0056] The invention also includes peptides that are conservative variations of those peptides exemplified herein. The term "conservative variation" as used herein denotes a polypeptide in which at least one amino acid is replaced 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 acid, or glutamine for asparagine, and the like. Neutral hydrophilic amino acids that can be substituted for one another include asparagine, glutamine, serine and threonine. The term "conservative variation" also encompasses a peptide having a substituted amino acid in place of an unsubstituted parent amino acid. Such substituted amino acids may include amino acids that have been methylated or amidated. Other substitutions will be known to those of skill in the art. In one aspect, antibodies raised to a substituted polypeptide will also specifically bind the unsubstituted polypeptide.

[0057] Peptides of the invention can be synthesized by commonly used methods such as those that include t-BOC or FMOC protection of alpha-amino groups. Both methods involve stepwise synthesis in which 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 such as those described by 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 a 1% acetic acid solution, which is then lyophilized to yield the crude material. The peptides can 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 eluate yield homogeneous peptide, which can then be characterized by standard techniques such as amino acid analysis, thin layer chromatography, high performance liquid chromatography, ultraviolet absorption spectroscopy, molar rotation, or measuring solubility. If desired, the peptides can be quantitated by the solid phase Edman degradation.

[0058] The invention also includes isolated nucleic acids (*e.g.*, DNA, cDNA, or RNA) encoding the peptides of the invention. Included are nucleic acids that encode analogs, mutants, conservative variations, and variants of the peptides described herein. The term "isolated" as used herein refers to a nucleic acid that is substantially free of proteins, lipids, and other nucleic acids with which an *in vivo*-produced nucleic acids naturally associated. Preferably, the nucleic acid is at least 70%, 80%, or preferably 90% pure by weight, and conventional methods for synthesizing nucleic acids *in vitro* can be used in lieu of *in vivo* methods. As used herein, "nucleic acid" refers to a polymer of deoxyribo-nucleotides or ribonucleotides, in the form of a separate fragment or as a component of a larger genetic construct (*e.g.*, by operably linking a promoter to a nucleic acid encoding a peptide of the invention). Numerous genetic constructs (*e.g.*, plasmids and other expression vectors) are known in the art and can be used to produce the peptides of the invention in cell-free systems or prokaryotic or eukaryotic (*e.g.*, yeast, insect, or mammalian) cells. By taking into account the degeneracy of the genetic code, one of ordinary skill in the art can readily synthesize nucleic acids encoding the polypeptides of the invention. The nucleic acids of the invention can readily be used in conventional molecular biology methods to produce the peptides of the invention.

[0059] DNA encoding the cationic peptides of the invention can be inserted into an "expression vector." The term "expression vector" refers to a genetic construct such as a plasmid, virus or other vehicle known in the art that can be engineered to contain a nucleic acid encoding a polypeptide of the invention. Such expression vectors are preferably plasmids that contain a promoter sequence that facilitates transcription of the inserted genetic sequence in a host cell. The expression vector typically contains an origin of replication, and a promoter, as well as polynucleotides that allow phenotypic selection of the transformed cells (*e.g.*, an antibiotic resistance polynucleotide). Various promoters, including inducible and constitutive promoters, can be utilized in the invention. Typically, the expression vector contains a replicon site and control sequences that are derived from a species compatible with the host cell.

[0060] Transformation or transfection of a recipient with a nucleic acid of the invention can be carried out using conventional techniques well known to those skilled in the art. For example, where the host cell is *E. coli*, competent cells that are capable of DNA uptake can be prepared using the $CaCl_2$, $MgCl_2$ or RbCl methods known in the art. Alternatively, physical means, such as electroporation or microinjection can be used. Electroporation allows transfer of a nucleic acid into a cell by high voltage electric impulse. Additionally, nucleic acids can be introduced into host cells by protoplast fusion, using methods well known in the art. Suitable methods for transforming eukaryotic cells, such as electroporation and lipofection, also are known.

[0061] "Host cells" or "Recipient cells" encompassed by of the invention are any cells in which the nucleic acids of the invention can be used to express the polypeptides of the invention. The term also includes any progeny of a recipient or host cell. Preferred recipient or host cells of the invention include *E. coli*, *S. aureus* and *P. aeruginosa*, although other Gram-negative and Gram-positive bacterial, fungal and mammalian cells and 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.

[0062] 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.

[0063] 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.

[0064] 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).

[0065] 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.

[0066] 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, sodium acetate, sodium citrate, 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.

[0067] The invention will now be described in greater detail by reference to the following non-limiting examples. While the invention has been described in detail with reference to certain preferred embodiments thereof, it will be understood that modifications and variations are within the spirit and scope of that which is described and claimed.

EXAMPLE 1
ANTI-SEPSIS/ANTI-INFLAMMATORY ACTIVITY

[0068] Polynucleotide arrays were utilized to determine the effect of cationic peptides on the transcriptional response of epithelial cells. The A549 human epithelial cell line was maintained in DMEM (Gibco) supplemented with 10 % fetal bovine serum (FBS, Medicorp). The A549 cells were plated in 100 mm tissue culture dishes at 2.5 x 10^6 cells/dish, cultured overnight and then incubated with 100 ng/ml *E. coli* O111:B4 LPS

(Sigma), without (control) or with 50 μg/ml peptide or medium alone for 4 h. After stimulation, the cells were washed once with diethyl pyrocarbonate-treated phosphate buffered saline (PBS), and detached from the dish using a cell scraper. Total RNA was isolated using RNAqueous (Ambion, Austin, TX). The RNA pellet was resuspended in RNase-free water containing Superase-In (RNase inhibitor; Ambion). DNA contamination was removed with DNA-free kit, Ambion). The quality of the RNA was assessed by gel electrophoresis on a 1% agarose gel.

[0069] The polynucleotide arrays used were the Human Operon arrays (identification number for the genome is PRHU04-S1), which consist of about 14,000 human oligos spotted in duplicate. Probes were prepared from 10 μg of total RNA and labeled with Cy3 or Cy5 labeled dUTP. The probes were purified and hybridized to printed glass slides overnight at 42ºC and washed. After washing, the image was captured using a Perkin Elmer array scanner. The image processing software (Imapolynucleotide 5.0, Marina Del Rey, CA) determines the spot mean intensity, median intensities, and background intensities. A "homemade" program was used to remove background. The program calculates the bottom 10 % intensity for each subgrid and subtracts this for each grid. Analysis was performed with Genespring software (Redwood City, CA). The intensities for each spot were normalized by taking the median spot intensity value from the population of spot values within a slide and comparing this value to the values of all slides in the experiment. The relative changes seen with cells treated with peptide compared to control cells can be found in Tables 1 and 2. These tables 2 reflect only those polynucleotides that demonstrated significant changes in expression of the 14,000 polynucleotides that were tested for altered expression. The data indicate that the peptides have a widespread ability to reduce the expression of polynucleotides that were induced by LPS.

[0070] In Table 1, the peptide, SEQ ID NO: 27 is shown to potently reduce the expression of many of the polynucleotides up-regulated by *E. coli* O111:B4 LPS as studied by polynucleotide microarrays. Peptide (50 μg/ml) and LPS (0.1 μg/ml) or LPS alone was incubated with the A549 cells for 4 h and the RNA was isolated. Five μg total RNA was used to make Cy3/Cy5 labeled cDNA probes and hybridized onto Human Operon arrays (PRHU04). The intensity of unstimulated cells is shown in the

third column of Table 1. The "Ratio: LPS/control" column refers to the intensity of polynucleotide expression in LPS simulated cells divided by in the intensity of unstimulated cells. The "Ratio: LPS+ ID 27/control" column refers to the intensity of polynucleotide expression in cells stimulated with LPS and peptide divided by unstimulated cells.

Table 1: Reduction, by peptide SEQ ID 27, of A549 human epithelial cell polynucleotide expression up-regulated by *E.coli* O111:B4 LPS

Accession Number[a]	Polynucleotide Gene Function	Control: Media only Intensity	Ratio: LPS/control	Ratio: LPS+ ID 27/control
AL031983	Unknown	0.032	302.8	5.1
L04510	ADP-ribosylation factor	0.655	213.6	1.4
D87451	ring finger protein 10	3.896	183.7	2.1
AK000869	hypothetical protein	0.138	120.1	2.3
U78166	Ric -like expressed in neurons	0.051	91.7	0.2
AJ001403	mucin 5 subtype B tracheobronchial	0.203	53.4	15.9
AB040057	serine/threonine protein kinase MASK	0.95	44.3	15.8
Z99756	Unknown	0.141	35.9	14.0
L42243	interferon	0.163	27.6	5.2

Accession Number[a]	Polynucleotide Gene Function	Control: Media only Intensity	Ratio: LPS/control	Ratio: LPS+ ID 27/control
	receptor 2			
NM_016216	RNA lariat debranching enzyme	6.151	22.3	10.9
AK001589	hypothetical protein	0.646	19.2	1.3
AL137376	Unknown	1.881	17.3	0.6
AB007856	FEM-1-like death receptor binding protein	2.627	15.7	0.6
AB007854	growth arrest-specific 7	0.845	14.8	2.2
AK000353	cytosolic ovarian carcinoma antigen 1	0.453	13.5	1.0
D14539	myeloid/lymphoid or mixed-lineage leukemia translocated to 1	2.033	11.6	3.1
X76785	integration site for Epstein-Barr virus	0.728	11.6	1.9
M54915	pim-1 oncogene	1.404	11.4	0.6
NM_006092	caspase recruitment domain 4	0.369	11.0	0.5
J03925	integrin_alpha M	0.272	9.9	4.2

Accession Number[a]	Polynucleotide Gene Function	Control: Media only Intensity	Ratio: LPS/control	Ratio: LPS+ ID 27/control
NM_001663	ADP-ribosylation factor 6	0.439	9.7	1.7
M23379	RAS p21 protein activator	0.567	9.3	2.8
K02581	thymidine kinase 1 soluble	3.099	8.6	3.5
U94831	transmembrane 9 superfamily member 1	3.265	7.1	1.5
X70394	zinc finger protein 146	1.463	6.9	1.7
AL137614	hypothetical protein	0.705	6.8	1.0
U43083	guanine nucleotide binding protein	0.841	6.6	1.6
AL137648	DKFZp434J1813 protein	1.276	6.5	0.8
AF085692	ATP-binding cassette sub-family C (CFTR/MRP) member 3	3.175	6.5	2.4
AK001239	hypothetical protein FLJ10377	2.204	6.4	1.3
NM_001679	ATPase Na+/K+ transporting beta	2.402	6.3	0.9

Accession Number[a]	Polynucleotide Gene Function	Control: Media only Intensity	Ratio: LPS/control	Ratio: LPS+ ID 27/control
	3 polypeptide			
L24804	unactive progesterone receptor	3.403	6.1	1.1
U15932	dual specificity phosphatase 5	0.854	6.1	2.1
M36067	ligase I DNA_ ATP-dependent	1.354	6.1	2.2
AL161951	Unknown	0.728	5.8	1.9
M59820	colony stimulating factor 3 receptor	0.38	5.7	2.0
AL050290	spermidine/ spermine N1-acetyltransferase	2.724	5.6	1.4
NM_002291	laminin_ beta 1	1.278	5.6	1.8
X06614	retinoic acid receptor_ alpha	1.924	5.5	0.8
AB007896	putative L-type neutral amino acid transporter	0.94	5.3	1.8
AL050333	DKFZP564B116 protein	1.272	5.3	0.6
AK001093	hypothetical protein	1.729	5.3	2.0
NM_016406	hypothetical protein	1.314	5.2	1.2
M86546	pre-B-cell	1.113	5.2	2.2

Accession Number[a]	Polynucleotide Gene Function	Control: Media only Intensity	Ratio: LPS/control	Ratio: LPS+ ID 27/control
	leukemia trans-cription factor 1			
X56777	zona pellucida glycoprotein 3A	1.414	5.0	1.4
NM_013400	replication initiation region protein	1.241	4.9	2.0
NM_002309	leukemia inhibitory factor	1.286	4.8	1.9
NM_001940	dentatorubral-pallidoluysian atrophy	2.034	4.7	1.2
U91316	cytosolic acyl coenzyme A thioester hydrolase	2.043	4.7	1.4
X76104	death-associated protein kinase 1	1.118	4.6	1.8
AF131838	Unknown	1.879	4.6	1.4
AL050348	Unknown	8.502	4.4	1.7
D42085	KIAA0095 gene product	1.323	4.4	1.2
X92896	Unknown	1.675	4.3	1.5
U26648	syntaxin 5A	1.59	4.3	1.4
X85750	monocyte to macrophage differentiation-associated	1.01	4.3	1.1

Accession Number[a]	Polynucleotide Gene Function	Control: Media only Intensity	Ratio: LPS/control	Ratio: LPS+ ID 27/control
D14043	CD164 antigen_ sialomucin	1.683	4.2	1.0
J04513	fibroblast growth factor 2	1.281	4.0	0.9
U19796	melanoma-associated antigen	1.618	4.0	0.6
AK000087	hypothetical protein	1.459	3.9	1.0
AK001569	hypothetical protein	1.508	3.9	1.2
AF189009	ubiquilin 2	1.448	3.8	1.3
U60205	sterol-C4-methyl oxidase-like	1.569	3.7	0.8
AK000562	hypothetical protein	1.166	3.7	0.6
AL096739	Unknown	3.66	3.7	0.5
AK000366	hypothetical protein	15.192	3.5	1.0
NM_006325	RAN member RAS oncogene family	1.242	3.5	1.4
X51688	cyclin A2	1.772	3.3	1.0
U34252	aldehyde dehydrogenase 9	1.264	3.3	1.2
NM_013241	FH1/FH2 domain-containing	1.264	3.3	0.6

Accession Number[a]	Polynucleotide Gene Function	Control: Media only Intensity	Ratio: LPS/control	Ratio: LPS+ ID 27/control
	protein			
AF112219	esterase D/formylglutathione hydrolase	1.839	3.3	1.1
NM_016237	anaphase-promoting complex subunit 5	2.71	3.2	0.9
AB014569	KIAA0669 gene product	2.762	3.2	0.2
AF151047	hypothetical protein	3.062	3.1	1.0
X92972	protein phosphatase 6 catalytic subunit	2.615	3.1	1.1
AF035309	proteasome 26S subunit ATPase 5	5.628	3.1	1.3
U52960	SRB7 homolog	1.391	3.1	0.8
J04058	electron-transfer-flavoprotein alpha polypeptide	3.265	3.1	1.2
M57230	interleukin 6 signal transducer	0.793	3.1	1.0
U78027	galactosidase_ alpha	3.519	3.1	1.1

Accession Number[a]	Polynucleotide Gene Function	Control: Media only Intensity	Ratio: LPS/control	Ratio: LPS+ ID 27/control
AK000264	Unknown	2.533	3.0	0.6
X80692	mitogen-activated protein kinase 6	2.463	2.9	1.3
L25931	lamin B receptor	2.186	2.7	0.7
X13334	CD14 antigen	0.393	2.5	1.1
M32315	tumor necrosis factor receptor superfamily member 1B	0.639	2.4	0.4
NM_004862	LPS-induced TNF-alpha factor	6.077	2.3	1.1
AL050337	interferon gamma receptor 1	2.064	2.1	1.0

[a]All Accession Numbers in Table 1 through Table 64 refer to GenBank Accession Numbers.

[0071] In Table 2, the cationic peptides at a concentration of 50 μg/ml were shown to potently reduce the expression of many of the polynucleotides up-regulated by 100 ng/ml *E. coli* O111:B4 LPS as studied by polynucleotide microarrays. Peptide and LPS or LPS alone was incubated with the A549 cells for 4 h and the RNA was isolated. 5 μg total RNA was used to make Cy3/Cy5 labeled cDNA probes and hybridized onto Human Operon arrays (PRHU04). The intensity of unstimulated cells is shown in the third column of Table 2. The "Ratio: LPS/control" column refers to the intensity of polynucleotide expression in LPS-simulated cells divided by in the intensity of unstimulated cells. The other columns refer to the intensity of

polynucleotide expression in cells stimulated with LPS and peptide divided by unstimulated cells.

[0072] Table 2: Human A549 Epithelial Cell Polynucleotide Expression up-regulated by E.coli O111:B4 LPS and reduced by Cationic Peptides

Accession Number	Gene	Control: Media only Intensity	Ratio: LPS/ control	Ratio: LPS+ ID 27/ control	Ratio: LPS+ID 16/ control	Ratio: LPS+ID 22/ control
AL031983	Unknown	0.03	302.8	5.06	6.91	0.31
L04510	ADP-ribosylation factor	0.66	213.6	1.4	2.44	3.79
D87451	ring finger protein	3.90	183.7	2.1	3.68	4.28
AK000869	hypothetical protein	0.14	120.1	2.34	2.57	2.58
U78166	Ric like	0.05	91.7	0.20	16.88	21.37
X03066	MHC class II DO beta	0.06	36.5	4.90	12.13	0.98
AK001904	hypothetical protein	0.03	32.8	5.93	0.37	0.37
AB037722	Unknown	0.03	21.4	0.30	0.30	2.36
AK001589	hypothetical protein	0.65	19.2	1.26	0.02	0.43
AL137376	Unknown	1.88	17.3	0.64	1.30	1.35
L19185	thioredoxin-dependent peroxide reductase 1	0.06	16.3	0.18	2.15	0.18
J05068	Transcobalamin I	0.04	15.9	1.78	4.34	0.83
AB007856	FEM-1-like death receptor binding	2.63	15.7	0.62	3.38	0.96

Accession Number	Gene	Control: Media only Intensity	Ratio: LPS/ control	Ratio: LPS+ ID 27/ control	Ratio: LPS+ID 16/ control	Ratio: LPS+ID 22/ control
	protein					
AK000353	cytosolic ovarian carcinoma ag 1	0.45	13.5	1.02	1.73	2.33
X16940	smooth muscle enteric actin γ2	0.21	11.8	3.24	0.05	2.26
M54915	pim-1 oncogene	1.40	11.4	0.63	1.25	1.83
AL122111	hypothetical protein	0.37	10.9	0.21	1.35	0.03
M95678	phospholipase C beta 2	0.22	7.2	2.38	0.05	1.33
AK001239	hypothetical protein	2.20	6.4	1.27	1.89	2.25
AC004849	Unknown	0.14	6.3	0.07	2.70	0.07
X06614	retinoic acid receptor_ alpha	1.92	5.5	0.77	1.43	1.03
AB007896	putative L-type neutral amino acid transporter	0.94	5.3	1.82	2.15	2.41
AB010894	BAI1-associated protein	0.69	5.0	1.38	1.03	1.80
U52522	partner of RAC1	1.98	2.9	1.35	0.48	1.38
AK001440	hypothetical protein	1.02	2.7	0.43	1.20	0.01
NM_001148	ankyrin 2_ neuronal	0.26	2.5	0.82	0.04	0.66
X07173	inter-alpha inhibitor H2	0.33	2.2	0.44	0.03	0.51
AF095687	brain and nasopharyngeal carcinoma susceptibility protein	0.39	2.1	0.48	0.03	0.98

Accession Number	Gene	Control: Media only Intensity	Ratio: LPS/ control	Ratio: LPS+ ID 27/ control	Ratio: LPS+ID 16/ control	Ratio: LPS+ID 22/ control
NM_016382	NK cell activation inducing ligand NAIL	0.27	2.1	0.81	0.59	0.04
AB023198	KIAA0981 protein	0.39	2.0	0.43	0.81	0.92

EXAMPLE 2

NEUTRALIZATION OF THE STIMULATION OF IMMUNE CELLS

[0073] The ability of compounds to neutralize the stimulation of immune cells by both Gram-negative and Gram-positive bacterial products was tested. Bacterial products stimulate cells of the immune system to produce inflammatory cytokines and when unchecked this can lead to sepsis. Initial experiments utilized the murine macrophage cell line RAW 264.7, which was obtained from the American Type Culture Collection, (Manassas, VA), the human epithelial cell line, A549, and primary macrophages derived from the bone marrow of BALB/c mice (Charles River Laboratories, Wilmington, MA). The cells from mouse bone marrow were cultured in 150-mm plates in Dulbecco's modified Eagle medium (DMEM; Life Technologies, Burlington, ON) supplemented with 20 % FBS (Sigma Chemical Co,St. Louis, MO) and 20 % L cell-conditioned medium as a source of M-CSF. Once macrophages were 60–80 % confluent, they were deprived of L cell-conditioned medium for 14–16 h to render the cells quiescent and then were subjected to treatments with 100 ng/ml LPS or 100 ng/ml LPS + 20 μg/ml peptide for 24 hours. The release of cytokines into the culture supernatant was determined by ELISA (R&D Systems, Minneapolis, MN). The cell lines, RAW 264.7 and A549, were maintained in DMEM supplemented with 10 % fetal calf serum. RAW 264.7 cells were seeded in 24 well plates at a density of 10^6 cells per well in DMEM and A549 cells were seeded in 24 well plates at a density of 10^5 cells per well in DMEM and both were incubated at 37°C in 5 % CO_2 overnight. DMEM was aspirated from cells grown overnight and replaced with fresh

medium. In some experiments, blood from volunteer human donors was collected (according to procedures accepted by UBC Clinical Research Ethics Board, certificate C00-0537) by venipuncture into tubes (Becton Dickinson, Franklin Lakes, NJ) containing 14.3 USP units heparin/ml blood. The blood was mixed with LPS with or without peptide in polypropylene tubes at 37°C for 6 h. The samples were centrifuged for 5 min at 2000 x g, the plasma was collected and then stored at –20°C until being analyzed for IL-8 by ELISA (R&D Systems). In the experiments with cells, LPS or other bacterial products were incubated with the cells for 6-24 hr at 37°C in 5 % $C0_2$. *S. typhimurium* LPS and *E. coli* 0111:B4 LPS were purchased from Sigma. Lipoteichoic acid (LTA) from *S. aureus* (Sigma) was resuspended in endotoxin free water (Sigma). The Limulus amoebocyte lysate assay (Sigma) was performed on LTA preparations to confirm that lots were not significantly contaminated by endotoxin. Endotoxin contamination was less than 1 ng/ml, a concentration that did not cause significant cytokine production in the RAW 264.7 cells. Non-capped lipoarabinomannan (AraLAM) was a gift from Dr. John T. Belisle of Colorado State University. The AraLAM from *Mycobacterium* was filter sterilized and the endotoxin contamination was found to be 3.75 ng per 1.0 mg of LAM as determined by Limulus Amebocyte assay. At the same time as LPS addition (or later where specifically described), cationic peptides were added at a range of concentrations. The supernatants were removed and tested for cytokine production by ELISA (R&D Systems). All assays were performed at least three times with similar results. To confirm the anti-sepsis activity *in vivo*, sepsis was induced by intraperitoneal injection of 2 or 3 μg of *E. coli* O111:B4 LPS in phosphate-buffered saline (PBS; pH 7.2) into galactosamine-sensitized 8- to 10- week-old female CD-1 or BALB/c mice. In experiments involving peptides, 200 μg in 100μl of sterile water was injected at separate intraperitoneal sites within 10 min of LPS injection. In other experiments, CD-1 mice were injected with 400 μg *E. coli* O111:B4 LPS and 10 min later peptide (200 μg) was introduced by intraperitoneal injection. Survival was monitored for 48 hours post injection.

[0074] Hyperproduction of TNF-α has been classically linked to development of sepsis. The three types of LPS, LTA or AraLAM used in this example represented

products released by both Gram-negative and Gram-positive bacteria. Peptide, SEQ ID NO: 1, was able to significantly reduce TNF-α production stimulated by *S. typhimurium, B. cepacia*, and *E. coli* O111:B4 LPS, with the former being affected to a somewhat lesser extent (Table 3). At concentrations as low as 1 μg/ml of peptide (0.25 nM) substantial reduction of TNF-α production was observed in the latter two cases. A different peptide, SEQ ID NO: 3 did not reduce LPS-induced production of TNF-α in RAW macrophage cells, demonstrating that this is not a uniform and predictable property of cationic peptides. Representative peptides from each Formula were also tested for their ability to affect TNF-α production stimulated by *E. coli* O111:B4 LPS (Table 4). The peptides had a varied ability to reduce TNF-α production although many of them lowered TNF-α by at least 60%.

[0075] At certain concentrations peptides SEQ ID NO: 1 and SEQ ID NO: 2, could also reduce the ability of bacterial products to stimulate the production of IL-8 by an epithelial cell line. LPS is a known potent stimulus of IL-8 production by epithelial cells. Peptides, at low concentrations (1-20 μg/ml), neutralized the IL-8 induction responses of epithelial cells to LPS (Table 5-7). Peptide SEQ ID 2 also inhibited LPS-induced production of IL-8 in whole human blood (Table 4). Conversely, high concentrations of peptide SEQ ID NO: 1 (50 to 100 μg/ml) actually resulted in increased levels of IL-8 (Table 5). This suggests that the peptides have different effects at different concentrations.

[0076] The effect of peptides on inflammatory stimuli was also demonstrated in primary murine cells, in that peptide SEQ ID NO: 1 significantly reduced TNF-α production (>90 %) by bone marrow-derived macrophages from BALB/c mice that had been stimulated with 100 ng/ml *E. coli* 0111:B4 LPS (Table 8). These experiments were performed in the presence of serum, which contains LPS-binding protein (LBP), a protein that can mediate the rapid binding of LPS to CD14. Delayed addition of SEQ ID NO: 1 to the supernatants of macrophages one hour after stimulation with 100 ng/ml *E. coli* LPS still resulted in substantial reduction (70 %) of TNF-α production (Table 9).

[0077] Consistent with the ability of SEQ ID NO: 1 to prevent LPS-induced production of TNF-α *in vitro*, certain peptides also protected mice against lethal shock induced by high concentrations of LPS. In some experiments, CD-1 mice were sensitized to LPS with a prior injection of galactosamine. Galactosamine-sensitized mice that were injected with 3 μg of *E. coli* 0111:B4 LPS were all killed within 4-6 hours. When 200 μg of SEQ ID NO: 1 was injected 15 min after the LPS, 50 % of the mice survived (Table 10). In other experiments when a higher concentration of LPS was injected into BALB/c mice with no D-galactosamine, peptide protected 100 % compared to the control group in which there was no survival (Table 13). Selected other peptides were also found to be protective in these models (Tables 11,12).

[0078] Cationic peptides were also able to lower the stimulation of macrophages by Gram-positive bacterial products such as *Mycobacterium* non-capped lipoarabinomannan (AraLAM) and *S. aureus* LTA. For example, SEQ ID NO: 1 inhibited induction of TNF-α in RAW 264.7 cells by the Gram-positive bacterial products, LTA (Table 14) and to a lesser extent AraLAM (Table 15). Another peptide, SEQ ID NO: 2, was also found to reduce LTA-induced TNF-α production by RAW 264.7 cells. At a concentration of 1 μg/ml SEQ ID NO: 1 was able to substantially reduce (>75 %) the induction of TNF-α production by 1 μg/ml *S. aureus* LTA. At 20 μg/ml SEQ ID NO: 1, there was >60 % inhibition of AraLAM induced TNF-α. Polymyxin B (PMB) was included as a control to demonstrate that contaminating endotoxin was not a significant factor in the inhibition by SEQ ID NO: 1 of AraLAM induced TNF-α. These results demonstrate that cationic peptides can reduce the pro-inflammatory cytokine response of the immune system to bacterial products.

[0079] Table 3: Reduction by SEQ ID 1 of LPS induced TNF-α production in RAW 264.7 cells. RAW 264.7 mouse macrophage cells were stimulated with 100 ng/ml *S. typhimurium* LPS, 100 ng/ml *B. cepacia* LPS and 100 ng/ml *E. coli* 0111:B4 LPS in the presence of the indicated concentrations of SEQ ID 1 for 6 hr. The concentrations of TNF-α released into the culture supernatants were determined by ELISA. 100 % represents the amount of TNF-α resulting from RAW 264.7 cells

incubated with LPS alone for 6 hours (*S. typhimurium* LPS = 34.5 ± 3.2 ng/ml, *B. cepacia* LPS = 11.6 ± 2.9 ng/ml, and *E. coli* 0111:B4 LPS = 30.8 ± 2.4 ng/ml). Background levels of TNF-α production by the RAW 264.7 cells cultured with no stimuli for 6 hours resulted in TNF-α levels ranging from 0.037 – 0.192 ng/ml. The data is from duplicate samples and presented as the mean of three experiments + standard error.

Amount of SEQ ID 1 (μg/ml)	**Inhibition of TNF-α (%)***		
	B. cepacia LPS	*E. coli* LPS	*S. typhimurium* LPS
0.1	8.5 ± 2.9	0.0 ± 0.6	0.0 ± 0
1	23.0 ± 11.4	36.6 ± 7.5	9.8 ± 6.6
5	55.4 ± 8	65.0 ± 3.6	31.1 ± 7.0
10	63.1 ± 8	75.0 ± 3.4	37.4 ± 7.5
20	71.7 ± 5.8	81.0 ± 3.5	58.5 ± 10.5
50	86.7 ± 4.3	92.6 ± 2.5	73.1 ± 9.1

[0080] Table 4: Reduction by Cationic Peptides of *E. coli* LPS induced TNF-α production in RAW 264.7 cells. RAW 264.7 mouse macrophage cells were stimulated with 100 ng/ml *E. coli* 0111:B4 LPS in the presence of the indicated concentrations of cationic peptides for 6 h. The concentrations of TNF-α released into the culture supernatants were determined by ELISA. Background levels of TNF-α production by the RAW 264.7 cells cultured with no stimuli for 6 hours resulted in TNF-α levels ranging from 0.037 – 0.192 ng/ml. The data is from duplicate samples and presented as the mean of three experiments + standard deviation.

Peptide (20 μg/ml)	**Inhibition of TNF-α (%)**
SEQ ID 5	65.6 ± 1.6
SEQ ID 6	59.8 ± 1.2
SEQ ID 7	50.6 ± 0.6
SEQ ID 8	39.3 ± 1.9

Peptide (20 μg/ml)	Inhibition of TNF-α (%)
SEQ ID 9	58.7 ± 0.8
SEQ ID 10	55.5 ± 0.52
SEQ ID 12	52.1 ± 0.38
SEQ ID 13	62.4 ± 0.85
SEQ ID 14	50.8 ± 1.67
SEQ ID 15	69.4 ± 0.84
SEQ ID 16	37.5 ± 0.66
SEQ ID 17	28.3 ± 3.71
SEQ ID 19	69.9 ± 0.09
SEQ ID 20	66.1 ± 0.78
SEQ ID 21	67.8 ± 0.6
SEQ ID 22	73.3 ± 0.36
SEQ ID 23	83.6 ± 0.32
SEQ ID 24	60.5 ± 0.17
SEQ ID 26	54.9 ± 1.6
SEQ ID 27	51.1 ± 2.8
SEQ ID 28	56 ± 1.1
SEQ ID 29	58.9 ± 0.005
SEQ ID 31	60.3 ± 0.6
SEQ ID 33	62.1 ± 0.08
SEQ ID 34	53.3 ± 0.9
SEQ ID 35	60.7 ± 0.76
SEQ ID 36	63 ± 0.24
SEQ ID 37	58.9 ± 0.67
SEQ ID 38	54 ± 1
SEQ ID 40	75 ± 0.45
SEQ ID 41	86 ± 0.37
SEQ ID 42	80.5 ± 0.76
SEQ ID 43	88.2 ± 0.65
SEQ ID 44	44.9 ± 1.5

Peptide (20 μg/ml)	Inhibition of TNF-α (%)
SEQ ID 45	44.7 ± 0.39
SEQ ID 47	36.9 ± 2.2
SEQ ID 48	64 ± 0.67
SEQ ID 49	86.9 ± 0.69
SEQ ID 53	46.5 ± 1.3
SEQ ID 54	64 ± 0.73

[0081] Table 5: Reduction by SEQ ID 1 of LPS induced IL-8 production in A549 cells. A549 cells were stimulated with increasing concentrations of SEQ ID 1 in the presence of LPS (100 ng/ml *E. coli* O111:B4) for 24 hours. The concentration of IL-8 in the culture supernatants was determined by ELISA. The background levels of IL-8 from cells alone was 0.172 ± 0.029 ng/ml. The data is presented as the mean of three experiments + standard error.

SEQ ID 1 (μg/ml)	Inhibition of IL-8 (%)
0.1	1 ± 0.3
1	32 ± 10
10	60 ± 9
20	47 ± 12
50	40 ± 13
100	0

[0082] Table 6: Reduction by SEQ ID 2 of *E. coli* LPS induced IL-8 production in A549 cells. Human A549 epithelial cells were stimulated with increasing concentrations of SEQ ID 2 in the presence of LPS (100 ng/ml *E. coli* O111:B4) for 24 hours. The concentration of IL-8 in the culture supernatants was determined by ELISA. The data is presented as the mean of three experiments + standard error.

Concentration of SEQ ID 2 (μg/ml)	Inhibition of IL-8 (%)
0.1	6.8 ± 9.6
1	12.8 ± 24.5
10	29.0 ± 26.0
50	39.8 ± 1.6
100	45.0 ± 3.5

[0083] Table 7: Reduction by SEQ ID 2 of *E. coli* LPS induced IL-8 in human blood. Whole human blood was stimulated with increasing concentrations of peptide and *E.coli* O111:B4 LPS for 4 hr. The human blood samples were centrifuged and the serum was removed and tested for IL-8 by ELISA. The data is presented as the average of 2 donors.

SEQ ID 2 (μg/ml)	IL-8 (pg/ml)
0	3205
10	1912
50	1458

[0084] Table 8: Reduction by SEQ ID 1 of *E. coli* LPS induced TNF-α production in murine bone marrow macrophages. BALB/c Mouse bone marrow-derived macrophages were cultured for either 6 h or 24 h with 100 ng/ml *E. coli* 0111:B4 LPS in the presence or absence of 20 μg/ml of peptide. The supernatant was collected and tested for levels of TNF-α by ELISA. The data represents the amount of TNF-α resulting from duplicate wells of bone marrow-derived macrophages incubated with LPS alone for 6 h (1.1 ± 0.09 ng/ml) or 24 h (1.7 ± 0.2 ng/ml). Background levels of TNF-α were 0.038 ± 0.008 ng/ml for 6 h and 0.06 ± 0.012 ng/ml for 24h.

SEQ ID 1 (μg/ml)	Production of TNF-α (ng/ml)	
	6 hours	24 hours
LPS alone	1.1	1.7
1	0.02	0.048
10	0.036	0.08
100	0.033	0.044
No LPS control	0.038	0.06

[0085] Table 9: Inhibition of *E. coli* LPS-induced TNF-α production by delayed addition of SEQ ID 1 to A549 cells. Peptide (20 μg/ml) was added at increasing time points to wells already containing A549 human epithelial cells and 100 ng/ml *E. coli* 0111:B4 LPS. The supernatant was collected after 6 hours and tested for levels of TNF-α by ELISA. The data is presented as the mean of three experiments + standard error.

Time of addition of SEQ ID 1 after LPS (min)	Inhibition of TNF-α (%)
0	98.3 ± 0.3
15	89.3 ± 3.8
30	83 ± 4.6
60	68 ± 8
90	53 ± 8

[0086] Table 10: Protection against lethal endotoxaemia in galactosamine-sensitized CD-1 mice by SEQ ID 1. CD-1 mice (9 weeks-old) were sensitized to endotoxin by three intraperitoneal injections of galactosamine (20 mg in 0.1 ml sterile PBS). Then endotoxic shock was induced by intraperitoneal injection of *E. coli* 0111:B4 LPS (3 μg in 0.1 ml PBS). Peptide, SEQ ID 1, (200 μg/mouse = 8mg/kg)

was injected at a separate intraperitoneal site 15 min after injection of LPS. The mice were monitored for 48 hours and the results were recorded.

D-Galactosamine treatment	*E. coli* 0111:B4 LPS	Peptide or buffer	Total mice	Survival post endotoxin shock
0	3 μg	PBS	5	5 (100%)
20 mg	3 μg	PBS	12	0 (0%)
20 mg	3 μg	SEQ ID 1	12	6 (50%)

[0087] Table 11: Protection against lethal endotoxaemia in galactosamine-sensitized CD-1 mice by Cationic Peptides. CD-1 mice (9 weeks-old) were sensitized to endotoxin by intraperitoneal injection of galactosamine (20 mg in 0.1 ml sterile PBS). Then endotoxic shock was induced by intraperitoneal injection of *E. coli* 0111:B4 LPS (2 μg in 0.1 ml PBS). Peptide (200 μg/mouse = 8mg/kg) was injected at a separate intraperitoneal site 15 min after injection of LPS. The mice were monitored for 48 hours and the results were recorded.

Peptide Treatment	*E. coli* 0111:B4 LPS added	Number of Mice	Survival (%)
Control (no peptide)	2 μg	5	0
SEQ ID 6	2 μg	5	40
SEQ ID 13	2 μg	5	20
SEQ ID 17	2 μg	5	40
SEQ ID 24	2 μg	5	0
SEQ ID 27	2 μg	5	20

[0088] Table 12: Protection against lethal endotoxaemia in galactosamine-sensitized BALB/c mice by Cationic Peptides. BALB/c mice (8 weeks-old) were sensitized to endotoxin by intraperitoneal injection of galactosamine (20 mg in 0.1 ml sterile PBS). Then endotoxic shock was induced by intraperitoneal injection of *E. coli* 0111:B4 LPS (2 μg in 0.1 ml PBS). Peptide (200 μg/mouse = 8mg/kg) was injected at a separate intraperitoneal site 15 min after injection of LPS. The mice were monitored for 48 hours and the results were recorded.

Peptide Treatment	***E. coli* 0111:B4 LPS added**	**Number of Mice**	**Survival (%)**
No peptide	2 μg	10	10
SEQ ID 1	2 μg	6	17
SEQ ID 3	2 μg	6	0
SEQ ID 5	2 μg	6	17
SEQ ID 6	2 μg	6	17
SEQ ID 12	2 μg	6	17
SEQ ID 13	2 μg	6	33
SEQ ID 15	2 μg	6	0
SEQ ID 16	2 μg	6	0
SEQ ID 17	2 μg	6	17
SEQ ID 23	2 μg	6	0
SEQ ID 24	2 μg	6	17
SEQ ID 26	2 μg	6	0
SEQ ID 27	2 μg	6	50
SEQ ID 29	2 μg	6	0
SEQ ID 37	2 μg	6	0
SEQ ID 38	2 μg	6	0
SEQ ID 41	2 μg	6	0
SEQ ID 44	2 μg	6	0
SEQ ID 45	2 μg	6	0

[0089] Table 13: Protection against lethal endotoxaemia in BALB/c mice by SEQ ID 1. BALB/c mice were injected intraperitoneal with 400 μg *E. coli* 0111:B4 LPS. Peptide (200 μg/mouse = 8mg/kg) was injected at a separate intraperitoneal site and the mice were monitored for 48 hours and the results were recorded.

Peptide Treatment	*E. coli* 0111:B4 LPS	Number of Mice	Survival (%)
No peptide	400 μg	5	0
SEQ ID 1	400 μg	5	100

[0090] Table 14: Peptide inhibition of TNF-α production induced by *S. aureus* LTA. RAW 264.7 mouse macrophage cells were stimulated with 1 μg/ml *S. aureus* LTA in the absence and presence of increasing concentrations of peptide. The supernatant was collected and tested for levels of TNF-α by ELISA. Background levels of TNF-α production by the RAW 264.7 cells cultured with no stimuli for 6 hours resulted in TNF-α levels ranging from 0.037 – 0.192 ng/ml. The data is presented as the mean of three or more experiments + standard error.

SEQ ID 1 added (μg/ml)	Inhibition of TNF-α (%)
0.1	44.5 ± 12.5
1	76.7 ± 6.4
5	91 ± 1
10	94.5 ± 1.5
20	96 ± 1

[0091] Table 15: Peptide inhibition of TNF-α production induced by *Mycobacterium* non-capped lipoarabinomannan. RAW 264.7 mouse macrophage cells were stimulated with 1 μg/ml AraLAM in the absence and presence of 20 μg/ml peptide or Polymyxin B. The supernatant was collected and tested for levels of TNF-α by ELISA. Background levels of TNF-α production by the RAW 264.7 cells

cultured with no stimuli for 6 hours resulted in TNF-α levels ranging from 0.037 – 0.192 ng/ml. The data is presented as the mean inhibition of three or more experiments + standard error.

Peptide (20 μg/ml)	Inhibition of TNF-α (%)
No peptide	0
SEQ ID 1	64 ± 5.9
Polymyxin B	15 ± 2

EXAMPLE 3

ASSESSMENT OF TOXICITY OF THE CATIONIC PEPTIDES

[0092] The potential toxicity of the peptides was measured in two ways. First, the Cytotoxicity Detection Kit (Roche) (Lactate dehydrogenase -LDH) Assay was used. It is a colorimetric assay for the quantification of cell death and cell lysis, based on the measurement of LDH activity released from the cytosol of damaged cells into the supernatant. LDH is a stable cytoplasmic enzyme present in all cells and it is released into the cell culture supernatant upon damage of the plasma membrane. An increase in the amount of dead or plasma membrane-damaged cells results in an increase of the LDH enzyme activity in the culture supernatant as measured with an ELISA plate reader, OD_{490}nm (the amount of color formed in the assay is proportional to the number of lysed cells). In this assay, human bronchial epithelial cells (16HBEo14, HBE) cells were incubated with 100 μg of peptide for 24 hours, the supernatant removed and tested for LDH. The other assay used to measure toxicity of the cationic peptides was the WST-1 assay (Roche). This assay is a colorimetric assay for the quantification of cell proliferation and cell viability, based on the cleavage of the tetrazolium salt WST-1 by mitochondrial dehydrogenases in viable cells (a non-radioactive alternative to the [^{3}H]-thymidine incorporation assay). In this assay, HBE cells were incubated with 100 μg of peptide for 24 hours, and then 10 μl/well Cell Proliferation Reagent WST-1 was added. The cells are incubated with the reagent and the plate is then measured with an ELISA plate reader, OD_{490}nm.

[0093] The results shown below in Tables 16 and 17 demonstrate that most of the peptides are not toxic to the cells tested. However, four of the peptides from Formula F (SEQ ID NOS: 40, 41, 42 and 43) did induce membrane damage as measured by both assays.

[0094] Table 16: Toxicity of the Cationic Peptides as Measured by the LDH Release Assay. Human HBE bronchial epithelial cells were incubated with 100 μg/ml peptide or Polymyxin B for 24 hours. LDH activity was assayed in the supernatant of the cell cultures. As a control for 100% LDH release, Triton X-100 was added. The data is presented as the mean ± standard deviation. Only peptides SEQ ID 40,41,42 and 43 showed any significant toxicity.

Treatment	LDH Release (OD_{490} nm)
No cells Control	0.6 ± 0.1
Triton X-100 Control	4.6 ± 0.1
No peptide control	1.0 ± 0.05
SEQ ID 1	1.18 ± 0.05
SEQ ID 3	1.05 ± 0.04
SEQ ID 6	0.97 ± 0.02
SEQ ID 7	1.01 ± 0.04
SEQ ID 9	1.6 ± 0.03
SEQ ID 10	1.04 ± 0.04
SEQ ID 13	0.93 ± 0.06
SEQ ID 14	0.99 ± 0.05
SEQ ID 16	0.91 ± 0.04
SEQ ID 17	0.94 ± 0.04
SEQ ID 19	1.08 ± 0.02
SEQ ID 20	1.05 ± 0.03
SEQ ID 21	1.06 ± 0.04
SEQ ID 22	1.29 ± 0.12
SEQ ID 23	1.26 ± 0.46
SEQ ID 24	1.05 ± 0.01

Treatment	LDH Release (OD_{490} nm)
SEQ ID 26	0.93 ± 0.04
SEQ ID 27	0.91 ± 0.04
SEQ ID 28	0.96 ± 0.06
SEQ ID 29	0.99 ± 0.02
SEQ ID 31	0.98 ± 0.03
SEQ ID 33	1.03 ± 0.05
SEQ ID 34	1.02 ± 0.03
SEQ ID 35	0.88 ± 0.03
SEQ ID 36	0.85 ± 0.04
SEQ ID 37	0.96± 0.04
SEQ ID 38	0.95± 0.02
SEQ ID 40	2.8 ± 0.5
SEQ ID 41	3.3 ± 0.2
SEQ ID 42	3.4 ± 0.2
SEQ ID 43	4.3 ± 0.2
SEQ ID 44	0.97 ± 0.03
SEQ ID 45	0.98 ± 0.04
SEQ ID 47	1.05 ± 0.05
SEQ ID 48	0.95 ± 0.05
SEQ ID 53	1.03 ± 0.06
Polymyxin B	1.21 ± 0.03

[0095] Table 17: Toxicity of the Cationic Peptides as Measured by the WST-1 Assay. HBE cells were incubated with 100 μg/ml peptide or Polymyxin B for 24 hours and cell viability was tested. The data is presented as the mean ± standard deviation. As a control for 100% LDH release, Triton X-100 was added. Only peptides SEQ ID 40,41,42 and 43 showed any significant toxicity.

Treatment	OD_{490} nm
No cells Control	0.24 ± 0.01
Triton X-100 Control	0.26 ± 0.01
No peptide control	1.63 ± 0.16
SEQ ID 1	1.62 ± 0.34
SEQ ID 3	1.35 ± 0.12
SEQ ID 10	1.22 ± 0.05
SEQ ID 6	1.81 ± 0.05
SEQ ID 7	1.78 ± 0.10
SEQ ID 9	1.69 ± 0.29
SEQ ID 13	1.23 ± 0.11
SEQ ID 14	1.25 ± 0.02
SEQ ID 16	1.39 ± 0.26
SEQ ID 17	1.60 ± 0.46
SEQ ID 19	1.42 ± 0.15
SEQ ID 20	1.61 ± 0.21
SEQ ID 21	1.28 ± 0.07
SEQ ID 22	1.33 ± 0.07
SEQ ID 23	1.14 ± 0.24
SEQ ID 24	1.27 ± 0.16
SEQ ID 26	1.42 ± 0.11
SEQ ID 27	1.63 ± 0.03
SEQ ID 28	1.69 ± 0.03
SEQ ID 29	1.75 ± 0.09
SEQ ID 31	1.84 ± 0.06
SEQ ID 33	1.75 ± 0.21
SEQ ID 34	0.96 ± 0.05
SEQ ID 35	1.00 ± 0.08
SEQ ID 36	1.58 ± 0.05
SEQ ID 37	1.67 ± 0.02
SEQ ID 38	1.83 ± 0.03

Treatment	OD_{490} nm
SEQ ID 40	0.46 ± 0.06
SEQ ID 41	0.40 ± 0.01
SEQ ID 42	0.39 ± 0.08
SEQ ID 43	0.46 ± 0.10
SEQ ID 44	1.49 ± 0.39
SEQ ID 45	1.54 ± 0.35
SEQ ID 47	1.14 ± 0.23
SEQ ID 48	0.93 ± 0.08
SEQ ID 53	1.51 ± 0.37
Polymyxin B	1.30 ± 0.13

EXAMPLE 4

POLYNUCLEOTIDE REGULATION BY CATIONIC PEPTIDES

[0096] Polynucleotide arrays were utilized to determine the effect of cationic peptides by themselves on the transcriptional response of macrophages and epithelial cells. Mouse macrophage RAW 264.7, Human Bronchial cells (HBE), or A549 human epithelial cells were plated in 150 mm tissue culture dishes at 5.6 x 10^6 cells/dish, cultured overnight and then incubated with 50 μg/ml peptide or medium alone for 4 h. After stimulation, the cells were washed once with diethyl pyrocarbonate-treated PBS, and detached from the dish using a cell scraper. Total RNA was isolated using Trizol (Gibco Life Technologies). The RNA pellet was resuspended in RNase-free water containing RNase inhibitor (Ambion, Austin, TX). The RNA was treated with DNaseI (Clontech, Palo Alto, CA) for 1 h at 37°C. After adding termination mix (0.1 M EDTA [pH 8.0], 1 mg/ml glycogen), the samples were extracted once with phenol: chloroform: isoamyl alcohol (25:24:1), and once with chloroform. The RNA was then precipitated by adding 2.5 volumes of 100% ethanol and $1/10^{th}$ volume sodium acetate, pH 5.2. The RNA was resuspended in RNase-free water with RNase inhibitor (Ambion) and stored at -70°C. The quality of the RNA was assessed by gel electrophoresis on a 1% agarose gel. Lack of genomic DNA contamination was assessed by using the isolated RNA as a template for PCR amplification with β-actin-

specific primers (5'-GTCCCTGTATGCCTCTGGTC-3' (SEQ ID NO: 55) and 5'-GATGTCACGCACGATTTCC-3' (SEQ ID NO: 56)). Agarose gel electrophoresis and ethidium bromide staining confirmed the absence of an amplicon after 35 cycles.

[0097] Atlas cDNA Expression Arrays (Clontech, Palo Alto, CA), which consist of 588 selected mouse cDNAs spotted in duplicate on positively charged membranes were used for early polynucleotide array studies (Tables 18,19). ^{32}P-radiolabeled cDNA probes prepared from 5 μg total RNA were incubated with the arrays overnight at 71°C. The filters were washed extensively and then exposed to a phosphoimager screen (Molecular Dynamics, Sunnyvale, CA) for 3 days at 4°C. The image was captured using a Molecular Dynamics PSI phosphoimager. The hybridization signals were analyzed using AtlasImage 1.0 Image Analysis software (Clontech) and Excel (Microsoft, Redmond, WA). The intensities for each spot were corrected for background levels and normalized for differences in probe labeling using the average values for 5 polynucleotides observed to vary little between the stimulation conditions: β-actin, ubiquitin, ribosomal protein S29, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and Ca^{2+} binding protein. When the normalized hybridization intensity for a given cDNA was less than 20, it was assigned a value of 20 to calculate the ratios and relative expression.

[0098] The next polynucleotide arrays used (Tables 21-26) were the Resgen Human cDNA arrays (identification number for the genome is PRHU03-S3), which consist of 7,458 human cDNAs spotted in duplicate. Probes were prepared from 15-20 μg of total RNA and labeled with Cy3 labeled dUTP. The probes were purified and hybridized to printed glass slides overnight at 42°C and washed. After washing, the image was captured using a Virtek slide reader. The image processing software (Imagene 4.1, Marina Del Rey, CA) determines the spot mean intensity, median intensities, and background intensities. Normalization and analysis was performed with Genespring software (Redwood City, CA). Intensity values were calculated by subtracting the mean background intensity from the mean intensity value determined by Imagene. The intensities for each spot were normalized by taking the median spot intensity value from the population of spot values within a slide and comparing this

value to the values of all slides in the experiment. The relative changes seen with cells treated with peptide compared to control cells can be found in the Tables below.

[0099] The other polynucleotide arrays used (Tables 27-35) were the Human Operon arrays (identification number for the genome is PRHU04-S1), which consist of about 14,000 human oligos spotted in duplicate. Probes were prepared from 10 μg of total RNA and labeled with Cy3 or Cy5 labeled dUTP. In these experiments, A549 epithelial cells were plated in 100 mm tissue culture dishes at 2.5 x 10^6 cells/dish. Total RNA was isolated using RNAqueous (Ambion). DNA contamination was removed with DNA-free kit (Ambion). The probes prepared from total RNA were purified and hybridized to printed glass slides overnight at 42ºC and washed. After washing, the image was captured using a Perkin Elmer array scanner. The image processing software (Imagene 5.0, Marina Del Rey, CA) determines the spot mean intensity, median intensities, and background intensities. An "in house" program was used to remove background. The program calculates the bottom 10% intensity for each subgrid and subtracts this for each grid. Analysis was performed with Genespring software (Redwood City, CA). The intensities for each spot were normalized by taking the median spot intensity value from the population of spot values within a slide and comparing this value to the values of all slides in the experiment. The relative changes seen with cells treated with peptide compared to control cells can be found in the Tables below.

[00100] Semi-quantitative RT-PCR was performed to confirm polynucleotide array results. 1 μg RNA samples were incubated with 1 μl oligodT (500 μg/ml) and 1 μl mixed dNTP stock at 1 mM, in a 12 μl volume with DEPC treated water at 65°C for 5 min in a thermocycler. 4 μl 5X First Strand buffer, 2 μl 0.1M DTT, and 1 μl RNaseOUT recombinant ribonuclease inhibitor (40 units/μl) were added and incubated at 42 °C for 2 min, followed by the addition of 1 μl (200 units) of Superscript II (Invitrogen, Burlington, ON). Negative controls for each RNA source were generated using parallel reactions in the absence of Superscript II. cDNAs were amplified in the presence of 5' and 3' primers (1.0 μM), 0.2 mM dNTP mixture, 1.5 mM MgCl, 1 U of *Taq* DNA polymerase (New England Biolabs, Missisauga, ON), and 1X PCR buffer. Each PCR was performed with a thermal cycler by using 30-40

cycles consisting of 30s of denaturation at 94 °C, 30s of annealing at either 52 °C or 55 °C and 40s of extension at 72 °C. The number of cycles of PCR was optimized to lie in the linear phase of the reaction for each primer and set of RNA samples. A housekeeping polynucleotide β-actin was amplified in each experiment to evaluate extraction procedure and to estimate the amount of RNA. The reaction product was visualized by electrophoresis and analyzed by densitometry, with relative starting RNA concentrations calculated with reference to β-actin amplification.

[00101] Table 18 demonstrates that SEQ ID NO: 1 treatment of RAW 264.7 cells up-regulated the expression of more than 30 different polynucleotides on small Atlas microarrays with selected known polynucleotides. The polynucleotides up-regulated by peptide, SEQ ID NO: 1, were mainly from two categories: one that includes receptors (growth, chemokine, interleukin, interferon, hormone, neurotransmitter), cell surface antigens and cell adhesion and another one that includes cell-cell communication (growth factors, cytokines, chemokines, interleukin, interferons, hormones), cytoskeleton, motility, and protein turnover. The specific polynucleotides up-regulated included those encoding chemokine MCP-3, the anti-inflammatory cytokine IL-10, macrophage colony stimulating factor, and receptors such as IL-1R-2 (a putative antagonist of productive IL-1 binding to IL-1R1), PDGF receptor B, NOTCH4, LIF receptor, LFA-1, TGFβ receptor 1, G-CSF receptor, and IFNγ receptor. The peptide also up-regulated polynucleotides encoding several metalloproteinases, and inhibitors thereof, including the bone morphogenetic proteins BMP-1, BMP-2, BMP-8a, TIMP2 and TIMP3. As well, the peptide up-regulated specific transcription factors, including JunD, and the YY and LIM-1 transcription factors, and kinases such as Etk1 and Csk demonstrating its widespread effects. It was also discovered from the polynucleotide array studies that SEQ ID NO: 1 down-regulated at least 20 polynucleotides in RAW 264.7 macrophage cells (Table 19). The polynucleotides down-regulated by peptide included DNA repair proteins and several inflammatory mediators such as MIP-1α, oncostatin M and IL-12. A number of the effects of peptide on polynucleotide expression were confirmed by RT-PCR (Table 20). The peptides, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 19, and SEQ ID NO: 1, and representative peptides from each of the formulas also altered the

transcriptional responses in a human epithelial cell line using mid-sized microarrays (7835 polynucleotides). The effect of SEQ ID NO: 1 on polynucleotide expression was compared in 2 human epithelial cell lines, A549 and HBE. Polynucleotides related to the host immune response that were up-regulated by 2 peptides or more by a ratio of 2-fold more than unstimulated cells are described in Table 21. Polynucleotides that were down-regulated by 2 peptides or more by a ratio of 2-fold more than unstimulated cells are described in Table 22. In Table 23 and Table 24, the human epithelial pro-inflammatory polynucleotides that are up- and down-regulated respectively are shown. In Table 25 and Table 26 the anti-inflammatory polynucleotides affected by cationic peptides are shown. The trend becomes clear that the cationic peptides up-regulate the anti-inflammatory response and down-regulate the pro-inflammatory response. It was very difficult to find a polynucleotide related to the anti-inflammatory response that was down-regulated (Table 26). The pro-inflammatory polynucleotides upregulated by cationic peptides were mainly polynucleotides related to migration and adhesion. Of the down-regulated pro-inflammatory polynucleotides, it should be noted that all the cationic peptides affected several toll-like receptor (TLR) polynucleotides, which are very important in signaling the host response to infectious agents. An important anti-inflammatory polynucleotide that was up-regulated by all the peptides is the IL-10 receptor. IL-10 is an important cytokine involved in regulating the pro-inflammatory cytokines. These polynucleotide expression effects were also observed using primary human macrophages as observed for peptide SEQ ID NO: 6 in Tables 27 and 28. The effect of representative peptides from each of the formulas on human epithelial cell expression of selected polynucleotides (out of 14,000 examined) is shown in Tables 31-37 below. At least 6 peptides from each formula were tested for their ability to alter human epithelial polynucleotide expression and indeed they had a wide range of stimulatory effects. In each of the formulas there were at least 50 polynucleotides commonly up-regulated by each of the peptides in the group.

[00102] Table 18: Polynucleotides up-regulated by peptide, SEQ ID NO: 1, treatment of RAW macrophage cells[a]. The cationic peptides at a concentration of 50 μg/ml were shown to potently induce the expression of several polynucleotides. Peptide was incubated with the RAW cells for 4 h and the RNA was isolated,

converted into labeled cDNA probes and hybridized to Atlas arrays. The intensity of unstimulated cells is shown in the third column. The "Ratio Peptide: Unstimulated" column refers to the intensity of polynucleotide expression in peptide-simulated cells divided by the intensity of unstimulated cells.

[00103] The changes in the normalized intensities of the housekeeping polynucleotides ranged from 0.8-1.2 fold, validating the use of these polynucleotides for normalization. When the normalized hybridization intensity for a given cDNA was less than 20, it was assigned a value of 20 to calculate the ratios and relative expression. The array experiments were repeated 3 times with different RNA preparations and the average fold change is shown above. Polynucleotides with a two fold or greater change in relative expression levels are presented.

Polynucleotide / Protein	**Polynucleotide Function**	**Unstimulated Intensity**	**Ratio peptide: Unstimulated[b]**	**Accession Number**
Etk1	Tyrosine-protein kinase receptor	20	43	M68513
PDGFRB	Growth factor receptor	24	25	X04367
	Corticotropin releasing factor receptor	20	23	X72305
NOTCH4	proto-oncopolynucleotide	48	18	M80456
IL-1R2	Interleukin receptor	20	16	X59769
MCP-3	Chemokine	56	14	S71251
BMP-1	Bone morpho-polynucleotidetic protein	20	14	L24755
Endothelin b receptor	Receptor	20	14	U32329
c-ret	Oncopolynucleotide precursor	20	13	X67812
LIFR	Cytokine receptor	20	12	D26177

Polynucleotide / Protein	Polynucleotide Function	Unstimulated Intensity	Ratio peptide: Unstimulated[b]	Accession Number
BMP-8a	Bone morpho-polynucleotidetic protein	20	12	M97017
Zfp92	Zinc finger protein 92	87	11	U47104
MCSF	Macrophage colony stimulating factor 1	85	11	X05010
GCSFR	Granulocyte colony-stimulating factor receptor	20	11	M58288
IL-8RB	Chemokine receptor	112	10	D17630
IL-9R	Interleukin receptor	112	6	M84746
Cas	Crk-associated substrate	31	6	U48853
p58/GTA	Kinase	254	5	M58633
CASP2	Caspase precursor	129	5	D28492
IL-1β precursor	Interleukin precursor	91	5	M15131
SPI2-2	Serine protease inhibitor	62	5	M64086
C5AR	Chemokine receptor	300	4	S46665
L-myc	Oncopolynucleotide	208	4	X13945
IL-10	Interleukin	168	4	M37897
p19ink4	cdk4 and cdk6 inhibitor	147	4	U19597
ATOH2	Atonal homolog 2	113	4	U29086
DNAse1	DNase	87	4	U00478
CXCR-4	Chemokine receptor	36	4	D87747
Cyclin D3	Cyclin	327	3	U43844
IL-7Rα	Interleukin receptor	317	3	M29697
POLA	DNA polymerase$_{\alpha}$	241	3	D17384
Tie-2	Oncopolynucleotide	193	3	S67051

Polynucleotide / Protein	Polynucleotide Function	Unstimulated Intensity	Ratio peptide: Unstimulated[b]	Accession Number
DNL1	DNA ligase I	140	3	U04674
BAD	Apoptosis protein	122	3	L37296
GADD45	DNA-damage-inducible protein	88	3	L28177
Sik	Src-related kinase	82	3	U16805
integrin$_\alpha$4	Integrin	2324	2	X53176
TGFβR1	Growth factor receptor	1038	2	D25540
LAMR1	Receptor	1001	2	J02870
Crk	Crk adaptor protein	853	2	S72408
ZFX	Chromosomal protein	679	2	M32309
Cyclin E1	Cylcin	671	2	X75888
POLD1	DNA polymerase subunit	649	2	Z21848
Vav	proto-oncopolynucleotide	613	2	X64361
YY (NF-E1)	Transcription factor	593	2	L13968
JunD	Transcription factor	534	2	J050205
Csk	c-src kinase	489	2	U05247
Cdk7	Cyclin-dependent kinase	475	2	U11822
MLC1A	Myosin light subunit isoform	453	2	M19436
ERBB-3	Receptor	435	2	L47240
UBF	Transcription factor	405	2	X60831
TRAIL	Apoptosis ligand	364	2	U37522
LFA-1	Cell adhesion receptor	340	2	X14951
SLAP	Src-like adaptor protein	315	2	U29056
IFNGR	Interferon gamma receptor	308	2	M28233
LIM-1	Transcription factor	295	2	Z27410
ATF2	Transcription factor	287	2	S76657

Polynucleotide / Protein	Polynucleotide Function	Unstimulated Intensity	Ratio peptide: Unstimulated[b]	Accession Number
FST	Follistatin precursor	275	2	Z29532
TIMP3	Protease inhibitor	259	2	L19622
RU49	Transcription factor	253	2	U41671
IGF-1Rα	Insulin-like growth factor receptor	218	2	U00182
Cyclin G2	Cyclin	214	2	U95826
fyn	Tyrosine-protein kinase	191	2	U70324
BMP-2	Bone morpho-polynucleotidetic protein	186	2	L25602
Brn-3.2 POU	Transcription factor	174	2	S68377
KIF1A	Kinesin family protein	169	2	D29951
MRC1	Mannose receptor	167	2	Z11974
PAI2	Protease inhibitor	154	2	X19622
BKLF	CACCC Box- binding protein	138	2	U36340
TIMP2	Protease inhibitor	136	2	X62622
Mas	Proto-oncopolynucleotide	131	2	X67735
NURR-1	Transcription factor	129	2	S53744

[00104] Table 19: Polynucleotides down-regulated by SEQ ID NO: 1 treatment of RAW macrophage cells[a]. The cationic peptides at a concentration of 50 μg/ml were shown to reduce the expression of several polynucleotides. Peptide was incubated with the RAW cells for 4 h and the RNA was isolated, converted into labeled cDNA probes and hybridized to Atlas arrays. The intensity of unstimulated cells is shown in the third column. The "Ratio Peptide: Unstimulated" column refers to the intensity of polynucleotide expression in peptide-simulated cells divided by the intensity of unstimulated cells. The array experiments were repeated 3 times with

different cells and the average fold change is shown below. Polynucleotides with an approximately two fold or greater change in relative expression levels are presented.

Polynucleotide / Protein	Polynucleotide Function	Unstimulated Intensity	Ratio peptide: Unstimulated	Accession Number
sodium channel	Voltage-gated ion channel	257	0.08	L36179
XRCC1	DNA repair protein	227	0.09	U02887
ets-2	Oncopolynucleotide	189	0.11	J04103
XPAC	DNA repair protein	485	0.12	X74351
EPOR	Receptor precursor	160	0.13	J04843
PEA 3	Ets-related protein	158	0.13	X63190
orphan receptor	Nuclear receptor	224	0.2	U11688
N-cadherin	Cell adhesion receptor	238	0.23	M31131
OCT3	Transcription factor	583	0.24	M34381
PLCβ	phospholipase	194	0.26	U43144
KRT18	Intermediate filament proteins	318	0.28	M11686
THAM	Enzyme	342	0.32	X58384
CD40L	CD40 ligand	66	0.32	X65453
CD86	T-lymphocyte antigen	195	0.36	L25606
oncostatin M	Cytokine	1127	0.39	D31942
PMS2 DNA	DNA repair protein	200	0.4	U28724
IGFBP6	Growth factor	1291	0.41	X81584
MIP-1β	Cytokine	327	0.42	M23503
ATBF1	AT motif-binding factor	83	0.43	D26046
nucleobindin	Golgi resident protein	367	0.43	M96823
bcl-x	Apoptosis protein	142	0.43	L35049
uromodulin	glycoprotein	363	0.47	L33406
IL-12 p40	Interleukin	601	0.48	M86671
MmRad52	DNA repair protein	371	0.54	Z32767

Polynucleotide / Protein	Polynucleotide Function	Unstimulated Intensity	Ratio peptide: Unstimulated	Accession Number
Tob1	Antiproliferative factor	956	0.5	D78382
Ung1	DNA repair protein	535	0.51	X99018
KRT19	Intermediate filament proteins	622	0.52	M28698
PLCγ	phospholipase	251	0.52	X95346
Integrin α_6	Cell adhesion receptor	287	0.54	X69902
GLUT1	Glucose transporter	524	0.56	M23384
CTLA4	immunoglobin superfamily	468	0.57	X05719
FRA2	Fos-related antigen	446	0.57	X83971
MTRP	Lysosome-associated protein	498	0.58	U34259

[00105] Table 20: Polynucleotide Expression changes in response to peptide, SEQ ID NO: 1, could be confirmed by RT-PCR. RAW 264.7 macrophage cells were incubated with 50 μg/ml of peptide or media only for 4 hours and total RNA isolated and subjected to semi-quantitative RT-PCR. Specific primer pairs for each polynucleotide were used for amplification of RNA. Amplification of β-actin was used as a positive control and for standardization. Densitometric analysis of RT-PCR products was used. The results refer to the relative fold change in polynucleotide expression of peptide treated cells compared to cells incubated with media alone. The data is presented as the mean ± standard error of three experiments.

Polynucleotide	Array Ratio-*	RT-PCR Ratio -*
CXCR-4	4.0 ± 1.7	4.1 ± 0.9
IL-8RB	9.5 ± 7.6	7.1 ± 1.4
MCP-3	13.5 ± 4.4	4.8 ± 0.88
IL-10	4.2 ± 2.1	16.6 ± 6.1

Polynucleotide	Array Ratio-*	RT-PCR Ratio -*
CD14	0.9 ± 0.1	0.8 ± 0.3
MIP-1B	0.42 ± 0.09	0.11 ± 0.04
XRCC1	0.12 ± 0.01	0.25 ± 0.093
MCP-1	Not on array	3.5 ± 1.4

[00106] Table 21: Polynucleotides up-regulated by peptide treatment of A549 epithelial cells[a]. The cationic peptides at concentrations of 50 μg/ml were shown to increase the expression of several polynucleotides. Peptide was incubated with the human A549 epithelial cells for 4 h and the RNA was isolated, converted into labeled cDNA probes and hybridized to Human cDNA arrays ID#PRHU03-S3. The intensity of polynucleotides in unstimulated cells is shown in the second column. The "Ratio Peptide: Unstimulated" columns refers to the intensity of polynucleotide expression in peptide-simulated cells divided by the intensity of unstimulated cells.

Polynucleotide/Protein	Unstimulated Intensity	Ratio Peptide: Unstimulated				Accession Number
		ID 2	ID 3	ID 19	ID 1	
IL-1 R antagonist homolog 1	0.00	3086	1856	870		AI167887
IL-10 R beta	0.53	2.5	1.6	1.9	3.1	AA486393
IL-11 R alpha	0.55	2.4	1.0	4.9	1.8	AA454657
IL-17 R	0.54	2.1	2.0	1.5	1.9	AW029299
TNF R superfamily, member 1B	0.28	18	3.0	15	3.6	AA150416
TNF R superfamily, member 5 (CD40LR)	33.71	3.0	0.02			H98636
TNF R superfamily, member 11b	1.00	5.3	4.50	0.8		AA194983
IL-8	0.55	3.6	17	1.8	1.1	AA102526
interleukin enhancer binding factor 2	0.75	1.3	2.3	0.8	4.6	AA894687
interleukin enhancer binding	0.41	2.7		5.3	2.5	R56553

Polynucleotide/Protein	Unstimulated Intensity	Ratio Peptide: Unstimulated				Accession Number
		ID 2	ID 3	ID 19	ID 1	
factor 1						
cytokine inducible SH2-containing protein	0.03	33	44	39	46	AA427521
IK cytokine, down-regulator of HLA II	0.50	3.1	2.0	1.7	3.3	R39227
cytokine inducible SH2-containing protein	0.03	33	44	39	46	AA427521
IK cytokine, down-regulator of HLA II	0.50	3.1	2.0	1.7	3.3	R39227
small inducible cytokine subfamily A (Cys-Cys), member 21	1.00	3.9			2.4	AI922341
TGFB inducible early growth response 2	0.90	2.4	2.1	0.9	1.1	AI473938
NK cell R	1.02	2.5	0.7	0.3	1.0	AA463248
CCR6	0.14	4.5	7.8	6.9	7.8	N57964
cell adhesion molecule	0.25	4.0	3.9	3.9	5.1	R40400
melanoma adhesion molecule	0.05	7. 9	20	43	29.1	AA497002
CD31	0.59	2.7	3.1	1.0	1.7	R22412
integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor	1.00	0.9	2.4	3.6	0.9	AA463257
integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 receptor)	0.94	0.8	2.5	1.9	1.1	AA424695
integrin, alpha E	0.01	180	120	28	81	AA425451
integrin, beta 1	0.47	2.1	2.1	7.0	2.6	W67174
integrin, beta 3	0.55	2.7	2.8	1.8	1.0	AA037229
integrin, beta 3	0.57	2.6	1.4	1.8	2.0	AA666269
integrin, beta 4	0.65	0.8	2.2	4.9	1.5	AA485668
integrin beta 4 binding protein	0.20	1.7	5.0	6.6	5.3	AI017019

Polynucleotide/Protein	Unstimulated Intensity	Ratio Peptide: Unstimulated				Accession Number
		ID 2	ID 3	ID 19	ID 1	
calcium and integrin binding protein	0.21	2.8	4.7	9.7	6.7	AA487575
disintegrin and metalloproteinase domain 8	0.46	3.1		2.2	3.8	AA279188
disintegrin and metalloproteinase domain 9	0.94	1.1	2.3	3.6	0.5	H59231
disintegrin and metalloproteinase domain 10	0.49	1.5	2.1	3.3	2.2	AA043347
disintegrin and metalloproteinase domain 23	0.44	1.9	2.3	2.5	4.6	H11006
cadherin 1, type 1, E-cadherin (epithelial)	0.42	8.1	2.2	2.4	7.3	H97778
cadherin 12, type 2 (N-cadherin 2)	0.11	13	26	9.5		AI740827
protocadherin 12	0.09	14.8	11.5	2.6	12.4	AI652584
protocadherin gamma subfamily C, 3	0.34	3.0	2.5	4.5	9.9	R89615
catenin (cadherin-associated protein), delta 1	0.86	1.2	2.2	2.4		AA025276
laminin R 1 (67kD, ribosomal protein SA)	0.50	0.4	2.0	4.4	3.0	AA629897
killer cell lectin-like receptor subfamily C, member 2	0.11	9.7	9.0	4.1	13.4	AA190627
killer cell lectin-like receptor subfamily C, member 3	1.00	3.2	1.0	0.9	1.3	W93370
killer cell lectin-like receptor subfamily G, member 1	0.95	2.3	1.7	0.7	1.1	AI433079
C-type lectin-like receptor-2	0.45	2.1	8.0	2.2	5.3	H70491
CSF 3 R	0.40	1.9	2.5	3.5	4.0	AA458507
macrophage stimulating 1 R	1.00	1.7	2.3	0.4	0.7	AA173454
BMP R type IA	0.72	1.9	2.8	0.3	1.4	W15390

Polynucleotide/Protein	Unstimulated Intensity	Ratio Peptide: Unstimulated				Accession Number
		ID 2	ID 3	ID 19	ID 1	
formyl peptide receptor 1	1.00	3.1	1.4	0.4		AA425767
CD2	1.00	2.6	0.9	1.2	0.9	AA927710
CD36	0.18	8.2	5.5	6.2	2.5	N39161
vitamin D R	0.78	2.5	1.3	1.1	1.4	AA485226
Human proteinase activated R-2	0.54	6.1	1.9	2.2		AA454652
prostaglandin E receptor 3 (subtype EP3)	0.25	4.1	4.9	3.8	4.9	AA406362
PDGF R beta polypeptide	1.03	2.5	1.0	0.5	0.8	R56211
VIP R 2	1.00	3.1			2.0	AI057229
growth factor receptor-bound protein 2	0.51	2.2	2.0	2.4	0.3	AA449831
Mouse Mammary Turmor Virus Receptor homolog	1.00	6.9		16		W93891
adenosine A2a R	0.41	3.1	1.8	4.0	2.5	N57553
adenosine A3 R	0.83	2.0	2.3	1.0	1.2	AA863086
T cell R delta locus	0.77	2.7	1.3		1.8	AA670107
prostaglandin E receptor 1 (subtype EP1)	0.65	7.2		6.0	1.5	AA972293
growth factor receptor-bound protein 14	0.34		3.0	6.3	2.9	R24266
Epstein-Barr virus induced polynucleotide 2	0.61	1.6	2.4		8.3	AA037376
complement component receptor 2	0.22	26	4.5	2.6	18.1	AA521362
endothelin receptor type A	0.07	12	14	14	16	AA450009
v-SNARE R	0.56	11	12	1.8		AA704511
tyrosine kinase, non-receptor, 1	0.12	7.8	8.5	10	8.7	AI936324
receptor tyrosine kinase-like orphan receptor 2	0.40	7.3	5.0	1.6	2.5	N94921

Polynucleotide/Protein	Unstimulated Intensity	Ratio Peptide: Unstimulated				Accession Number
		ID 2	ID 3	ID 19	ID 1	
protein tyrosine phosphatase, non-receptor type 3	1.02	1.0	13.2	0.5	0.8	AA682684
protein tyrosine phosphatase, non-receptor type 9	0.28	3.5	4.0	0.9	5.3	AA434420
protein tyrosine phosphatase, non-receptor type 11	0.42	2.9	2.4	2.2	3.0	AA995560
protein tyrosine phosphatase, non-receptor type 12	1.00	2.3	2.2	0.8	0.5	AA446259
protein tyrosine phosphatase, non-receptor type 13	0.58	1.7	2.4	3.6	1.7	AA679180
protein tyrosine phosphatase, non-receptor type 18	0.52	3.2	0.9	1.9	6.5	AI668897
protein tyrosine phosphatase, receptor type, A	0.25	4.0	2.4	16.8	12.8	H82419
protein tyrosine phosphatase, receptor type, J	0.60	3.6	3.2	1.6	1.0	AA045326
protein tyrosine phosphatase, receptor type, T	0.73	1.2	2.8	3.0	1.4	R52794
protein tyrosine phosphatase, receptor type, U	0.20	6.1	1.2	5.6	5.0	AA644448
protein tyrosine phosphatase, receptor type, C-associated protein	1.00	5.1			2.4	AA481547
phospholipase A2 receptor 1	0.45	2.8	2.2	1.9	2.2	AA086038
MAP kinase-activated protein kinase 3	0.52	2.1	2.7	1.1	1.9	W68281
MAP kinase kinase 6	0.10	18	9.6		32	H07920
MAP kinase kinase 5	1.00	3.0	5.2	0.8	0.2	W69649
MAP kinase 7	0.09		11.5	12	33	H39192
MAP kinase 12	0.49	2.1	1.7	2.2	2.0	AI936909
G protein-coupled receptor 4	0.40	3.7	3.0	2.4	2.5	AI719098

Polynucleotide/Protein	Unstimulated Intensity	Ratio Peptide: Unstimulated				Accession Number
		ID 2	ID 3	ID 19	ID 1	
G protein-coupled receptor 49	0.05		19	19	27	AA460530
G protein-coupled receptor 55	0.08	19	15	12		N58443
G protein-coupled receptor 75	0.26	5.2	3.1	7.1	3.9	H84878
G protein-coupled receptor 85	0.20	6.8	5.4	4.9	5.0	N62306
regulator of G-protein signalling 20	0.02	48	137	82		AI264190
regulator of G-protein signalling 6	0.27		3.7	8.9	10.6	R39932
BCL2-interacting killer (apoptosis-inducing)	1.00	1.9		5.2		AA291323
apoptosis inhibitor 5	0.56	2.8	1.6	2.4	1.8	AI972925
caspase 6, apoptosis-related cysteine protease	0.79	0.7	2.6	1.3	2.8	W45688
apoptosis-related protein PNAS-1	0.46	2.2	1.4	2.3	2.9	AA521316
caspase 8, apoptosis-related cysteine protease	0.95	2.2	1.0	0.6	2.0	AA448468

[00107] Table 22: Polynucleotides down-regulated by peptide treatment of A549 epithelial cells[a]. The cationic peptides at concentrations of 50 μg/ml were shown to decrease the expression of several polynucleotides. Peptide was incubated with the human A549 epithelial cells for 4 h and the RNA was isolated, converted into labeled cDNA probes and hybridized to Human cDNA arrays ID#PRHU03-S3. The intensity of polynucleotides in unstimulated cells is shown in the second column. The "Ratio Peptide: Unstimulated" columns refers to the intensity of polynucleotide expression in peptide-simulated cells divided by the intensity of unstimulated cells.

Polynucleotide/Protein	Unstimulated Intensity	Ratio Peptide: Unstimulated				Accession Number
		ID 2	ID 3	ID 19	ID 1	
TLR 1	3.22	0.35	0.31	0.14	0.19	AI339155
TLR 2	2.09	0.52	0.31	0.48	0.24	T57791
TLR 5	8.01	0.12	0.39			N41021
TLR 7	5.03	0.13	0.11	0.20	0.40	N30597
TNF receptor-associated factor 2	0.82	1.22	0.45	2.50	2.64	T55353
TNF receptor-associated factor 3	3.15	0.15		0.72	0.32	AA504259
TNF receptor superfamily, member 12	4.17	0.59	0.24		0.02	W71984
TNF R superfamily, member 17	2.62		0.38	0.55	0.34	AA987627
TRAF and TNF receptor-associated protein	1.33	0.75	0.22	0.67	0.80	AA488650
IL-1 receptor, type I	1.39	0.34	0.72	1.19	0.34	AA464526
IL-2 receptor, alpha	2.46	0.41	0.33	0.58		AA903183
IL-2 receptor, gamma (severe combined immunodeficiency)	3.34	0.30	0.24		0.48	N54821
IL-12 receptor, beta 2	4.58	0.67	0.22			AA977194
IL-18 receptor 1	1.78	0.50	0.42	0.92	0.56	AA482489
TGF beta receptor III	2.42	0.91	0.24	0.41	0.41	H62473
leukotriene b4 receptor (chemokine receptor-like 1)	1.00		1.38	4.13	0.88	AI982606
small inducible cytokine subfamily A (Cys-Cys), member 18	2.26	0.32		0.44	1.26	AA495985
small inducible cytokine subfamily A (Cys-Cys), member 20	2.22	0.19	0.38	0.45	0.90	AI285199
small inducible cytokine subfamily A (Cys-Cys),	2.64	0.38	0.31	1.53		AA916836

Polynucleotide/Protein	Unstimulated Intensity	Ratio Peptide: Unstimulated				Accession Number
		ID 2	ID 3	ID 19	ID 1	
member 23						
small inducible cytokine subfamily B (Cys-X-Cys), member 6 (granulocyte chemotactic protein 2)	3.57	0.11	0.06	0.28	0.38	AI889554
small inducible cytokine subfamily B (Cys-X-Cys), member 10	2.02	0.50	1.07	0.29	0.40	AA878880
small inducible cytokine A3 (homologous to mouse Mip-1a)	2.84	1.79	0.32	0.35		AA677522
cytokine-inducible kinase	2.70	0.41	0.37	0.37	0.34	AA489234
complement component C1q receptor	1.94	0.46	0.58	0.51	0.13	AI761788
cadherin 11, type 2, OB-cadherin (osteoblast)	2.00	0.23	0.57	0.30	0.50	AA136983
cadherin 3, type 1, P-cadherin (placental)	2.11	0.43	0.53	0.10	0.47	AA425217
cadherin, EGF LAG seven-pass G-type receptor 2, flamingo (Drosophila) homolog	1.67	0.42	0.41	1.21	0.60	H39187
cadherin 13, H-cadherin (heart)	1.78	0.37	0.40	0.56	0.68	R41787
selectin L (lymphocyte adhesion molecule 1)	4.43	0.03	0.23	0.61		H00662
vascular cell adhesion molecule 1	1.40	0.20	0.72	0.77	0.40	H16591
intercellular adhesion molecule 3	1.00	0.12	0.31	2.04	1.57	AA479188
integrin, alpha 1	2.42	0.41	0.26		0.56	AA450324

Polynucleotide/Protein	Unstimulated Intensity	Ratio Peptide: Unstimulated				Accession Number
		ID 2	ID 3	ID 19	ID 1	
integrin, alpha 7	2.53	0.57	0.39	0.22	0.31	AA055979
integrin, alpha 9	1.16	0.86	0.05	0.01	2.55	AA865557
integrin, alpha 10	1.00	0.33	0.18	1.33	2.25	AA460959
integrin, beta 5	1.00	0.32	1.52	1.90	0.06	AA434397
integrin, beta 8	3.27	0.10	1.14	0.31	0.24	W56754
disintegrin and metalloproteinase domain 18	2.50	0.40	0.29	0.57	0.17	AI205675
disintegrin-like and metalloprotease with thrombospondin type 1 motif, 3	2.11	0.32	0.63	0.47	0.35	AA398492
disintegrin-like and metalloprotease with thrombospondin type 1 motif, 5	1.62	0.39	0.42	1.02	0.62	AI375048
T-cell receptor interacting molecule	1.00	0.41	1.24	1.41	0.45	AI453185
diphtheria toxin receptor (heparin-binding epidermal growth factor-like growth factor)	1.62	0.49	0.85	0.62	0.15	R45640
vasoactive intestinal peptide receptor 1	2.31	0.43	0.31	0.23	0.54	H73241
Fc fragment of IgG, low affinity IIIb, receptor for (CD16)	3.85	-0.20	0.26	0.76	0.02	H20822
Fc fragment of IgG, low affinity IIb, receptor for (CD32)	1.63	0.27	0.06	1.21	0.62	R68106
Fc fragment of IgE, high affinity I, receptor for; alpha	1.78	0.43	0.00	0.56	0.84	AI676097

Polynucleotide/Protein	Unstimulated Intensity	Ratio Peptide: Unstimulated				Accession Number
		ID 2	ID 3	ID 19	ID 1	
polypeptide						
leukocyte immunoglobulin-like receptor, subfamily A	2.25	0.44	0.05	0.38	0.99	N63398
leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 3	14.21			1.10	0.07	AI815229
leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 4	2.31	0.75	0.43	0.19	0.40	AA076350
leukocyte immunoglobulin-like receptor, subfamily B	1.67	0.35	0.60	0.18	0.90	H54023
peroxisome proliferative activated receptor, alpha	1.18	0.38	0.85	0.87	0.26	AI739498
protein tyrosine phosphatase, receptor type, f polypeptide (PTPRF), interacting protein (liprin), α1	2.19	0.43		1.06	0.46	N49751
protein tyrosine phosphatase, receptor type, C	1.55	0.44	0.64	0.30	0.81	H74265
protein tyrosine phosphatase, receptor type, E	2.08	0.23	0.37	0.56	0.48	AA464542
protein tyrosine phosphatase, receptor type, N polypeptide 2	2.27	0.02	0.44		0.64	AA464590
protein tyrosine phosphatase, receptor type, H	2.34	0.11	0.43	0.24	0.89	AI924306
protein tyrosine phosphatase, receptor-type, Z polypeptide 1	1.59	0.63	0.34	0.72	0.35	AA476461
protein tyrosine phosphatase, non-receptor type 21	1.07	0.94	0.43	0.25	1.13	H03504

Polynucleotide/Protein	Unstimulated Intensity	Ratio Peptide: Unstimulated				Accession Number
		ID 2	ID 3	ID 19	ID 1	
MAP kinase 8 interacting protein 2	1.70	0.07	0.85	0.47	0.59	AA418293
MAP kinase kinase kinase 4	1.27	0.37	0.79	1.59	-5.28	AA402447
MAP kinase kinase kinase 14	1.00	0.34	0.66	2.10	1.49	W61116
MAP kinase 8 interacting protein 2	2.90	0.16	0.35	0.24	0.55	AI202738
MAP kinase kinase kinase 12	1.48	0.20	0.91	0.58	0.68	AA053674
MAP kinase kinase kinase kinase 3	2.21	0.45	0.20	1.03	0.41	AA043537
MAP kinase kinase kinase 6	2.62	0.37	0.38		0.70	AW084649
MAP kinase kinase kinase kinase 4	1.04	0.96	0.09	0.29	2.79	AA417711
MAP kinase kinase kinase 11	1.53	0.65	0.41	0.99	0.44	R80779
MAP kinase kinase kinase 10	1.32	1.23	0.27	0.50	0.76	H01340
MAP kinase 9	2.54	0.57	0.39	0.16	0.38	AA157286
MAP kinase kinase kinase 1	1.23	0.61	0.42	0.81	1.07	AI538525
MAP kinase kinase kinase 8	0.66	1.52	1.82	9.50	0.59	W56266
MAP kinase-activated protein kinase 3	0.52	2.13	2.68	1.13	1.93	W68281
MAP kinase kinase 2	0.84	1.20	3.35	0.02	1.31	AA425826
MAP kinase kinase kinase 7	1.00	0.97		1.62	7.46	AA460969
MAP kinase 7	0.09		11.45	11.80	33.43	H39192
MAP kinase kinase 6	0.10	17.83	9.61		32.30	H07920
regulator of G-protein signalling 5	3.7397	0.27	0.06	0.68	0.18	AA668470
regulator of G-protein signalling 13	1.8564	0.54	0.45	0.07	1.09	H70047
G protein-coupled receptor	1.04	1.84	0.16	0.09	0.96	R91916
G protein-coupled receptor 17	1.78	0.32	0.56	0.39	0.77	AI953187
G protein-coupled receptor kinase 7	2.62		0.34	0.91	0.38	AA488413

Polynucleotide/Protein	Unstimulated Intensity	Ratio Peptide: Unstimulated				Accession Number
		ID 2	ID 3	ID 19	ID 1	
orphan seven-transmembrane receptor, chemokine related	7.16	1.06	0.10	0.11	0.14	AI131555
apoptosis antagonizing transcription factor	1.00	0.28	2.50	1.28	0.19	AI439571
caspase 1, apoptosis-related cysteine protease (interleukin 1, beta, convertase)	2.83	0.44		0.33	0.35	T95052
programmed cell death 8 (apoptosis-inducing factor)	1.00	1.07	0.35	1.94	0.08	AA496348

[00108] Table 23: Pro-inflammatory polynucleotides up-regulated by peptide treatment of A549 cells. The cationic peptides at concentrations of 50 μg/ml were shown to increase the expression of certain pro-inflammatory polynucleotides (data is a subset of Table 21). Peptide was incubated with the human A549 epithelial cells for 4 h and the RNA was isolated, converted into labeled cDNA probes and hybridized to Human cDNA arrays ID#PRHU03-S3. The intensity of polynucleotides in unstimulated cells is shown in the second column. The "Ratio Peptide: Unstimulated" columns refers to the intensity of polynucleotide expression in peptide-simulated cells divided by the intensity of unstimulated cells.

Polynucleotide/Protein and function	Unstim. Intensity	Ratio Peptide: Unstimulated				Accession Number
		ID 2	ID 3	ID 19	ID 1	
IL-11 Rα; Receptor for pro-inflammatory cytokine, inflammation	0.55	2.39	0.98	4.85	1.82	AA454657
IL-17 R; Receptor for IL-17, an inducer of cytokine production in epithelial cells	0.54	2.05	1.97	1.52	1.86	AW029299

Polynucleotide/Protein and function	Unstim. Intensity	Ratio Peptide: Unstimulated				Accession Number
		ID 2	ID 3	ID 19	ID 1	
small inducible cytokine subfamily A, member 21; a chemokine	1.00	3.88			2.41	AI922341
CD31; Leukocyte and cell to cell adhesion (PECAM)	0.59	2.71	3.13	1.01	1.68	R22412
CCR6; Receptor for chemokine MIP-3α	0.14	4.51	7.75	6.92	7.79	N57964
integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor; Adhesion to leukocytes	1.00	0.89	2.44	3.62	0.88	AA463257
integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 receptor); Leukocyte Adhesion	0.94	0.79	2.51	1.88	1.07	AA424695
integrin, alpha E; Adhesion	0.01	179.33	120.12	28.48	81.37	AA425451
integrin, beta 4; Leukocyte adhesion	0.65	0.79	2.17	4.94	1.55	AA485668
C-type lectin-like receptor-2;Leukocyte adhesion	0.45	2.09	7.92	2.24	5.29	H70491

[00109] Table 24: Pro-inflammatory polynucleotides down-regulated by peptide treatment of A549 cells. The cationic peptides at concentrations of 50 μg/ml were shown to decrease the expression of certain pro-inflammatory polynucleotides (data is a subset of Table 22). Peptide was incubated with the human A549 epithelial cells for 4 h and the RNA was isolated, converted into labeled cDNA probes and hybridized to Human cDNA arrays ID#PRHU03-S3. The intensity of polynucleotides in unstimulated cells is shown in the second column. The "Ratio Peptide: Unstimulated" columns refers to the intensity of polynucleotide expression in peptide-simulated cells divided by the intensity of unstimulated cells.

Polynucleotide/Protein; Function	Unstim Intensity	Ratio Peptide:Unstimulated				Accession Number
		ID 2	ID 3	ID 19	ID 1	
Toll-like receptor (TLR) 1; Response to gram positive bacteria	3.22	0.35	0.31	0.14	0.19	AI339155
TLR 2; Response to gram positive bacteria and yeast	2.09	0.52	0.31	0.48	0.24	T57791
TLR 5; May augment other TLR responses, Responsive to flagellin	8.01	0.12	0.39			N41021
TLR 7: Putative host defence mechanism	5.03	0.13	0.11	0.20	0.40	N30597
TNF receptor-associated factor 2; Inflammation	0.82	1.22	0.45	2.50	2.64	T55353
TNF receptor-associated factor 3; Inflammation	3.15	0.15		0.72	0.32	AA504259
TNF receptor superfamily, member 12; Inflammation	4.17	0.59	0.24		0.02	W71984
TNF R superfamily, member 17; Inflammation	2.62		0.38	0.55	0.34	AA987627
TRAF and TNF receptor-associated protein; TNF signalling	1.33	0.75	0.22	0.67	0.80	AA488650
small inducible cytokine subfamily A, member 18; Chemokine	2.26	0.32		0.44	1.26	AA495985
small inducible cytokine subfamily A, member 20; Chemokine	2.22	0.19	0.38	0.45	0.90	AI285199
small inducible cytokine subfamily A, member 23; Chemokine	2.64	0.38	0.31	1.53		AA916836
small inducible cytokine subfamily B, member 6 (granulocyte chemotactic protein); Chemokine	3.57	0.11	0.06	0.28	0.38	AI889554
small inducible cytokine subfamily B, member 10; Chemokine	2.02	0.50	1.07	0.29	0.40	AA878880
small inducible cytokine A3 (homologous to mouse Mip-1α);	2.84	1.79	0.32	0.35		AA677522

Polynucleotide/Protein; Function	Unstim Intensity	Ratio Peptide:Unstimulated				Accession Number
		ID 2	ID 3	ID 19	ID 1	
Chemokine						
IL-12 receptor, beta 2; Interleukin and Interferon receptor	4.58	0.67	0.22			AA977194
IL-18 receptor 1; Induces IFN-γ	1.78	0.50	0.42	0.92	0.56	AA482489
selectin L (lymphocyte adhesion molecule 1); Leukocyte adhesion	4.43	0.03	0.23	0.61		H00662
vascular cell adhesion molecule 1; Leukocyte adhesion	1.40	0.20	0.72	0.77	0.40	H16591
intercellular adhesion molecule 3; Leukocyte adhesion	1.00	0.12	0.31	2.04	1.57	AA479188
integrin, alpha 1; Leukocyte adhesion	2.42	0.41	0.26		0.56	AA450324

[00110] Table 25: Anti-inflammatory polynucleotides up-regulated by peptide treatment of A549 cells. The cationic peptides at concentrations of 50 μg/ml were shown to increase the expression of certain anti-inflammatory polynucleotides (data is a subset of Table 21). Peptide was incubated with the human A549 epithelial cells for 4 h and the RNA was isolated, converted into labeled cDNA probes and hybridized to Human cDNA arrays ID#PRHU03-S3. The intensity of polynucleotides in unstimulated cells is shown in the second column. The "Ratio Peptide: Unstimulated" columns refers to the intensity of polynucleotide expression in peptide-simulated cells divided by the intensity of unstimulated cells.

Polynucleotide/Protein; Function	Unstim Intensity	Ratio Peptide: Unstimulated				Accession Number
		ID 2	ID 3	ID 19	ID 1	
IL-1 R antagonist homolog 1; Inhibitor of septic shock	0.00	3085.96	1855.90	869.57		AI167887
IL-10 R beta; Receptor for cytokine synthesis inhibitor	0.53	2.51	1.56	1.88	3.10	AA486393
TNF R, member 1B; Apoptosis	0.28	17.09	3.01	14.93	3.60	AA150416

Polynucleotide/Protein; Function	Unstim Intensity	Ratio Peptide: Unstimulated				Accession Number
		ID 2	ID 3	ID 19	ID 1	
TNF R, member 5; Apoptosis (CD40L)	33.71	2.98	0.02			H98636
TNF R, member 11b; Apoptosis	1.00	5.29	4.50	0.78		AA194983
IK cytokine, down-regulator of HLA II; Inhibits antigen presentation	0.50	3.11	2.01	1.74	3.29	R39227
TGFB inducible early growth response 2; anti-inflammatory cytokine	0.90	2.38	2.08	0.87	1.11	AI473938
CD2; Adhesion molecule, binds LFAp3	1.00	2.62	0.87	1.15	0.88	AA927710

[00111] Table 26: Anti-inflammatory polynucleotides down-regulated by peptide treatment of A549 cells. The cationic peptides at concentrations of 50 μg/ml were shown to increase the expression of certain anti-inflammatory polynucleotides (data is a subset of Table 21). Peptide was incubated with the human A549 epithelial cells for 4 h and the RNA was isolated, converted into labeled cDNA probes and hybridized to Human cDNA arrays ID#PRHU03-S3. The intensity of polynucleotides in unstimulated cells is shown in the second column. The "Ratio Peptide: Unstimulated" columns refers to the intensity of polynucleotide expression in peptide-simulated cells divided by the intensity of unstimulated cells.

Polynucleotide/Protein; Function	Unstim Intensity	Ratio Peptide: Unstimulated				Accession Number
		ID 2	ID 3	ID 19	ID 1	
MAP kinase 9	2.54	0.57	0.39	0.16	0.38	AA157286

[00112] Table 27: Polynucleotides up-regulated by SEQ ID NO: 6, in primary human macrophages. The peptide SEQ ID NO: 6 at a concentration of 50 μg/ml was shown to increase the expression of many polynucleotides. Peptide was incubated with the human macrophages for 4 h and the RNA was isolated, converted into labeled cDNA probes and hybridized to Human Operon arrays (PRHU04). The intensity of polynucleotides in unstimulated cells is shown in the second column. The "Ratio peptide treated : Control" columns refer to the intensity of polynucleotide expression in peptide-simulated cells divided by the intensity of unstimulated cells.

Gene (Accession Number)	Control: Unstimulated cells	Ratio peptide treated:control
proteoglycan 2 (Z26248)	0.69	9.3
Unknown (AK001843)	26.3	8.2
phosphorylase kinase alpha 1 (X73874)	0.65	7.1
actinin, alpha 3 (M86407)	0.93	6.9
DKFZP586B2420 protein (AL050143)	0.84	5.9
Unknown (AL109678)	0.55	5.6
transcription factor 21 (AF047419)	0.55	5.4
Unknown (A433612)	0.62	5.0
chromosome condensation 1-like (AF060219)	0.69	4.8
Unknown (AL137715)	0.66	4.4
apoptosis inhibitor 4 (U75285)	0.55	4.2
TERF1 (TRF1)-interacting nuclear factor 2 (NM_012461)	0.73	4.2
LINE retrotransposable element 1 (M22333)	6.21	4.0
1-acylglycerol-3-phosphate O-acyltransferase 1 (U56417)	0.89	4.0
Vacuolar proton-ATPase, subunit D; V-ATPase, subunit D (X71490)	1.74	4.0

KIAA0592 protein (AB011164)	0.70	4.0
potassium voltage-gated channel KQT-like subfamily member 4 (AF105202)	0.59	3.9
CDC14 homolog A (AF000367)	0.87	3.8
histone fold proteinCHRAC17 (AF070640)	0.63	3.8
Cryptochrome 1 (D83702)	0.69	3.8
pancreatic zymogen granule membrane associated protein (AB035541)	0.71	3.7
Sp3 transcription factor (X68560)	0.67	3.6
hypothetical protein FLJ20495 (AK000502)	0.67	3.5
E2F transcription factor 5, p130-binding (U31556)	0.56	3.5
hypothetical protein FLJ20070 (AK000077)	1.35	3.4
glycoprotein IX (X52997)	0.68	3.4
KIAA1013 protein (AB023230)	0.80	3.4
eukaryotic translation initiation factor 4A, isoform 2 (AL137681)	2.02	3.4
FYN-binding protein (AF198052)	1.04	3.3
guanine nucleotide binding protein, gamma transducing activity polypeptide 1 (U41492)	0.80	3.3
glypican 1 (X54232)	0.74	3.2
mucosal vascular addressin cell adhesion molecule 1 (U43628)	0.65	3.2
lymphocyte antigen (M38056)	0.70	3.2
H1 histone family, member 4 (M60748)	0.81	3.0
translational inhibitor protein p14.5 (X95384)	0.78	3.0

hypothetical protein FLJ20689 (AB032978)	1.03	2.9
KIAA1278 protein (AB03104)	0.80	2.9
unknown (AL031864)	0.95	2.9
chymotrypsin-like protease (X71877)	3.39	2.9
calumenin (NM_001219)	2.08	2.9
protein kinase, cAMP-dependent, regulatory, type I, beta (M65066)	7.16	2.9
POU domain, class 4, transcription factor 2 (U06233)	0.79	2.8
POU domain, class 2, associating factor 1 (Z49194)	1.09	2.8
KIAA0532 protein (AB011104)	0.84	2.8
unknown (AF068289)	1.01	2.8
unknown (AL117643)	0.86	2.7
cathepsin E (M84424)	15.33	2.7
matrix metalloproteinase 23A (AF056200)	0.73	2.7
interferon receptor 2 (L42243)	0.70	2.5
MAP kinase kinase 1 (L11284)	0.61	2.4
protein kinase C, alpha (X52479)	0.76	2.4
c-Cbl-interacting protein (AF230904)	0.95	2.4
c-fos induced growth factor (Y12864)	0.67	2.3
cyclin-dependent kinase inhibitor 1B (S76988)	0.89	2.2
zinc finger protein 266 (X78924)	1.67	2.2
MAP kinase 14 (L35263)	1.21	2.2
KIAA0922 protein (AB023139)	0.96	2.1
bone morphogenetic protein 1 (NM_006129)	1.10	2.1
NADH dehydrogenase 1 alpha	1.47	2.1

subcomplex, 10 (AF087661)		
bone morphogenetic protein receptor, type IB (U89326)	0.50	2.1
interferon regulatory factor 2 (NM 002199)	1.46	2.0
protease, serine, 21 (AB031331)	0.89	2.0

[00113] Table 28: Polynucleotides down-regulated by SEQ ID NO: 6, in primary human macrophages. The peptide SEQ ID NO: 6 at a concentration of 50 μg/ml was shown to increase the expression of many polynucleotides. Peptide was incubated with the human macrophages for 4 h and the RNA was isolated, converted into labeled cDNA probes and hybridized to Human Operon arrays (PRHU04). The intensity of polynucleotides in unstimulated cells is shown in the second column. The "Ratio of Peptide: Control" columns refer to the intensity of polynucleotide expression in peptide-simulated cells divided by the intensity of unstimulated cells.

Gene (Accession Number)	Control: Unstimulated cells	Ratio peptide treated:control
Unknown (AL049263)	17	0.06
integrin-linked kinase (U40282)	2.0	0.13
KIAA0842 protein (AB020649)	1.1	0.13
Unknown (AB037838)	13	0.14
Granulin (AF055008)	8.6	0.14
glutathione peroxidase 3 (NM_002084)	1.2	0.15
KIAA0152 gene product (D63486)	0.9	0.17
TGFB1-induced anti-apoptotic factor 1 (D86970)	0.9	0.19
disintegrin protease (Y13323)	1.5	0.21
proteasome subunit beta type 7 (D38048)	0.7	0.22
cofactor required for Sp1 transcriptional activation subunit 3 (AB033042)	0.9	0.23
TNF receptor superfamily, member 14 (U81232)	0.8	0.26
proteasome 26S subunit non-ATPase 8 (D38047)	1.1	0.28
proteasome subunit beta type, 4 (D26600)	0.7	0.29
TNF receptor superfamily member 1B (M32315)	1.7	0.29

cytochrome c oxidase subunit Vic (X13238)	3.3	0.30
S100 calcium-binding protein A4 (M80563)	3.8	0.31
proteasome subunit alpha type, 6 (X59417)	2.9	0.31
proteasome 26S subunit non-ATPase, 10 (AL031177)	1.0	0.32
MAP kinase kinase kinase 2 (NM_006609)	0.8	0.32
ribosomal protein L11 (X79234)	5.5	0.32
matrix metalloproteinase 14 (Z48481)	1.0	0.32
proteasome subunit beta type, 5 (D29011)	1.5	0.33
MAP kinase-activated protein kinase 2 (U12779)	1.5	0.34
caspase 3 (U13737)	0.5	0.35
jun D proto-oncogene (X56681)	3.0	0.35
proteasome 26S subunit, ATPase, 3 (M34079)	1.3	0.35
IL-1 receptor-like 1 (AB012701)	0.7	0.35
interferon alpha-inducible protein (AB019565)	13	0.35
SDF receptor 1 (NM_012428)	1.6	0.35
Cathepsin D (M63138)	46	0.36
MAP kinase kinase 3 (D87116)	7.4	0.37
TGF, beta-induced, (M77349)	1.8	0.37
TNF receptor superfamily, member 10b (AF016266)	1.1	0.37
proteasome subunit beta type, 6 (M34079)	1.3	0.38
nuclear receptor binding protein (NM_013392)	5.2	0.38
Unknown (AL050370)	1.3	0.38
protease inhibitor 1 alpha-1-antitrypsin (X01683)	0.7	0.40
proteasome subunit alpha type, 7 (AF054185)	5.6	0.40
LPS-induced TNF-alpha factor (NM_004862)	5.3	0.41
transferrin receptor (X01060)	14	0.42
proteasome 26S subunit non-ATPase 13 (AB009398)	1.8	0.44
MAP kinase kinase 5 (U25265)	1.3	0.44
Cathepsin L (X12451)	15	0.44
IL-1 receptor-associated kinase 1 (L76191)	1.7	0.45
MAP kinase kinase kinase kinase 2 (U07349)	1.1	0.46
peroxisome proliferative activated receptor delta (AL022721)	2.2	0.46
TNF superfamily, member 15 (AF039390)	16	0.46

defender against cell death 1 (D15057)	3.9	0.46
TNF superfamily member 10 (U37518)	287	0.46
cathepsin H (X16832)	14	0.47
protease inhibitor 12 (Z81326)	0.6	0.48
proteasome subunit alpha type, 4 (D00763)	2.6	0.49
proteasome 26S subunit ATPase, 1 (L02426)	1.8	0.49
proteasome 26S subunit ATPase, 2 (D11094)	2.1	0.49
caspase 7 (U67319)	2.4	0.49
matrix metalloproteinase 7 (Z11887)	2.5	0.49

[00114] Table 29: Polynucleotides up-regulated by SEQ ID NO: 1, in HBE cells. The peptide SEQ ID NO: 1 at a concentration of 50 μg/ml was shown to increase the expression of many polynucleotides. Peptide was incubated with the human HBE epithelial cells for 4 h and the RNA was isolated, converted into labeled cDNA probes and hybridized to Human Operon arrays (PRHU04). The intensity of polynucleotides in unstimulated cells is shown in the second column. The "Ratio Peptide: Control" columns refer to the intensity of polynucleotide expression in peptide-simulated cells divided by the intensity of unstimulated cells.

Accession Number	Gene	Control: Unstimulated cells	Ratio peptide treated:control
AL110161	Unknown	0.22	5218.3
AF131842	Unknown	0.01	573.1
AJ000730	solute carrier family	0.01	282.0
Z25884	chloride channel 1	0.01	256.2
M93426	protein tyrosine phosphatase receptor-type,zeta	0.01	248.7
X65857	olfactory receptor, family 1, subfamily D,member 2	0.01	228.7
M55654	TATA box binding protein	0.21	81.9
AK001411	hypothetical protein	0.19	56.1
D29643	dolichyl-	1.56	55.4

Accession Number	Gene	Control: Unstimulated cells	Ratio peptide treated:control
	diphosphooligosaccharide-protein glycosyltransferase		
AF006822	myelin transcription factor 2	0.07	55.3
AL117601	Unknown	0.05	53.8
AL117629	DKFZP434C245 protein	0.38	45.8
M59465	tumor necrosis factor,alpha-induced protein 3	0.50	45.1
AB013456	aquaporin 8	0.06	41.3
AJ131244	SEC24 related gene family, member A	0.56	25.1
AL110179	Unknown	0.87	24.8
AB037844	Unknwon	1.47	20.6
Z47727	polymerase II polypeptide K	0.11	20.5
AL035694	Unknown	0.81	20.4
X68994	H.sapiens CREB gene	0.13	19.3
AJ238379	hypothetical protein	1.39	18.5
NM_003519	H2B histone family member	0.13	18.3
U16126	glutamate receptor, ionotropic kainate 2	0.13	17.9
U29926	adenosine monophosphate deaminase	0.16	16.3
AK001160	hypothetical protein	0.39	14.4
U18018	ets variant gene 4	0.21	12.9
D80006	KIAA0184 protein	0.21	12.6
AK000768	hypothetical protein	0.30	12.3
X99894	insulin promoter factor 1,	0.26	12.0
AL031177	Unknown	1.09	11.2
AF052091	unknown	0.28	10.9

Accession Number	Gene	Control: Unstimulated cells	Ratio peptide treated:control
L38928	5,10-methenyltetrahydrofolate synthetase	0.22	10.6
AL117421	unknown	0.89	10.1
AL133606	hypothetical protein	0.89	9.8
NM_016227	membrane protein CH1	0.28	9.6
NM_006594	adaptor-related protein complex 4	0.39	9.3
U54996	ZW10 homolog,protein	0.59	9.3
AJ007557	potassium channel,	0.28	9.0
AF043938	muscle RAS oncogene	1.24	8.8
AK001607	unknown	2.74	8.7
AL031320	peroxisomal biogenesis factor 3	0.31	8.4
D38024	unknown	0.31	8.3
AF059575	LIM homeobox TF	2.08	8.2
AF043724	hepatitis A virus cellular receptor 1	0.39	8.1
AK002062	hypothetical protein	2.03	8.0
L13436	natriuretic peptide receptor	0.53	7.8
U33749	thyroid transcription factor 1	0.36	7.6
AF011792	cell cycle progression 2 protein	0.31	7.6
AK000193	hypothetical protein	1.18	6.8
AF039022	exportin, tRNA	0.35	6.8
M17017	interleukin 8	0.50	6.7
AF044958	NADH dehydrogenase	0.97	6.5
U35246	vacuolar protein sorting	0.48	6.5
AK001326	tetraspan 3	1.59	6.5
M55422	Krueppel-related zinc finger protein	0.34	6.4
U44772	palmitoyl-protein thioesterase	1.17	6.3

Accession Number	Gene	Control: Unstimulated cells	Ratio peptide treated:control
AL117485	hypothetical protein	0.67	5.9
AB037776	unknown	0.75	5.7
AF131827	unknown	0.69	5.6
AL137560	unknown	0.48	5.2
X05908	annexin A1	0.81	5.1
X68264	melanoma adhesion molecule	0.64	5.0
AL161995	neurturin	0.86	4.9
AF037372	cytochrome c oxidase	0.48	4.8
NM_016187	bridging integrator 2	0.65	4.8
AL137758	unknown	0.57	4.8
U59863	TRAF family member-associated NFKB activator	0.46	4.7
Z30643	chloride channel Ka	0.70	4.7
D16294	acetyl-Coenzyme A acyltransferase 2	1.07	4.6
AJ132592	zinc finger protein 281	0.55	4.6
X82324	POU domain TF	1.73	4.5
NM_016047	CGI-110 protein	1.95	4.5
AK001371	hypothetical protein	0.49	4.5
M60746	H3 histone family member D	3.05	4.5
AB033071	hypothetical protein	4.47	4.4
AB002305	KIAA0307 gene product	1.37	4.4
X92689	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 3	0.99	4.4
AL049543	glutathione peroxidase 5	1.62	4.3
U43148	patched homolog	0.96	4.3
M67439	dopamine receptor D5	2.61	4.2

Accession Number	Gene	Control: Unstimulated cells	Ratio peptide treated:control
U09850	zinc finger protein 143	0.56	4.2
L20316	glucagon receptor	0.75	4.2
AB037767	a disintegrin-like and metalloprotease	0.69	4.2
NM_017433	myosin IIIA	99.20	4.2
D26579	a disintegrin and metalloprotease domain 8	0.59	4.1
L10333	reticulon 1	1.81	4.1
AK000761	unknown	1.87	4.1
U91540	NK homeobox family 3, A	0.80	4.1
Z17227	interleukin 10 receptor, beta	0.75	4.0

[00115] Table 30: Polynucleotides down-regulated by Peptide (50 μg/ml), SEQ ID NO: 1, in HBE cells. The peptide SEQ ID NO: 1 at a concentration of 50 μg/ml was shown to decrease the expression of many polynucleotides. Peptide was incubated with the human A549 epithelial cells for 4 h and the RNA was isolated, converted into labeled cDNA probes and hybridized to Human Operon arrays (PRHU04). The intensity of polynucleotides in unstimulated cells is shown in the third column. The "Ratio Peptide: Control" columns refer to the intensity of polynucleotide expression in peptide-simulated cells divided by the intensity of unstimulated cells.

Accession Number	Gene	Control: Unstimulated Cells	Ratio SEQ ID NO:1- treated: control
AC004908	Unknown	32.4	0.09
S70622	G1 phase-specific gene	43.1	0.10

Accession Number	Gene	Control: Unstimulated Cells	Ratio SEQ ID NO:1- treated: control
Z97056	DEAD/H box polypeptide	12.8	0.11
AK002056	hypothetical protein	11.4	0.12
L33930	CD24 antigen	28.7	0.13
X77584	thioredoxin	11.7	0.13
NM_014106	PRO1914 protein	25.0	0.14
M37583	H2A histone family member	22.2	0.14
U89387	polymerase (RNA) II polypeptide D	10.2	0.14
D25274	ras-related C3 botulinum toxin substrate 1	10.3	0.15
J04173	phosphoglycerate mutase 1	11.4	0.15
U19765	zinc finger protein 9	8.9	0.16
X67951	proliferation-associated gene A	14.1	0.16
AL096719	profilin 2	20.0	0.16
AF165217	tropomodulin 4	14.6	0.16
NM_014341	mitochondrial carrier homolog 1	11.1	0.16
AL022068	Unknown	73.6	0.17
X69150	ribosomal protein S18	42.8	0.17
AL031577	Unknown	35.0	0.17
AL031281	Unknown	8.9	0.17
AF090094	Human mRNA for ornithine decarboxylase antizyme,	10.3	0.17
AL022723	HLA-G histocompatibility antigen, class I, G	20.6	0.18
U09813	ATP synthase, H+ transporting mitochondrial F0 complex	9.8	0.18
AF000560	Homo sapiens TTF-I interacting peptide 20	20.2	0.19

Accession Number	Gene	Control: Unstimulated Cells	Ratio SEQ ID NO:1- treated: control
NM_016094	HSPC042 protein	67.2	0.19
AF047183	NADH dehydrogenase	7.5	0.19
D14662	anti-oxidant protein 2 (non-selenium glutathione peroxidase, acidic calcium-independent phospholipas	8.1	0.19
X16662	annexin A8	8.5	0.19
U14588	paxillin	11.3	0.19
AL117654	DKFZP586D0624 protein	12.6	0.20
AK001962	hypothetical protein	7.7	0.20
L41559	6-pyruvoyl-tetrahydropterin synthase/dimerization cofactor of hepatocyte nuclear factor 1 alpha	9.1	0.20
NM_016139	16.7Kd protein	21.0	0.21
NM_016080	CGI-150 protein	10.7	0.21
U86782	26S proteasome-associated pad1 homolog	6.7	0.21
AJ400717	tumor protein, translationally-controlled 1	9.8	0.21
X07495	homeo box C4	31.0	0.21
AL034410	Unknown	7.3	0.22
X14787	thrombospondin 1	26.2	0.22
AF081192	purine-rich element binding protein B	6.8	0.22
D49489	protein disulfide isomerase-related protein	11.0	0.22
NM_014051	PTD011 protein	9.3	0.22
AK001536	Unknown	98.0	0.22

Accession Number	Gene	Control: Unstimulated Cells	Ratio SEQ ID NO:1- treated: control
X62534	high-mobility group protein 2	9.5	0.22
AJ005259	endothelial differentiation-related factor 1	6.7	0.22
NM_000120	epoxide hydrolase 1, microsomal	10.0	0.22
M38591	S100 calcium-binding protein A10	23.9	0.23
AF071596	immediate early response 3	11.5	0.23
X16396	methylene tetrahydrofolate dehydrogenase	8.3	0.23
AK000934	ATPase inhibitor precursor	7.6	0.23
AL117612	Unknown	10.7	0.23
AF119043	transcriptional intermediary factor 1 gamma	7.3	0.23
AF037066	solute carrier family 22 member 1-like antisense	7.6	0.23
AF134406	cytochrome c oxidase subunit	13.3	0.23
AE000661	Unknown	9.2	0.24
AL157424	synaptojanin 2	7.2	0.24
X56468	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein,	7.2	0.24
U39318	ubiquitin-conjugating enzyme E2D 3	10.7	0.24
AL034348	Unknown	24.4	0.24
D26600	proteasome subunit beta type 4	11.4	0.24
AB032987	Unknown	16.7	0.24
J04182	lysosomal-associated membrane protein 1	7.4	0.24
X78925	zinc finger protein 267	16.1	0.25

Accession Number	Gene	Control: Unstimulated Cells	Ratio SEQ ID NO:1- treated: control
NM_000805	gastrin	38.1	0.25
U29700	anti-Mullerian hormone receptor, type II	12.0	0.25
Z98200	Unknown	13.4	0.25
U07857	signal recognition particle	10.3	0.25
L05096	Homo sapiens ribosomal protein L39	25.3	0.25
AK001443	hypothetical protein	7.5	0.25
K03515	glucose phosphate isomerase	6.2	0.25
X57352	interferon induced transmembrane protein 3	7.5	0.26
J02883	colipase pancreatic	5.7	0.26
M24069	cold shock domain protein	6.3	0.26
AJ269537	chondroitin-4-sulfotransferase	60.5	0.26
AL137555	Unknown	8.5	0.26
U89505	RNA binding motif protein 4	5.5	0.26
U82938	CD27-binding protein	7.5	0.26
X99584	SMT3 homolog 1	12.8	0.26
AK000847	Unknown	35.8	0.27
NM_014463	Lsm3 protein	7.8	0.27
AL133645	Unknown	50.8	0.27
X78924	zinc finger protein 266	13.6	0.27
NM_004304	anaplastic lymphoma kinase	15.0	0.27
X57958	ribosomal protein L7	27.9	0.27
U63542	Unknown	12.3	0.27
AK000086	hypothetical protein	8.3	0.27
X57138	H2A histone family member N	32.0	0.27
AB023206	KIAA0989 protein	6.5	0.27

Accession Number	Gene	Control: Unstimulated Cells	Ratio SEQ ID NO:1- treated: control
AB021641	gonadotropin inducible transcriptn repressor-1	5.5	0.28
AF050639	NADH dehydrogenase	5.5	0.28
M62505	complement component 5 receptor 1	7.5	0.28
X64364	basigin	5.8	0.28
AJ224082	Unknown	22.5	0.28
AF042165	cytochrome c oxidase	20.4	0.28
AK001472	anillin	10.9	0.28
X86428	protein phosphatase 2A subunit	12.7	0.28
AF227132	candidate taste receptor T2R5	5.1	0.28
Z98751	Unknown	5.3	0.28
D21260	clathrin heavy polypeptide	8.3	0.28
AF041474	actin-like 6	15.1	0.28
NM_005258	GTP cyclohydrolase I protein	7.6	0.28
L20859	solute carrier family 20	9.6	0.29
Z80783	H2B histone family member	9.0	0.29
AB011105	laminin alpha 5	7.1	0.29
AL008726	protective protein for beta-galactosidase	5.2	0.29
D29012	proteasome subunit	12.6	0.29
X63629	cadherin 3 P-cadherin	6.8	0.29
X02419	plasminogen activator urokinase	12.9	0.29
X13238	cytochrome c oxidase	8.0	0.29
X59798	cyclin D1	12.7	0.30
D78151	proteasome 26S subunit	7.6	0.31
AF054185	proteasome subunit	18.8	0.31
J03890	surfactant pulmonary-associated	5.5	0.32

Accession Number	Gene	Control: Unstimulated Cells	Ratio SEQ ID NO:1- treated: control
	protein C		
M34079	proteasome 26S subunit,	5.2	0.33

[00116] Table 31: Up-regulation of Polynucleotide expression in A549 cells induced by Formula A Peptides. The peptides at a concentration of 50 μg/ml were shown to increase the expression of many polynucleotides. Peptide was incubated with the human A549 epithelial cells for 4 h and the RNA was isolated, converted into labeled cDNA probes and hybridized to Human Operon arrays (PRHU04). The intensity of polynucleotides in control, unstimulated cells are shown in the second and third columns for labeling of cDNA with the dyes Cy3 and Cy5 respectively. The "ID#: Control" columns refer to the intensity of polynucleotide expression in peptide-simulated cells divided by the intensity of unstimulated cells.

Accession Number	Gene	control-Cy3	control-Cy5	ID 5: control	ID 6: control	ID 7: control	ID 8: control	ID 9: control	ID 10: control
U12472	glutathione S-transferase	0.09	0.31	13.0	3.5	4.5	7.0	4.3	16.4
X66403	cholinergic receptor	0.17	0.19	7.8	9.9	6.0	6.4	5.0	15.7
AK001932	unknown	0.11	0.25	19.4	4.6	9.9	7.6	8.1	14.5
X58079	S100 calcium-binding protein	0.14	0.24	12.2	7.6	8.1	4.3	4.5	13.2
U18244	solute carrier family 1	0.19	0.20	6.1	9.7	11.9	5.0	3.7	10.6
U20648	zinc finger protein	0.16	0.13	5.3	6.2	5.6	3.1	6.8	9.5
AB037832	unknown	0.10	0.29	9.0	4.2	9.4	3.1	2.6	8.7
AC002542	unknown	0.15	0.07	10.5	15.7	7.8	10.1	11.7	8.2
M89796	membrane-spanning 4-	0.15	0.14	2.6	6.1	7.6	3.5	13.3	8.1

Accession Number	Gene	control-Cy3	control-Cy5	ID 5: control	ID 6: control	ID 7: control	ID 8: control	ID 9: control	ID 10: control
	domains, subfamily A								
AF042163	cytochrome c oxidase	0.09	0.19	3.9	3.2	7.6	6.3	4.9	7.9
AL032821	Vanin 2	0.41	0.23	2.5	5.2	3.2	2.1	4.0	7.9
U25341	melatonin receptor 1B	0.04	0.24	33.1	5.1	23.3	6.6	4.1	7.6
U52219	G protein-coupled receptor	0.28	0.20	2.1	6.2	6.9	2.4	3.9	7.1
X04506	apolipoprotein B	0.29	0.32	7.9	3.4	3.3	4.8	2.6	7.0
AB011138	ATPase type IV	0.12	0.07	3.5	12.9	6.6	6.4	21.3	6.9
AF055018	unknown	0.28	0.22	3.8	6.9	5.0	2.3	3.1	6.8
AK002037	hypothetical protein	0.08	0.08	2.9	7.9	14.1	7.9	20.1	6.5
AK001024	guanine nucleotide-binding protein	0.16	0.11	7.7	11.9	5.0	10.3	6.0	6.3

Accession Number	Gene	control-Cy3	control-Cy5	ID 5: control	ID 6: control	ID 7: control	ID 8: control	ID 9: control	ID 10: control
AF240467	TLR-7	0.11	0.10	20.4	9.0	3.4	9.4	12.9	6.1
AF105367	glucagon-like peptide 2 receptor	0.15	0.35	23.2	2.6	3.0	10.6	2.9	5.7
AL009183	TNFR superfamily, member 9	0.46	0.19	10.6	4.7	3.7	2.8	6.5	5.7
X54380	pregnancy-zone protein	0.23	0.08	4.7	11.9	7.2	12.7	3.8	5.5
AL137736	unknown	0.22	0.15	2.1	7.2	3.3	7.1	4.6	5.5
X05615	thyroglobulin	0.28	0.42	6.3	2.7	7.7	2.4	3.1	5.4
D28114	myelin-associated protein	0.24	0.08	2.5	15.9	13.0	7.1	13.7	5.4
AK000358	microfibrillar-associated protein 3	0.28	0.28	8.7	4.2	7.2	3.2	2.4	5.3

Accession Number	Gene	control-Cy3	control-Cy5	ID 5: control	ID 6: control	ID 7: control	ID 8: control	ID 9: control	ID 10: control
AK001351	unknown	0.12	0.22	3.9	7.6	8.7	3.9	2.3	5.2
U79289	unknown	0.14	0.27	2.5	2.7	2.8	2.0	4.3	5.1
AB014546	ring finger protein	0.12	0.34	6.8	2.4	4.1	2.7	2.0	5.0
AL117428	DKFZP434A236 protein	0.10	0.07	2.8	16.1	12.8	9.7	14.2	4.9
AL050378	unknown	0.41	0.14	3.5	8.7	11.7	3.5	7.0	4.9
AJ250562	transmembrane 4 superfamily member 2	0.13	0.10	5.2	5.7	14.2	3.8	10.3	4.8
NM_001756	corticosteroid binding globulin	0.28	0.13	4.0	7.9	6.5	14.9	5.6	4.8
AL137471	hypothetical protein	0.29	0.05	3.7	18.0	6.2	7.2	16.3	4.7
M19684	protease inhibitor 1	0.41	0.14	3.5	4.6	5.4	2.8	9.4	4.7
NM_001963	epidermal	0.57	0.05	3.4	6.2	1.8	32.9	14.7	4.4

Accession Number	Gene	control-Cy3	control-Cy5	ID 5: control	ID 6: control	ID 7: control	ID 8: control	ID 9: control	ID 10: control
	growth factor								
NM_000910	neuropeptide Y receptor	0.62	0.36	3.1	2.7	2.3	2.6	3.1	4.4
AF022212	Rho GTPase activating protein 6	0.19	0.02	9.0	45.7	25.6	12.4	72.2	4.4
AK001674	cofactor required for Sp1	0.11	0.13	8.4	6.5	7.9	4.5	7.4	4.3
U51920	signal recognition particle	0.23	0.27	3.4	3.8	2.1	4.1	8.8	4.2
AK000576	hypothetical protein	0.27	0.06	4.4	14.7	7.4	14.1	8.6	4.2
AL080073	unknown	0.17	0.20	21.6	3.9	4.3	8.8	2.6	4.1
U59628	paired box gene 9	0.34	0.06	3.4	14.1	5.4	7.9	4.9	4.1
U90548	butyrophilin,	0.41	0.31	2.3	4.7	5.5	6.8	3.4	4.1

Accession Number	Gene	control-Cy3	control-Cy5	ID 5: control	ID 6: control	ID 7: control	ID 8: control	ID 9: control	ID 10: control
	subfamily 3, member A3								
M19673	cystatin SA	0.43	0.26	2.3	8.5	4.5	2.5	4.1	3.8
AL161972	ICAM 2	0.44	0.37	2.0	3.6	2.0	2.7	5.5	3.8
X54938	inositol 1,4,5-trisphosphate 3-kinase A	0.32	0.22	3.9	3.3	6.2	3.1	4.4	3.7
AB014575	KIAA0675 gene product	0.04	0.13	46.2	4.5	10.2	8.0	6.2	3.4
M83664	MHC II, DP beta 1	0.57	0.29	2.9	2.1	2.0	3.1	6.6	3.4
AK000043	hypothetical protein	0.34	0.14	2.7	7.1	3.7	9.4	8.8	3.3
U60666	testis specific leucine rich repeat protein	0.21	0.11	9.9	9.0	4.1	5.5	13.0	3.3
AK000337	hypothetical	0.49	0.19	4.3	5.1	4.7	10.6	7.1	3.3

Accession Number	Gene	control-Cy3	control-Cy5	ID 5: control	ID 6: control	ID 7: control	ID 8: control	ID 9: control	ID 10: control
	protein								
AF050198	putative mitochondrial space protein	0.34	0.15	7.0	6.3	3.6	5.6	11.9	3.3
AJ251029	odorant-binding protein 2A	0.28	0.12	4.4	9.4	7.2	8.8	7.1	3.2
X74142	forkhead box G1B	0.12	0.33	19.5	4.5	8.4	6.4	4.4	3.2
AB029033	KIAA1110 protein	0.35	0.24	3.1	2.2	5.6	5.2	3.1	3.1
D85606	cholecystokinin A receptor	0.51	0.14	4.3	3.9	4.6	3.5	7.2	3.1
X84195	acylphosphatase 2 muscle type	0.32	0.19	4.8	3.7	5.0	11.2	9.8	3.0
U57971	ATPase Ca++ transporting plasma	0.29	0.13	2.2	7.9	1.8	6.3	4.8	3.0

Accession Number	Gene	control-Cy3	control-Cy5	ID 5: control	ID 6: control	ID 7: control	ID 8: control	ID 9: control	ID 10: control
	membrane 3								
J02611	apolipoprotein D	0.28	0.10	2.8	11.0	3.7	10.3	8.4	3.0
AF071510	lecithin retinol acyltransferase	0.07	0.05	7.9	3.8	11.7	46.0	16.3	3.0
AF131757	unknown	0.10	0.08	4.8	9.0	44.3	9.3	10.7	3.0
L10717	IL2-inducible T-cell kinase	0.45	0.21	2.5	4.9	2.8	10.9	4.5	2.9
L32961	4-aminobutyrate aminotransferase	0.64	0.32	3.6	2.9	3.2	5.3	2.3	2.9
NM_003631	poly (ADP-ribose) glycohydrolase	0.46	0.41	9.7	3.9	4.1	3.8	2.8	2.7
AF098484	pronapsin A	0.28	0.14	3.7	3.7	5.6	11.6	3.7	2.5
NM_009589	arylsulfatase D	0.73	0.16	3.2	5.6	6.0	48.6	7.2	2.4
M14764	TNFR superfamily, member 16	0.49	0.15	2.3	3.5	10.6	13.6	6.8	2.2

Accession Number	Gene	control-Cy3	control-Cy5	ID 5: control	ID 6: control	ID 7: control	ID 8: control	ID 9: control	ID 10: control
AL035250	endothelin 3	0.52	0.14	2.1	7.3	4.8	4.5	3.7	2.2
M97925	defensin, alpha 5, Paneth cell-specific	0.33	0.07	4.0	14.7	7.8	9.4	3.5	2.1
D43945	transcription factor EC	0.46	0.19	6.6	2.9	8.2	4.0	3.5	2.1
D16583	histidine decarboxylase	0.46	0.09	3.2	13.8	4.2	8.8	13.7	2.1

[00117] Table 32: Up-regulation of Polynucleotide expression in A549 cells induced by Formula B Peptides The peptides at a concentration of 50 μg/ml were shown to increase the expression of many polynucleotides. Peptide was incubated with the human A549 epithelial cells for 4 h and the RNA was isolated, converted into labeled cDNA probes and hybridized to Human Operon arrays (PRHU04). The intensity of polynucleotides in control, unstimulated cells are shown in the second and third columns for labeling of cDNA with the dyes Cy3 and Cy5 respectively. The "ID#: Control" columns refer to the intensity of polynucleotide expression in peptide-simulated cells divided by the intensity of unstimulated cells.

Accession Number	Gene	control-Cy3	control-Cy5	ID 12: control	ID 13: control	ID 14: control	ID 15: control	ID 16: control	ID 17: control
AL157466	unknown	0.05	0.06	18.0	21.4	16.7	5.2	6.8	8.6
AB023215	KIAA0998 protein	0.19	0.07	14.8	10.6	7.9	14.4	6.6	16.1
AL031121	unknown	0.24	0.09	14.1	5.7	3.8	5.5	2.8	4.6
NM_016331	zinc finger protein	0.16	0.08	12.8	7.2	11.0	5.3	11.2	9.7
M14565	cytochrome P450	0.16	0.12	10.6	12.5	5.0	3.6	10.1	6.3
U22492	G protein-coupled receptor 8	0.28	0.07	10.4	8.9	4.8	10.8	6.6	3.6
U76010	solute carrier family 30	0.14	0.07	9.7	18.6	3.7	4.8	5.6	8.9
AK000685	unknown	0.51	0.10	9.0	3.1	2.8	3.9	15.3	3.0
AF013620	Immunoglobulin heavy variable 4-4	0.19	0.18	8.5	2.6	6.2	5.7	8.2	3.8
AL049296	unknown	0.61	0.89	8.1	3.2	2.7	3.2	2.7	2.0
AB006622	KIAA0284 protein	0.47	0.28	7.5	5.0	2.8	11.1	5.5	4.6
X04391	CD5 antigen	0.22	0.13	7.2	16.7	2.7	7.7	6.1	5.9
AK000067	hypothetical protein	0.80	0.35	7.1	4.6	2.1	3.2	8.5	2.2
AF053712	TNF superfamily_	0.17	0.08	6.9	17.7	3.0	6.2	12.3	5.2

Accession Number	Gene	control-Cy3	control-Cy5	ID 12: control	ID 13: control	ID 14: control	ID 15: control	ID 16: control	ID 17: control
	member 11								
X58079	S100 calcium-binding protein A1	0.14	0.24	6.7	6.7	5.9	6.5	5.3	2.5
M91036	hemoglobin_ gamma A	0.48	0.36	6.7	14.2	2.1	2.9	2.7	4.8
AF055018	unknown	0.28	0.22	6.3	10.7	2.7	2.6	4.6	6.5
L17325	pre-T/NK cell associated protein	0.19	0.29	6.1	4.4	6.5	4.7	4.0	4.0
D45399	phosphodiesterase	0.21	0.18	6.1	4.6	5.0	2.8	10.8	4.0
AB023188	KIAA0971 protein	0.29	0.13	5.9	10.6	3.6	3.4	10.6	7.2
NM_012177	F-box protein	0.26	0.31	5.9	5.5	3.8	2.8	3.0	6.8
D38550	E2F TF 3	0.43	0.39	5.8	3.4	2.1	4.5	2.5	2.4
AL050219	unknown	0.26	0.04	5.7	17.0	3.1	9.2	30.3	16.1
AL137540	unknown	0.67	0.79	5.5	3.2	3.9	10.9	2.9	2.3
D50926	KIAA0136 protein	0.57	0.21	5.4	5.6	2.0	3.3	4.4	3.2
AL137658	unknown	0.31	0.07	5.4	12.1	2.6	10.8	3.9	8.6
U21931	fructose-	0.48	0.14	5.4	4.1	2.9	3.6	6.0	3.2

Accession Number	Gene	control-Cy3	control-Cy5	ID 12: control	ID 13: control	ID 14: control	ID 15: control	ID 16: control	ID 17: control
	bisphosphatase 1								
AK001230	DKFZP586D211 protein	0.43	0.26	5.0	4.6	2.1	2.2	2.5	2.7
AL137728	unknown	0.67	0.47	5.0	5.9	2.2	6.8	5.9	2.1
AB022847	unknown	0.39	0.24	4.5	2.2	3.5	4.3	3.8	3.7
X75311	mevalonate kinase	0.67	0.22	4.3	4.0	2.0	8.3	4.0	5.1
AK000946	DKFZP566C243 protein	0.36	0.29	4.1	3.8	3.9	5.4	25.8	2.7
AB023197	KIAA0980 protein	0.25	0.30	4.0	8.3	2.1	8.8	2.2	4.9
AB014615	fibroblast growth factor 8	0.19	0.07	3.9	3.3	7.0	3.4	2.2	7.7
X04014	unknown	0.29	0.16	3.8	2.5	2.2	3.0	5.5	3.1
U76368	solute carrier family 7	0.46	0.17	3.8	3.8	2.8	3.2	4.2	3.0
AB032436	unknown	0.14	0.21	3.8	2.7	6.1	3.2	4.5	2.6
AB020683	KIAA0876 protein	0.37	0.21	3.7	4.2	2.2	5.3	2.9	9.4
NM_012126	carbohydrate	0.31	0.20	3.7	5.2	3.2	3.4	3.9	2.5

Accession Number	Gene	control-Cy3	control-Cy5	ID 12: control	ID 13: control	ID 14: control	ID 15: control	ID 16: control	ID 17: control
	sulfotransferase 5								
AK002037	hypothetical protein	0.08	0.08	3.7	17.1	4.6	12.3	11.0	8.7
X78712	glycerol kinase pseudogene 2	0.17	0.19	3.6	2.5	4.5	5.3	2.2	3.3
NM_014178	HSPC156 protein	0.23	0.12	3.5	8.4	2.9	6.9	14.4	5.5
AC004079	homeo box A2	0.31	0.11	3.5	7.0	2.1	2.0	7.3	9.1
AL080182	unknown	0.51	0.21	3.4	3.5	2.2	2.1	2.9	2.4
M91036	hemoglobin gamma G	0.22	0.02	3.4	26.3	5.8	6.8	30.4	21.6
AJ000512	serum/glucocorticoid regulated kinase	0.27	0.43	3.3	2.1	4.9	2.3	3.9	2.7
AK002140	hypothetical protein	0.28	0.14	3.3	9.9	2.8	2.1	16.6	7.2
AL137284	unknown	0.22	0.04	3.3	7.2	4.1	6.0	12.2	3.7
Z11898	POU domain_ class 5 TF 1	0.12	0.29	3.2	3.7	8.2	2.5	6.6	2.2
AB017016	brain-specific protein	0.27	0.29	3.1	2.8	2.5	2.8	3.3	5.5

Accession Number	Gene	control-Cy3	control-Cy5	ID 12: control	ID 13: control	ID 14: control	ID 15: control	ID 16: control	ID 17: control
X54673	Solute-carrier family 6	0.34	0.08	2.9	12.0	2.2	10.4	7.4	5.9
AL033377	unknown	0.40	0.22	2.6	2.6	2.6	2.3	4.5	2.2
X85740	CCR4	0.34	0.05	2.6	2.3	2.6	2.5	12.5	5.2
AB010419	core-binding factor	0.59	0.20	2.5	12.8	2.0	2.8	2.9	5.9
AL109726	uknown	0.14	0.15	2.3	9.0	4.3	4.4	2.6	3.7
NM_012450	sulfate transporter 1	0.15	0.10	2.2	3.1	8.2	9.9	4.7	5.9
J04599	biglycan	0.39	0.30	2.1	3.3	6.6	2.2	2.7	5.4
AK000266	hypothetical protein	0.49	0.35	2.1	3.5	3.5	6.6	4.3	4.0

[00118] Table 33: Up-regulation of Polynucleotide expression in A549 cells induced by Formula C Peptides. The peptides at a concentration of 50 μg/ml were shown to increase the expression of many polynucleotides. Peptide was incubated with the human A549 epithelial cells for 4 h and the RNA was isolated, converted into labeled cDNA probes and hybridized to Human Operon arrays (PRHU04). The intensity of polynucleotides in control, unstimulated cells are shown in the second and third columns for labeling of cDNA with the dyes Cy3 and Cy5 respectively. The "ID#: Control" columns refer to the intensity of polynucleotide expression in peptide-simulated cells divided by the intensity of unstimulated cells.

Accession Number	Gene	control-Cy3	control-Cy5	ID 19: control	ID 20: control	ID 21: control	ID 22: control	ID 23: control	ID 24: control
NM_014139	sodium channel voltage-gated,	0.04	0.05	31.6	25.2	18.0	9.7	22.2	11.2
X84003	TATA box binding protein	0.47	0.07	31.8	12.7	2.5	2.8	18.0	14.2
AF144412	lens epithelial cell protein	0.25	0.07	23.9	8.0	6.8	3.4	16.2	3.5
AL080107	unknown	0.11	0.06	17.8	34.4	12.4	6.2	5.4	7.9
AF052116	unknown	0.34	0.07	15.5	3.9	9.2	3.0	6.9	2.7
AB033063	unknown	0.46	0.13	15.2	10.3	4.0	2.6	7.2	11.2
AK000258	hypothetical protein	0.27	0.07	13.9	8.0	3.5	3.4	26.5	11.5
NM_006963	zinc finger protein	0.10	0.08	12.8	6.8	6.2	5.9	17.2	1241.2
NM_014099	PRO1768 protein	0.30	0.06	12.3	17.4	5.4	5.4	19.5	3.4
AK000996	hypothetical protein	0.17	0.07	10.0	8.0	9.7	7.4	20.7	16.3
M81933	cell division cycle 25A	0.13	0.21	8.8	7.8	19.6	15.6	4.8	3.8
AF181286	unknown	0.05	0.22	8.8	2.7	12.0	35.6	5.9	2.3
AJ272208	IL-1R accessory	0.22	0.17	8.8	2.9	5.0	3.2	9.8	7.3

Accession Number	Gene	control-Cy3	control-Cy5	ID 19: control	ID 20: control	ID 21: control	ID 22: control	ID 23: control	ID 24: control
	protein-like 2								
AF030555	fatty-acid-Coenzyme A ligase	0.10	0.39	8.7	2.2	11.3	9.9	3.0	2.1
AL050125	unknown	0.23	0.07	8.6	14.3	5.2	2.8	18.7	8.3
AB011096	KIAA0524 protein	0.21	0.08	8.5	24.4	4.7	6.8	10.4	7.5
J03068	N-acylaminoacyl-peptide hydrolase	0.54	0.21	8.3	2.4	2.2	4.1	3.0	6.0
M33906	MHC class II, DQ alpha 1	0.14	0.08	7.6	4.5	15.2	6.1	7.5	7.9
AJ272265	secreted phosphoprotein	0.21	0.09	7.6	9.0	3.3	4.9	18.8	14.5
J00210	interferon alpha 13	0.41	0.07	7.2	15.0	2.8	3.1	11.0	4.3
AK001952	hypothetical protein	0.42	0.21	6.9	4.9	2.5	3.1	7.6	4.5
X54131	protein tyrosine phosphatase, receptor type,	0.09	0.20	6.4	6.5	7.7	15.0	5.6	4.1
AF064493	LIM binding domain	0.46	0.14	5.9	5.6	2.2	2.9	8.5	5.8

Accession Number	Gene	control-Cy3	control-Cy5	ID 19: control	ID 20: control	ID 21: control	ID 22: control	ID 23: control	ID 24: control
	2								
AL117567	DKFZP566O084 protein	0.44	0.22	5.8	3.3	2.9	2.3	5.7	14.9
L40933	phosphoglucomutase 5	0.16	0.03	5.6	11.0	4.8	3.5	8.5	76.3
M27190	regenerating islet-derived 1 alpha	0.19	0.28	5.3	3.0	3.8	3.6	5.8	3.6
AL031121	unknown	0.24	0.09	5.3	3.8	3.2	3.9	3.0	27.9
U27655	regulator of G-protein signalling	0.24	0.29	5.0	9.0	4.5	8.3	4.2	4.5
AB037786	unknown	0.12	0.03	4.7	54.1	2.8	2.3	2.2	11.0
X73113	myosin-binding protein C	0.29	0.13	4.7	6.5	6.0	2.4	6.7	6.3
AB010962	matrix metalloproteinase	0.08	0.12	4.7	6.2	2.4	4.7	10.9	4.2
AL096729	unknown	0.36	0.13	4.7	7.7	3.2	2.4	6.3	6.2
AB018320	Arg/Abl-interacting	0.16	0.18	4.6	7.1	3.0	3.3	5.8	8.9

Accession Number	Gene	control-Cy3	control-Cy5	ID 19: control	ID 20: control	ID 21: control	ID 22: control	ID 23: control	ID 24: control
	protein								
AK001024	guanine nucleotide-binding protein	0.16	0.11	4.6	2.0	9.8	2.6	7.6	14.1
AJ275355	unknown	0.15	0.08	4.6	17.3	5.4	9.2	5.1	5.5
U21931	fructose-bisphosphatase 1	0.48	0.14	4.6	4.3	2.6	2.1	8.4	9.6
X66403	cholinergic receptor	0.17	0.19	4.4	9.0	10.9	9.3	5.1	6.7
X67734	contactin 2	0.25	0.09	4.3	6.8	3.1	5.8	7.9	8.4
U92981	unknown	0.20	0.23	4.3	3.2	4.8	5.6	5.4	6.3
X68879	empty spiracles homolog 1	0.05	0.08	4.3	2.0	12.3	2.7	5.6	4.7
AL137362	unknown	0.22	0.22	4.2	4.1	2.7	4.1	9.3	4.2
NM_001756	corticosteroid binding globulin	0.28	0.13	4.1	10.6	3.9	2.7	10.3	5.5
U80770	unknown	0.31	0.14	4.1	4.1	23.3	2.7	7.0	10.1
AL109792	unknown	0.16	0.19	4.0	4.5	4.3	8.8	8.7	3.9
X65962	cytochrome P-450	0.33	0.05	3.8	25.3	5.7	5.1	19.8	12.0

Accession Number	Gene	control-Cy3	control-Cy5	ID 19: control	ID 20: control	ID 21: control	ID 22: control	ID 23: control	ID 24: control
AK001856	unknown	0.40	0.21	3.8	7.0	2.6	3.1	2.9	7.8
AL022723	MHC, class I, F	0.55	0.18	3.7	5.7	4.4	2.3	3.3	5.2
D38449	putative G protein coupled receptor	0.18	0.09	3.5	11.1	13.3	5.8	4.8	5.2
AL137489	unknown	0.74	0.26	3.3	2.9	2.6	3.3	2.5	5.4
AB000887	small inducible cytokine subfamily A	0.76	0.18	3.3	5.0	2.6	2.4	5.9	10.3
NM_012450	sulfate transporter 1	0.15	0.10	3.3	9.0	10.0	10.9	4.6	8.7
U86529	glutathione S-transferase zeta 1	0.55	0.15	3.2	6.8	4.4	2.3	9.3	5.1
AK001244	unknown	0.79	0.31	3.2	5.5	2.3	2.3	3.9	2.8
AL133602	unknown	0.16	0.21	3.1	7.8	8.7	2.6	4.1	5.6
AB033080	cell cycle progression 8 protein	0.31	0.31	3.1	4.6	3.0	3.5	2.2	4.2
AF023466	putative glycine-N-	0.27	0.18	3.1	5.0	4.2	7.4	10.1	3.8

Accession Number	Gene	control-Cy3	control-Cy5	ID 19: control	ID 20: control	ID 21: control	ID 22: control	ID 23: control	ID 24: control
	acyltransferase								
AL117457	cofilin 2	0.68	0.53	3.0	4.6	3.3	2.4	7.4	3.4
AC007059	unknown	0.37	0.35	3.0	5.7	3.1	2.4	2.6	2.4
U60179	growth hormone receptor	0.34	0.21	2.9	3.5	2.3	3.1	8.0	4.7
M37238	phospholipase C, gamma 2	0.60	0.36	2.9	2.0	3.2	2.1	2.9	4.6
L22569	cathepsin B	0.32	0.12	2.9	2.1	6.2	3.0	13.1	16.7
M80359	MAP/microtubule affinity-regulating kinase 3	0.37	0.76	2.9	3.1	6.1	7.6	2.1	3.3
S70348	Integrin beta 3	0.58	0.31	2.6	4.8	4.1	2.6	2.6	2.6
L13720	growth arrest-specific 6	0.36	0.26	2.4	2.5	6.8	4.8	3.9	3.7
AL049423	unknown	0.33	0.30	2.4	3.7	3.8	2.8	2.9	3.4
AL050201	unknown	0.68	0.29	2.2	3.1	3.7	3.0	3.0	2.2
AF050078	growth arrest	0.87	0.33	2.1	8.4	2.5	2.2	2.6	4.4

Accession Number	Gene	control-Cy3	control-Cy5	ID 19: control	ID 20: control	ID 21: control	ID 22: control	ID 23: control	ID 24: control
	specific 11								
AK001753	hypothetical protein	0.53	0.28	2.1	5.0	2.2	2.8	3.6	4.6
X05323	unknown	0.39	0.13	2.1	7.8	2.6	2.4	21.5	3.5
AB014548	KIAA0648 protein	0.61	0.30	2.0	2.4	4.8	3.4	4.9	3.9

[00119] Table 34: Up-regulation of Polynucleotide expression in A549 cells induced by Formula D Peptides. The peptides at a concentration of 50 μg/ml were shown to increase the expression of many polynucleotides. Peptide was incubated with the human A549 epithelial cells for 4 h and the RNA was isolated, converted into labeled cDNA probes and hybridized to Human Operon arrays (PRHU04). The intensity of polynucleotides in control, unstimulated cells are shown in the second and third columns for labeling of cDNA with the dyes Cy3 and Cy5 respectively. The "ID#: Control" columns refer to the intensity of polynucleotide expression in peptide-simulated cells divided by the intensity of unstimulated cells.

Accession Number	Gene	control -Cy3	control-Cy5	ID 26: control	ID 27: control	ID 28: control	ID 29: control	ID 30: control	ID 31: control
U68018	MAD	0.13	0.71	11.2	2.2	8.0	2.3	6.7	25.6

Accession Number	Gene	control -Cy3	control-Cy5	ID 26: control	ID 27: control	ID 28: control	ID 29: control	ID 30: control	ID 31: control
	homolog 2								
NM_016015	CGI-68 protein	0.92	1.59	2.3	2.3	3.5	3.7	3.4	22.9
AF071510	lecithin retinol acyltransferase	0.07	0.05	15.4	10.3	5.3	44.1	2.1	21.2
AC005154	unkown	0.17	1.13	2.7	7.2	12.6	6.4	3.3	20.6
M81933	cell division cycle 25A	0.13	0.21	4.3	3.1	3.2	4.3	5.6	18.2
AF124735	LIM HOX gene 2	0.17	0.21	2.1	4.4	5.9	5.2	7.6	17.0
AL110125	unknown	0.30	0.08	5.0	2.7	6.8	10.2	2.8	12.0
NM_004732	potassium voltage-gated channel	0.15	0.16	7.6	4.0	3.4	2.2	2.9	11.4
AF030555	fatty-acid-Coenzyme A	0.10	0.39	10.5	2.2	6.4	3.0	5.1	10.7

Accession Number	Gene	control -Cy3	control-Cy5	ID 26: control	ID 27: control	ID 28: control	ID 29: control	ID 30: control	ID 31: control
	ligase_ long-chain 4								
AF000237	1-acylglycerol-3-phosphate O-acyltransferase 2	1.80	2.37	3.4	2.5	2.4	2.1	3.7	9.9
AL031588	hypothetical protein	0.40	0.26	5.8	20.2	2.8	4.7	5.6	9.1
AL080077	unknown	0.15	0.21	2.4	2.0	11.9	3.8	2.3	8.7
NM_014366	putative nucleotide binding protein_ estradiol-induced	0.90	2.52	2.4	4.3	2.4	2.6	3.0	8.6

Accession Number	Gene	control -Cy3	control-Cy5	ID 26: control	ID 27: control	ID 28: control	ID 29: control	ID 30: control	ID 31: control
AB002359	phosphoribosylformylglycinamidine synthase	0.81	2.12	3.2	2.7	5.5	2.5	2.8	6.9
U33547	MHC class II antigen HLA-DRB6 mRNA_	0.14	0.16	2.5	5.3	4.5	5.0	3.1	6.6
AL133051	unknown	0.09	0.07	7.7	6.3	5.4	23.1	5.4	6.5
AK000576	hypothetical protein	0.27	0.06	7.1	9.3	5.0	6.9	2.9	6.2
AF042378	spindle pole body protein	0.36	0.39	3.3	3.0	9.5	4.5	3.4	6.2
AF093265	Homer neuronal immediate early gene_ 3	0.67	0.53	2.7	13.3	6.5	5.0	2.9	6.2

Accession Number	Gene	control -Cy3	control-Cy5	ID 26: control	ID 27: control	ID 28: control	ID 29: control	ID 30: control	ID 31: control
D80000	Segregation of mitotic chromosomes 1	1.01	1.56	3.6	2.5	4.9	3.2	6.3	6.1
AF035309	proteasome 26S subunit ATPase 5	3.61	4.71	2.7	6.6	5.2	4.9	2.7	6.0
M34175	adaptor-related protein complex 2 beta 1 subunit	4.57	5.13	3.2	3.1	4.0	4.6	2.7	6.0
AB020659	KIAA0852 protein	0.18	0.37	4.1	7.6	5.7	4.8	2.5	5.7
NM_004862	LPS-induced TNF-alpha factor	2.61	3.36	3.8	4.8	4.1	4.9	3.2	5.6
U00115	zinc finger	0.51	0.07	18.9	2.2	3.5	7.2	21.2	5.6

Accession Number	Gene	control -Cy3	control-Cy5	ID 26: control	ID 27: control	ID 28: control	ID 29: control	ID 30: control	ID 31: control
	protein 51								
AF088868	fibrousheathin II	0.45	0.20	4.7	10.0	3.2	6.4	6.0	5.6
AK001890	unknown	0.42	0.55	2.4	3.5	3.6	2.3	2.2	5.6
AL137268	KIAA0759 protein	0.49	0.34	3.8	2.3	5.0	3.5	3.3	5.4
X63563	polymerase II polypeptide B	1.25	1.68	2.5	8.1	3.4	4.8	5.2	5.4
D12676	CD36 antigen	0.35	0.39	2.9	3.4	2.6	2.2	3.5	5.3
AK000161	hypothetical protein	1.06	0.55	3.4	8.7	2.1	6.7	2.9	5.1
AF052138	unknown	0.64	0.51	2.9	2.8	2.7	5.2	3.6	5.0
AL096803	unknown	0.36	0.03	20.1	18.3	3.7	19.3	16.1	4.9
S49953	DNA-binding transcriptional activator	0.70	0.15	3.7	4.0	2.1	6.6	4.0	4.8
X89399	RAS p21	0.25	0.10	8.5	14.9	4.8	18.6	4.3	4.8

Accession Number	Gene	control -Cy3	control-Cy5	ID 26: control	ID 27: control	ID 28: control	ID 29: control	ID 30: control	ID 31: control
	protein activator								
AJ005273	antigenic determinant of recA protein	0.70	0.10	7.6	11.1	2.8	9.9	12.0	4.6
AK001154	hypothetical protein	1.70	0.96	2.4	4.4	2.9	8.9	2.4	4.5
AL133605	unknown	0.26	0.15	12.4	4.2	4.4	3.3	3.3	4.1
U71092	G protein-coupled receptor 24	0.53	0.06	19.0	9.1	2.2	12.0	3.3	4.1
AF074723	RNA polymerase II transcriptional regulation mediator	0.67	0.54	4.0	3.2	3.1	3.4	6.0	4.0
AL137577	unknown	0.32	0.12	31.4	6.2	5.3	10.1	25.3	3.9

Accession Number	Gene	control -Cy3	control-Cy5	ID 26: control	ID 27: control	ID 28: control	ID 29: control	ID 30: control	ID 31: control
AF151043	hypothetical protein	0.48	0.35	2.6	2.2	2.0	3.3	2.2	3.8
AF131831	unknown	0.67	0.81	2.1	7.0	3.5	3.2	3.9	3.7
D50405	histone deacetylase 1	1.52	2.62	3.1	7.2	2.9	4.1	2.8	3.7
U78305	protein phosphatase 1D	1.21	0.20	4.7	13.0	3.5	5.9	4.2	3.7
AL035562	paired box gene 1	0.24	0.01	30.2	81.9	5.6	82.3	6.2	3.7
U67156	mitogen-activated protein kinase kinase kinase 5	1.15	0.30	6.6	3.0	2.2	2.3	2.5	3.6
AL031121	unknown	0.24	0.09	5.2	3.7	2.3	6.5	9.1	3.6
U13666	G protein-	0.34	0.14	3.8	5.4	3.1	3.3	2.8	3.6

Accession Number	Gene	control -Cy3	control-Cy5	ID 26: control	ID 27: control	ID 28: control	ID 29: control	ID 30: control	ID 31: control
	coupled receptor 1								
AB018285	KIAA0742 protein	0.53	0.13	14.9	13.9	5.9	18.5	15.2	3.5
D42053	site-1 protease	0.63	0.40	2.6	7.1	5.6	9.2	2.6	3.5
AK001135	Sec23-interacting protein p125	0.29	0.53	5.7	4.5	3.4	2.6	11.3	3.4
AL137461	unknown	0.25	0.02	23.8	9.0	2.7	59.2	12.5	3.3
NM_006963	zinc finger protein 22	0.10	0.08	3.2	7.6	3.7	7.9	11.2	3.2
AL137540	unknown	0.67	0.79	3.9	2.6	5.6	4.2	3.5	3.1
AL137718	unknown	0.95	0.18	4.7	8.0	4.0	13.3	3.0	3.1
AF012086	RAN binding protein 2-like 1	1.20	0.59	4.6	4.0	2.0	4.6	3.6	3.1
S57296	HER2/neu	0.59	0.17	7.3	12.1	2.3	20.0	22.2	3.0

Accession Number	Gene	control -Cy3	control-Cy5	ID 26: control	ID 27: control	ID 28: control	ID 29: control	ID 30: control	ID 31: control
	receptor								
NM_013329	GC-rich sequence DNA-binding factor candidate	0.16	0.08	6.9	14.3	9.7	3.3	7.2	3.0
AF038664	UDP-Gal:betaGlcN Ac beta 1_4-galactosyltran sferase	0.15	0.03	13.4	22.2	5.4	15.8	17.6	3.0
AF080579	Homo sapiens integral membrane protein	0.34	1.03	3.3	3.0	6.7	2.1	2.9	2.9
AK001075	hypothetical protein	0.67	0.10	2.1	2.6	2.6	8.9	2.2	2.9

Accession Number	Gene	control -Cy3	control-Cy5	ID 26: control	ID 27: control	ID 28: control	ID 29: control	ID 30: control	ID 31: control
AB011124	KIAA0552 gene product	0.46	0.04	9.6	72.0	6.0	33.9	13.6	2.9
J03068	N-acylaminoacyl-peptide hydrolase	0.54	0.21	2.2	5.0	2.4	5.2	3.6	2.8
D87120	osteoblast protein	0.87	0.87	2.2	2.0	4.7	2.3	2.0	2.8
AB006537	IL-1R accessory protein	0.17	0.07	2.9	7.0	14.5	5.3	6.6	2.8
L34587	transcription elongation factor B	2.49	1.23	2.2	16.3	5.0	15.8	5.5	2.7
D31891	SET domain_ bifurcated_1	1.02	0.29	3.9	6.0	4.3	4.9	6.6	2.7
D00760	proteasome	4.97	4.94	4.1	2.6	2.0	2.8	2.7	2.7

Accession Number	Gene	control -Cy3	control-Cy5	ID 26: control	ID 27: control	ID 28: control	ID 29: control	ID 30: control	ID 31: control
	subunit_ alpha type_ 2								
AC004774	distal-less homeo box 5	0.25	0.12	2.3	6.3	3.8	5.2	5.2	2.6
AL024493	unknown	1.46	0.54	4.8	13.5	2.1	11.6	6.8	2.6
AB014536	copine III	1.80	1.29	3.2	9.5	3.8	6.8	2.6	2.6
X59770	IL-1R type II	0.59	0.16	9.6	4.7	3.9	3.2	4.9	2.5
AF052183	unknown	0.65	0.76	4.0	3.7	2.3	5.0	3.0	2.5
AK000541	hypothetical protein	0.92	0.27	4.5	13.9	3.6	18.1	4.3	2.5
U88528	cAMP responsive element binding protein	1.37	0.86	3.1	5.4	2.1	2.8	2.1	2.4
M97925	defensin alpha 5_ Paneth	0.33	0.07	4.6	35.9	2.0	7.8	6.5	2.4

Accession Number	Gene	control -Cy3	control-Cy5	ID 26: control	ID 27: control	ID 28: control	ID 29: control	ID 30: control	ID 31: control
	cell-specific								
NM_013393	cell division protein FtsJ	1.38	0.94	3.1	5.8	2.1	4.2	2.6	2.3
X62744	MHC class II DM alpha	0.86	0.32	4.0	4.7	2.3	2.9	6.1	2.3
AF251040	putative nuclear protein	0.64	0.30	6.7	3.4	2.9	3.9	5.7	2.2
AK000227	hypothetical protein	1.49	0.43	3.4	7.1	2.3	3.3	9.1	2.1
U88666	SFRS protein kinase 2	1.78	0.37	3.4	5.9	2.6	8.4	6.1	2.0

[00120] Table 35: Up-regulation of Polynucleotide expression in A549 cells induced by Formula E Peptides. The peptides at a concentration of 50 μg/ml were shown to increase the expression of many polynucleotides. Peptide was incubated with the human A549 epithelial cells for 4 h and the RNA was isolated, converted into labeled cDNA probes and hybridized to Human Operon arrays (PRHU04). The intensity of polynucleotides in control, unstimulated cells are shown in the second and third columns for labeling of

cDNA with the dyes Cy3 and Cy5 respectively. The "ID#: Control" columns refer to the intensity of polynucleotide expression in peptide-simulated cells divided by the intensity of unstimulated cells.

Accession Number	Gene	control-Cy3	control-Cy5	ID 33: control	ID 34: control	ID 35: control	ID 36: control	ID 37: control	ID 38: control
AL049689	Novel human mRNA	0.25	0.05	2.7	26.5	3.3	21.7	5.4	37.9
AK000576	hypothetical protein	0.27	0.06	3.0	19.1	3.9	23.0	3.1	28.3
X74837	mannosidase, alpha class 1A member 1	0.10	0.07	5.6	10.0	10.8	12.3	12.0	19.9
AK000258	hypothetical protein	0.27	0.07	14.0	11.1	7.9	16.1	6.2	18.9
X89067	transient receptor	0.20	0.14	3.7	2.2	2.4	2.6	8.0	18.1
AL137619	unknown	0.16	0.08	6.3	6.7	10.8	10.5	7.9	16.5
NM_003445	zinc finger protein	0.17	0.07	4.0	23.6	2.9	13.6	4.3	14.4
X03084	complement	0.36	0.15	2.4	3.1	2.9	7.7	3.4	13.7

Accession Number	Gene	control-Cy3	control-Cy5	ID 33: control	ID 34: control	ID 35: control	ID 36: control	ID 37: control	ID 38: control
	component 1								
U27330	fucosyltransferase 5	0.39	0.08	2.4	2.5	2.6	12.1	3.5	13.0
AF070549	unknown	0.16	0.09	2.7	4.7	7.9	10.3	4.2	12.6
AB020335	sel-1 -like	0.19	0.24	2.9	2.6	2.0	7.3	4.7	12.4
M26901	renin	0.09	0.12	14.9	2.2	7.3	12.0	20.8	12.0
Y07828	ring finger protein	0.09	0.06	9.0	26.6	8.9	16.0	3.6	11.6
AK001848	hypothetical protein	0.21	0.07	6.2	8.2	2.7	5.2	5.5	10.9
NM_016331	zinc finger protein	0.16	0.08	7.6	5.1	7.0	25.5	5.5	10.9
U75330	neural cell adhesion molecule 2	0.42	0.08	2.5	3.6	2.0	5.8	6.2	9.9
AB037826	unknown	0.16	0.11	3.8	6.0	3.4	13.4	6.0	9.8
M34041	adrenergic alpha-	0.30	0.13	4.5	4.5	3.7	8.6	5.6	9.8

Accession Number	Gene	control-Cy3	control-Cy5	ID 33: control	ID 34: control	ID 35: control	ID 36: control	ID 37: control	ID 38: control
	2B- receptor								
D38449	putative G protein coupled receptor	0.18	0.09	2.3	25.8	11.7	2.3	3.2	9.5
AJ250562	transmembrane 4 superfamily member 2	0.13	0.10	10.0	8.4	2.2	8.1	16.3	9.1
AK001807	hypothetical protein	0.18	0.12	4.2	5.3	4.6	3.2	4.0	8.3
AL133051	unknown	0.09	0.07	5.1	13.6	6.0	9.1	2.2	8.2
U43843	Neuro-d4 homolog	0.61	0.10	2.0	6.4	2.3	16.6	2.2	8.1
NM_013227	aggrecan 1	0.28	0.15	7.5	3.1	2.5	6.9	8.5	7.8
AF226728	somatostatin receptor-interacting protein	0.23	0.17	7.0	3.6	3.1	5.5	3.5	7.7

Accession Number	Gene	control-Cy3	control-Cy5	ID 33: control	ID 34: control	ID 35: control	ID 36: control	ID 37: control	ID 38: control
AK001024	guanine nucleotide-binding protein	0.16	0.11	3.9	12.3	2.7	7.4	3.3	7.0
AC002302	unknown	0.13	0.14	16.1	5.8	5.8	2.6	9.6	6.2
AB007958	unknown	0.17	0.27	2.0	2.3	11.3	3.3	3.0	6.1
AF059293	cytokine receptor-like factor 1	0.19	0.22	3.6	2.5	10.2	3.8	2.7	5.9
V01512	v-fos	0.27	0.21	6.7	3.7	13.7	9.3	3.7	5.4
U82762	sialyltransferase 8	0.23	0.15	3.2	6.5	2.7	9.2	5.7	5.4
U44059	thyrotrophic embryonic factor	0.05	0.13	22.9	7.1	12.5	7.4	9.7	5.4
X05323	antigen identified by monoclonal antibody	0.39	0.13	4.3	2.5	2.2	7.4	2.8	5.1
U72671	ICAM 5,	0.25	0.14	5.3	2.7	3.7	10.0	3.2	4.8
AL133626	hypothetical	0.26	0.25	2.2	4.2	2.9	3.0	2.6	4.7

Accession Number	Gene	control-Cy3	control-Cy5	ID 33: control	ID 34: control	ID 35: control	ID 36: control	ID 37: control	ID 38: control
	protein								
X96401	MAX binding protein	0.31	0.29	6.9	2.3	4.9	3.1	2.9	4.6
AL117533	unknown	0.05	0.26	8.2	2.7	11.1	2.5	11.9	4.5
AK001550	hypothetical protein	0.10	0.30	8.0	2.0	4.9	2.1	7.8	4.5
AB032436	Homo sapiens BNPI mRNA	0.14	0.21	5.1	2.2	9.1	4.5	6.4	4.4
AL035447	hypothetical protein	0.28	0.23	4.3	3.7	8.7	5.2	3.7	4.2
U09414	zinc finger protein	0.28	0.25	4.0	2.2	4.7	3.3	7.2	4.2
AK001256	unknown	0.09	0.08	5.3	6.5	31.1	12.7	6.4	4.1
L14813	carboxyl ester lipase-like	0.64	0.21	2.7	6.2	3.1	2.1	3.4	3.9
AF038181	unknowan	0.06	0.18	34.1	6.4	4.5	8.7	11.3	3.9
NM_001486	glucokinase	0.21	0.08	3.0	2.2	6.5	12.4	5.7	3.9

Accession Number	Gene	control-Cy3	control-Cy5	ID 33: control	ID 34: control	ID 35: control	ID 36: control	ID 37: control	ID 38: control
AB033000	hypothetical protein	0.24	0.22	3.4	3.3	7.1	5.5	4.5	3.8
AL117567	DKFZP566O084 protein	0.44	0.22	2.2	2.7	3.9	4.0	4.5	3.7
NM_012126	carbohydrate sulfotransferase 5	0.31	0.20	5.5	5.4	3.8	5.5	2.6	3.5
AL031687	unknown	0.16	0.27	5.9	2.6	3.4	2.3	4.9	3.5
X04506	apolipoprotein B	0.29	0.32	5.4	4.4	6.9	5.5	2.1	3.5
NM_006641	CCR 9	0.35	0.11	3.3	3.3	2.2	16.5	2.3	3.5
Y00970	acrosin	0.12	0.14	8.2	8.8	3.1	6.2	17.5	3.4
X67098	rTS beta protein	0.19	0.26	2.4	3.1	7.8	3.5	4.4	3.3
U51990	pre-mRNA splicing factor	0.56	0.19	2.2	3.0	2.8	13.7	2.9	3.0
AF030555	fatty-acid-Coenzyme A	0.10	0.39	3.5	6.9	13.3	4.4	7.5	2.9
AL009183	TNFR superfamily,	0.46	0.19	6.0	4.1	2.8	8.6	2.6	2.8

Accession Number	Gene	control-Cy3	control-Cy5	ID 33: control	ID 34: control	ID 35: control	ID 36: control	ID 37: control	ID 38: control
	member 9								
AF045941	sciellin	0.16	0.21	11.6	2.4	2.8	2.2	4.1	2.8
AF072756	A kinase anchor protein 4	0.33	0.07	2.5	5.3	3.9	32.7	2.3	2.7
X78678	ketohexokinase	0.10	0.20	18.0	3.5	4.1	2.5	14.6	2.6
AL031734	unknown	0.03	0.39	43.7	2.3	41.7	4.0	10.8	2.5
D87717	KIAA0013 gene product	0.35	0.42	4.2	2.3	3.6	2.6	2.9	2.5
U01824	solute carrier family 1	0.42	0.29	4.8	2.3	4.2	7.1	4.2	2.4
AF055899	solute carrier family 27	0.14	0.31	9.5	12.3	7.4	4.7	6.6	2.3
U22526	lanosterol synthase	0.09	0.45	4.1	3.4	10.4	2.2	17.9	2.3
AB032963	unknown	0.19	0.34	6.3	6.1	2.9	2.1	5.7	2.2
NM_015974	lambda-crystallin	0.17	0.25	11.4	2.8	5.9	2.4	5.8	2.2
X82200	stimulated trans-	0.23	0.15	8.2	3.4	3.0	2.8	11.3	2.2

Accession Number	Gene	control-Cy3	control-Cy5	ID 33: control	ID 34: control	ID 35: control	ID 36: control	ID 37: control	ID 38: control
	acting factor								
AL137522	unknown	0.12	0.26	12.1	3.7	12.6	6.9	4.3	2.2
Z99916	crystallin, beta B3	0.28	0.65	2.5	2.1	3.6	2.2	2.6	2.1
AF233442	ubiquitin specific protease 21	0.41	0.31	2.6	3.6	3.6	4.5	3.4	2.1
AK001927	hypothetical protein	0.24	0.52	7.6	5.6	5.0	2.5	4.1	2.0

[00121] Table 36: Up-regulation of Polynucleotide expression in A549 cells induced by Formula F Peptides. The peptides at a concentration of 50 μg/ml were shown to increase the expression of many polynucleotides. Peptide was incubated with the human A549 epithelial cells for 4 h and the RNA was isolated, converted into labeled cDNA probes and hybridized to Human Operon arrays (PRHU04). The intensity of polynucleotides in control, unstimulated cells are shown in the second and third columns for labeling of cDNA with the dyes Cy3 and Cy5 respectively. The "Ratio ID#: Control" columns refer to the intensity of polynucleotide expression in peptide-simulated cells divided by the intensity of unstimulated cells.

Accession Number	Gene	control-Cy3	control-Cy5	Ratio ID 40: control	Ratio ID 42: control	Ratio ID 43: control	Ratio ID 44: control	Ratio ID 45: control
AF025840	polymerase epsilon 2	0.34	0.96	3.4	2.0	2.0	2.1	4.3
AF132495	CGI-133 protein	0.83	0.67	3.0	2.2	2.6	2.8	5.1
AL137682	hypothetical protein	0.73	0.40	2.0	5.3	4.8	2.9	8.2
U70426	regulator of G-protein signalling 16	0.23	0.25	3.1	3.0	5.3	3.1	12.2
AK001135	Sec23-interacting protein p125	0.29	0.53	3.2	2.6	3.3	14.4	5.2
AB023155	KIAA0938 protein	0.47	0.21	2.7	4.8	8.1	4.2	10.4
AB033080	cell cycle progression 8 protein	0.31	0.31	4.4	2.2	5.9	4.3	6.9
AF061836	Ras association domain family 1	0.29	0.31	3.2	2.5	11.1	18.8	6.8

Accession Number	Gene	control-Cy3	control-Cy5	Ratio ID 40: control	Ratio ID 42: control	Ratio ID 43: control	Ratio ID 44: control	Ratio ID 45: control
AK000298	hypothetical protein	0.48	0.27	3.3	2.2	7.1	5.6	7.7
L75847	zinc finger protein	0.35	0.52	3.2	3.0	4.0	3.0	3.9
X97267	protein tyrosine phosphatase	0.19	0.24	4.1	9.3	2.4	4.2	8.3
Z11933	POU domain class 3 TF 2	0.09	0.23	8.7	2.5	3.6	4.3	8.2
AB037744	unknown	0.37	0.57	2.6	2.9	2.7	3.0	3.1
U90908	unknown	0.12	0.16	11.8	7.7	3.4	7.8	11.2
AL050139	unknown	0.29	0.60	5.2	2.4	3.3	3.0	2.8
AB014615	fibroblast growth factor 8	0.19	0.07	5.4	3.5	8.5	3.2	22.7
M28825	CD1A antigen	0.51	0.36	4.1	2.6	2.0	4.6	4.4
U27330	fucosyltransferase 5	0.39	0.08	3.3	2.1	24.5	8.2	19.3
NM_0069	zinc finger protein	0.10	0.08	10.4	12.6	12.3	29.2	20.5

Accession Number	Gene	control-Cy3	control-Cy5	Ratio ID 40: control	Ratio ID 42: control	Ratio ID 43: control	Ratio ID 44: control	Ratio ID 45: control
63								
AF093670	peroxisomal biogenesis factor	0.44	0.53	4.0	2.6	2.6	4.3	2.9
AK000191	hypothetical protein	0.50	0.18	2.3	3.6	4.4	2.2	8.2
AB022847	unknown	0.39	0.24	2.1	6.9	4.5	2.8	6.2
AK000358	microfibrillar-associated protein 3	0.28	0.28	5.7	2.0	3.5	5.2	5.2
X74837	mannosidase_ alpha class 1A	0.10	0.07	13.1	18.4	23.6	16.3	20.8
AF053712	TNF superfamily_ member 11	0.17	0.08	11.3	9.3	13.4	10.6	16.6
AL133114	DKFZP586P2421 protein	0.11	0.32	8.5	3.4	4.9	5.3	4.3
AF049703	E74-like factor 5	0.22	0.24	5.1	6.0	3.3	2.7	5.4

Accession Number	Gene	control-Cy3	control -Cy5	Ratio ID 40: control	Ratio ID 42: control	Ratio ID 43: control	Ratio ID 44: control	Ratio ID 45: control
AL137471	hypothetical protein	0.29	0.05	4.0	15.0	10.1	2.7	25.3
AL035397	unknown	0.33	0.14	2.3	2.8	10.6	4.6	9.3
AL035447	hypothetical protein	0.28	0.23	3.8	6.8	2.7	3.0	5.7
X55740	CD73	0.41	0.61	2.1	3.3	2.9	3.2	2.1
NM_004909	taxol resistance associated gene 3	0.20	0.22	3.9	2.9	6.5	3.2	5.6
AF233442	ubiquitin specific protease	0.41	0.31	2.9	4.7	2.7	3.5	3.9
U92980	unknown	0.83	0.38	4.2	4.1	4.8	2.3	3.1
AF105424	myosin heavy polypeptide-like	0.30	0.22	2.8	3.3	4.4	2.3	5.3
M26665	histatin 3	0.29	0.26	7.9	3.5	4.6	3.5	4.5
AF083898	neuro-oncological ventral antigen 2	0.20	0.34	18.7	3.8	2.2	3.6	3.5

Accession Number	Gene	control-Cy3	control-Cy5	Ratio ID 40: control	Ratio ID 42: control	Ratio ID 43: control	Ratio ID 44: control	Ratio ID 45: control
AJ009771	ariadne_ Drosophila_ homolog of	0.33	0.06	2.3	17.6	15.9	2.5	20.3
AL022393	hypothetical protein P1	0.05	0.33	32.9	2.4	3.0	69.4	3.4
AF039400	chloride channel_ calcium activated_ family member 1	0.11	0.19	8.4	2.9	5.1	18.1	5.9
AJ012008	dimethylarginine dimethylaminohydrolase 2	0.42	0.43	5.1	3.3	3.2	6.2	2.6
AK000542	hypothetical protein	0.61	0.24	2.1	4.5	5.0	3.7	4.4
AL133654	unknown	0.27	0.40	2.8	2.1	2.5	2.5	2.6
AL137513	unknown	0.43	0.43	6.4	3.2	3.8	2.3	2.3
U05227	GTP-binding	0.38	0.36	5.0	3.1	3.1	2.2	2.8

Accession Number	Gene	control-Cy3	control-Cy5	Ratio ID 40: control	Ratio ID 42: control	Ratio ID 43: control	Ratio ID 44: control	Ratio ID 45: control
	protein							
D38449	putative G protein coupled receptor	0.18	0.09	5.8	6.7	6.7	9.1	10.4
U80770	unknown	0.31	0.14	3.9	3.8	6.6	3.1	6.8
X61177	IL-5R alpha	0.40	0.27	2.6	4.4	9.8	8.1	3.6
U35246	vacuolar protein sorting 45A	0.15	0.42	5.8	2.8	2.6	4.5	2.2
AB017016	brain-specific protein p25 alpha	0.27	0.29	6.0	2.6	3.4	3.1	3.1
X82153	cathepsin K	0.45	0.20	4.2	5.2	4.8	4.4	4.6
AC005162	probable carboxypeptidase precursor	0.12	0.28	11.9	3.4	6.8	18.7	3.2
AL137502	unknown	0.22	0.16	3.9	4.9	7.3	3.9	5.3
U66669	3-hydroxyisobutyryl	0.30	0.40	10.3	3.5	5.2	2.3	2.1

Accession Number	Gene	control-Cy3	control-Cy5	Ratio ID 40: control	Ratio ID 42: control	Ratio ID 43: control	Ratio ID 44: control	Ratio ID 45: control
	-Coenzyme A hydrolase							
AK000102	unknown	0.39	0.30	2.8	5.3	5.2	4.1	2.8
AF034970	docking protein 2	0.28	0.05	3.3	8.5	15.7	4.0	17.3
AK000534	hypothetical protein	0.13	0.29	6.8	2.3	4.0	20.6	2.9
J04599	biglycan	0.39	0.30	4.0	3.7	4.0	4.8	2.8
AL133612	unknown	0.62	0.33	2.7	3.4	5.2	3.0	2.5
D10495	protein kinase C delta	0.18	0.10	12.0	20.7	8.7	6.8	8.1
X58467	cytochrome P450	0.07	0.24	15.4	4.7	7.9	34.4	3.4
AF131806	unknown	0.31	0.25	2.6	3.4	5.7	7.0	3.2
AK000351	hypothetical protein	0.34	0.13	4.0	6.9	5.5	2.8	6.3
AF075050	hypothetical protein	0.55	0.09	2.7	17.8	5.1	2.2	8.3

Accession Number	Gene	contr ol-Cy3	control -Cy5	Ratio ID 40: control	Ratio ID 42: control	Ratio ID 43: control	Ratio ID 44: control	Ratio ID 45: control
AK000566	hypothetical protein unknown	0.15	0.35	6.7	2.2	6.8	6.4	2.1
U43328	cartilage linking protein 1	0.44	0.19	2.5	6.2	6.9	7.8	3.8
AF045941	sciellin	0.16	0.21	6.8	7.5	4.8	6.9	3.4
U27655	regulator of G-protein signalling 3	0.24	0.29	5.5	4.9	2.9	4.9	2.4
AK000058	hypothetical protein	0.25	0.15	5.0	9.7	16.4	2.7	4.5
AL035364	hypothetical protein	0.32	0.26	4.4	4.2	7.3	2.8	2.6
AK001864	unknown	0.40	0.25	3.7	3.7	4.6	3.2	2.6
AB015349	unknown	0.14	0.24	10.5	2.8	3.7	8.0	2.7
V00522	MHC class II DR beta 3	0.62	0.22	4.8	3.9	4.7	2.5	3.0

Accession Number	Gene	control-Cy3	control-Cy5	Ratio ID 40: control	Ratio ID 42: control	Ratio ID 43: control	Ratio ID 44: control	Ratio ID 45: control
U75330	neural cell adhesion molecule 2	0.42	0.08	2.1	9.6	13.2	3.3	7.8
NM_007199	IL-1R-associated kinase M	0.15	0.25	8.7	7.8	8.6	16.1	2.5
D30742	calcium/calmodulin-dependent protein kinase IV	0.28	0.09	6.2	28.7	7.4	2.4	6.8
X05978	cystatin A	0.63	0.17	2.7	4.8	9.4	2.2	3.6
AF240467	TLR-7	0.11	0.10	13.8	13.3	4.7	7.7	4.9

[00122] Table 37: Up-regulation of Polynucleotide expression in A549 cells induced by Formula G and additional Peptides. The peptides at a concentration of 50 μg/ml were shown to increase the expression of many polynucleotides. Peptide was incubated with the human A549 epithelial cells for 4 h and the RNA was isolated, converted into labelled cDNA probes and hybridised to Human Operon arrays (PRHU04). The intensity of polynucleotides in control, unstimulated cells are shown in the second and third columns for labelling

of cDNA with the dyes Cy3 and Cy5 respectively. The "Ratio ID#: Control" columns refer to the intensity of polynucleotide expression in peptide-simulated cells divided by the intensity of unstimulated cells. Accession numbers and gene designations are U00115, zinc finger protein; M91036, hemoglobin gamma G;K000070, hypothetical protein; AF055899, solute carrier family 27; AK001490, hypothetical protein; X97674, nuclear receptor coactivator 2; AB022847, unknown; AJ275986, transcription factor; D10495, protein kinase C, delta; L36642, EphA7; M31166, pentaxin-related gene; AF176012, unknown; AF072756, A kinase anchor protein 4; NM_014439, IL-1 Superfamily z; AJ271351, putative transcriptional regulator; AK000576, hypothetical protein; AJ272265, secreted phosphoprotein 2; AL122038, hypothetical protein; AK000307, hypothetical protein; AB029001, KIAA1078 protein; U62437, cholinergic receptor; AF064854, unknown; AL031588, hypothetical protein; X89399, RAS p21 protein activator; D45399, phosphodiesterase; AB037716, hypothetical protein; X79981, cadherin 5; AF034208, RIG-like 7-1; AL133355, chromosome 21 open reading frame 53; NM_016281, STE20-like kinase; AF023614, transmembrane activator and CAML interactor; AF056717, ash2-like; AB029039, KIAA1116 protein; J03634, inhibin, beta A; U80764, unknown; AB032963, unknown; X82835, sodium channel, voltage-gated, type IX

Accession Number	control-Cy3	control-Cy5	ID 53: control	ID 54: control	ID 47: control	ID 48: control	ID 49: control	ID 50: control	ID 51: control	ID 52: control
U00115	0.51	0.07	27.4	7.3	2.4	3.1	4.8	8.3	3.5	20.0
M91036	0.22	0.02	39.1	32.5	5.2	2.2	37.0	6.0	16.2	18.0
AK000070	0.36	0.18	3.8	7.6	2.6	15.1	12.2	9.9	17.2	15.3
AF055899	0.14	0.31	6.7	3.7	9.7	10.0	2.2	16.7	5.4	14.8
AK001490	0.05	0.02	14.1	35.8	3.2	28.6	25.0	20.2	56.5	14.1
X97674	0.28	0.28	3.2	3.7	4.0	10.7	3.3	3.1	4.0	13.2

Accession Number	control-Cy3	control-Cy5	ID 53: control	ID 54: control	ID 47: control	ID 48: control	ID 49: control	ID 50: control	ID 51: control	ID 52: control
AB022847	0.39	0.24	4.1	4.4	4.5	2.7	3.7	10.4	5.0	11.3
AJ275986	0.26	0.35	5.8	2.3	5.7	2.2	2.5	9.7	4.3	11.1
D10495	0.18	0.10	8.0	3.4	4.6	2.0	6.9	2.5	12.7	10.3
L36642	0.26	0.06	5.8	14.2	2.6	4.1	8.9	3.4	6.5	6.6
M31166	0.31	0.12	4.8	3.8	12.0	3.6	9.8	2.4	8.8	6.4
AF176012	0.45	0.26	3.1	2.9	2.8	2.6	2.3	6.9	3.0	5.8
AF072756	0.33	0.07	9.9	9.3	4.4	4.3	3.2	4.9	11.9	5.4
NM_014439	0.47	0.07	12.0	7.1	3.3	3.3	4.7	5.9	5.0	5.4
AJ271351	0.46	0.12	3.4	3.5	2.3	4.7	2.3	2.7	6.9	5.2
AK000576	0.27	0.06	7.4	15.7	2.9	4.7	9.0	2.4	8.2	5.1
AJ272265	0.21	0.09	6.2	7.9	2.3	3.7	10.3	4.5	4.6	4.7
AL122038	0.46	0.06	6.7	4.5	2.6	4.3	16.4	6.5	26.6	4.6
AK000307	0.23	0.09	3.7	4.0	4.3	3.2	5.3	2.9	13.1	4.4
AB029001	0.52	0.21	14.4	4.3	4.6	4.4	4.8	21.9	3.2	4.2
U62437	0.38	0.13	12.6	6.5	4.2	6.7	2.2	3.7	4.8	3.9
AF064854	0.15	0.16	2.6	2.9	6.2	8.9	14.4	5.0	9.1	3.9
AL031588	0.40	0.26	8.3	5.2	2.8	3.3	5.3	9.0	5.6	3.4

Accession Number	control-Cy3	control-Cy5	ID 53: control	ID 54: control	ID 47: control	ID 48: control	ID 49: control	ID 50: control	ID 51: control	ID 52: control
X89399	0.25	0.10	15.8	12.8	7.4	4.2	16.7	6.9	12.7	3.3
D45399	0.21	0.18	3.0	4.7	3.3	4.4	8.7	5.3	5.1	3.3
AB037716	0.36	0.40	5.1	7.5	2.6	2.1	3.5	3.1	2.4	2.8
X79981	0.34	0.10	4.7	7.2	3.2	4.6	6.5	5.1	5.8	2.7
AF034208	0.45	0.24	2.7	10.9	2.1	3.7	2.3	5.9	2.2	2.5
AL133355	0.22	0.23	2.3	3.4	7.3	2.7	3.3	4.3	2.8	2.5
NM_016281	0.40	0.19	6.6	10.6	2.1	2.8	5.0	11.2	10.6	2.5
AF023614	0.11	0.42	2.2	2.2	6.0	7.5	5.0	2.7	2.0	2.4
AF056717	0.43	0.62	4.3	3.2	5.1	4.0	4.6	9.7	3.1	2.2
AB029039	0.79	0.49	2.7	3.3	3.7	2.0	2.3	2.4	4.8	2.2
J03634	0.40	0.12	3.7	2.3	2.3	4.0	10.5	4.1	9.1	2.2
U80764	0.31	0.18	2.3	7.4	4.2	2.3	5.1	3.3	8.8	2.1
AB032963	0.19	0.34	4.0	7.3	5.0	3.0	2.9	6.7	3.8	2.1
X82835	0.25	0.38	2.0	2.7	2.9	7.7	3.3	3.1	3.5	2.0

EXAMPLE 5

INDUCTION OF CHEMOKINES IN CELL LINES, WHOLE HUMAN BLOOD, AND IN MICE BY PEPTIDES

[00123] The murine macrophage cell line RAW 264.7, THP-1 cells (human monocytes), a human epithelial cell line (A549), human bronchial epithelial cells (16HBEo14), and whole human blood were used. HBE cells were grown in MEM with Earle's. THP-1 cells were grown and maintained in RPMI 1640 medium. The RAW and A549 cell lines were maintained in DMEM supplemented with 10% fetal calf serum. The cells were seeded in 24 well plates at a density of 10^6 cells per well in DMEM (see above) and A549 cells were seeded in 24 well plates at a density of 10^5 cells per well in DMEM (see above) and both were incubated at 37°C in 5 % CO_2 overnight. DMEM was aspirated from cells grown overnight and replaced with fresh medium. After incubation of the cells with peptide, the release of chemokines into the culture supernatant was determined by ELISA (R&D Systems, Minneapolis, MN).

[00124] Animal studies were approved by the UBC Animal Care Committee (UBC ACC # A01-0008). BALB/c mice were purchased from Charles River Laboratories and housed in standard animal facilities. Age, sex and weight matched adult mice were anaesthetized with an intraperitoneal injection of Avertin (4.4 mM 2-2-2-tribromoethanol, 2.5% 2-methyl-2-butanol, in distilled water), using 200 µl per 10 g body weight. The instillation was performed using a non-surgical, intratracheal instillation method adapted from Ho and Furst 1973. Briefly, the anaesthetized mouse was placed with its upper teeth hooked over a wire at the top of a support frame with its jaw held open and a spring pushing the thorax forward to position the pharynx, larynx and trachea in a vertical straight line. The airway was illuminated externally and an intubation catheter was inserted into the clearly illuminated tracheal lumen. Twenty-µl of peptide suspension or sterile water was placed in a well at the proximal end of the catheter and gently instilled into the trachea with 200 µl of air. The animals were maintained in an upright position for 2 minutes after instillation to allow the

fluid to drain into the respiratory tree. After 4 hours the mice were euthanaised by intraperitoneal injection of 300 mg/kg of pentobarbital. The trachea was exposed; an intravenous catheter was passed into the proximal trachea and tied in place with suture thread. Lavage was performed by introducing 0.75 ml sterile PBS into the lungs via the tracheal cannula and then after a few seconds, withdrawing the fluid. This was repeated 3 times with the same sample of PBS. The lavage fluid was placed in a tube on ice and the total recovery volume per mouse was approximately 0.5 ml. The bronchoalveolar lavage (BAL) fluid was centrifuged at 1200 rpm for 10 min, the clear supernatant removed and tested for TNF-α and MCP-1 by ELISA.

[00125] The up-regulation of chemokines by cationic peptides was confirmed in several different systems. The murine MCP-1, a homologue of the human MCP-1, is a member of the β(C-C) chemokine family. MCP-1 has been demonstrated to recruit monocytes, NK cells and some T lymphocytes. When RAW 264.7 macrophage cells and whole human blood from 3 donors were stimulated with increasing concentrations of peptide, SEQ ID NO: 1, they produced significant levels of MCP-1 in their supernatant, as judged by ELISA (Table 36). RAW 264.7 cells stimulated with peptide concentrations ranging from 20-50 μg/ml for 24 hr produced significant levels of MCP-1 (200-400 pg/ml above background). When the cells (24h) and whole blood (4h) were stimulated with 100 μg/ml of LL-37, high levels of MCP-1 were produced.

[00126] The effect of cationic peptides on chemokine induction was also examined in a completely different cell system, A549 human epithelial cells. Interestingly, although these cells produce MCP-1 in response to LPS, and this response could be antagonized by peptide; there was no production of MCP-1 by A549 cells in direct response to peptide, SEQ ID NO: 1. Peptide SEQ ID NO: 1 at high concentrations, did however induce production of IL-8, a neutrophil specific chemokine (Table 37). Thus, SEQ ID NO: 1 can induce a different spectrum of responses from different cell types and at different concentrations. A number of peptides from each of the formula groups were tested for their ability to induce IL-8 in A549 cells (Table 38). Many of these peptides at a low concentration, 10 μg/ml induced IL-8 above background levels. At high concentrations (100 μg/ml) SEQ ID NO: 13 was also found to induce

IL-8 in whole human blood (Table 39). Peptide SEQ ID NO: 2 also significantly induced IL-8 in HBE cells (Table 40) and undifferentiated THP-1 cells (Table 41).

[00127] BALB/c mice were given SEQ ID NO: 1 or endotoxin-free water by intratracheal instillation and the levels of MCP-1 and TNF-α examined in the bronchioalveolar lavage fluid after 3-4 hr. It was found that the mice treated with 50 μg/ml peptide, SEQ ID NO: 1 produced significantly increased levels of MCP-1 over mice given water or anesthetic alone (Table 42). This was not a pro-inflammatory response to peptide, SEQ ID NO: 1 since peptide did not significantly induce more TNF-α than mice given water or anesthetic alone. peptide, SEQ ID NO: 1 was also found not to significantly induce TNF-α production by RAW 264.7 cells and bone marrow-derived macrophages treated with peptide, SEQ ID NO: 1 (up to 100 μg/ml) (Table 43). Thus, peptide, SEQ ID NO: 1 selectively induces the production of chemokines without inducing the production of inflammatory mediators such as TNF-α. This illustrates the dual role of peptide, SEQ ID NO: 1 as a factor that can block bacterial product-induced inflammation while helping to recruit phagocytes that can clear infections.

[00128] Table 38: Induction of MCP-1 in RAW 264.7 cells and whole human blood. RAW 264.7 mouse macrophage cells or whole human blood were stimulated with increasing concentrations of LL-37 for 4 hr. The human blood samples were centrifuged and the serum was removed and tested for MCP-1 by ELISA along with the supernatants from the RAW 264.7 cells. The RAW cell data presented is the mean of three or more experiments ± standard error and the human blood data represents the mean ± standard error from three separate donors.

Peptide, SEQ ID NO: 1 (μg/ml)	**Monocyte chemoattractant protein (MCP)-1 (pg/ml)***	
	RAW cells	**Whole blood**
0	135.3 ± 16.3	112.7 ± 43.3
10	165.7 ± 18.2	239.3 ± 113.3

Peptide, SEQ ID NO: 1 (μg/ml)	Monocyte chemoattractant protein (MCP)-1 (pg/ml)*	
	RAW cells	Whole blood
50	367 ± 11.5	371 ± 105
100	571 ± 17.4	596 ± 248.1

[00129] Table 39: Induction of IL-8 in A549 cells and whole human blood. A549 cells or whole human blood were stimulated with increasing concentrations of peptide for 24 and 4 hr respectively. The human blood samples were centrifuged and the serum was removed and tested for IL-8 by ELISA along with the supernatants from the A549 cells. The A549 cell data presented is the mean of three or more experiments ± standard error and the human blood data represents the mean ± standard error from three separate donors.

Peptide, SEQ ID NO: 1 (μg/ml)	IL-8 (pg/ml)	
	A549 cells	Whole blood
0	172 ± 29.1	660.7 ± 126.6
1	206.7 ± 46.1	
10	283.3 ± 28.4	945.3 ± 279.9
20	392 ± 31.7	
50	542.3 ± 66.2	1160.3 ± 192.4
100	1175.3 ± 188.3	

[00130] Table 40: Induction of IL-8 in A549 cells by Cationic peptides. A549 human epithelial cells were stimulated with 10 μg of peptide for 24 hr. The supernatant was removed and tested for IL-8 by ELISA.

Peptide (10 ug/ml)	IL-8 (ng/ml)
No peptide	0.164

Peptide (10 ug/ml)	IL-8 (ng/ml)
LPS, no peptide	0.26
SEQ ID NO: 1	0.278
SEQ ID NO: 6	0.181
SEQ ID NO: 7	0.161
SEQ ID NO: 9	0.21
SEQ ID NO: 10	0.297
SEQ ID NO: 13	0.293
SEQ ID NO: 14	0.148
SEQ ID NO: 16	0.236
SEQ ID NO: 17	0.15
SEQ ID NO: 19	0.161
SEQ ID NO: 20	0.151
SEQ ID NO: 21	0.275
SEQ ID NO: 22	0.314
SEQ ID NO: 23	0.284
SEQ ID NO: 24	0.139
SEQ ID NO: 26	0.201
SEQ ID NO: 27	0.346
SEQ ID NO: 28	0.192
SEQ ID NO: 29	0.188
SEQ ID NO: 30	0.284
SEQ ID NO: 31	0.168
SEQ ID NO: 33	0.328
SEQ ID NO: 34	0.315
SEQ ID NO: 35	0.301
SEQ ID NO: 36	0.166
SEQ ID NO: 37	0.269
SEQ ID NO: 38	0.171
SEQ ID NO: 40	0.478
SEQ ID NO: 41	0.371

Peptide (10 ug/ml)	IL-8 (ng/ml)
SEQ ID NO: 42	0.422
SEQ ID NO: 43	0.552
SEQ ID NO: 44	0.265
SEQ ID NO: 45	0.266
SEQ ID NO: 47	0.383
SEQ ID NO: 48	0.262
SEQ ID NO: 49	0.301
SEQ ID NO: 50	0.141
SEQ ID NO: 51	0.255
SEQ ID NO: 52	0.207
SEQ ID NO: 53	0.377
SEQ ID NO: 54	0.133

[00131] Table 41: Induction by Peptide of IL-8 in human blood. Whole human blood was stimulated with increasing concentrations of peptide for 4 hr . The human blood samples were centrifuged and the serum was removed and tested for IL-8 by ELISA. The data shown is the average 2 donors.

SEQ ID NO: 3 (μg/ml)	IL-8 (pg/ml)
0	85
10	70
100	323

[00132] Table 42: Induction of IL-8 in HBE cells. Increasing concentrations of the peptide were incubated with HBE cells for 8 h, the supernantant removed and tested for IL-8. The data is presented as the mean of three or more experiments ± standard error.

SEQ ID NO: 2 (μg/ml)	IL-8 (pg/ml)
0	552 ± 90
0.1	670 ± 155
1	712 ± 205
10	941 ± 15
50	1490 ± 715

[00133] Table 43: Induction of IL-8 in undifferentiated THP-1 cells. The human monocyte THP-1 cells were incubated with indicated concentrations of peptide for 8 hr. The supernatant was removed and tested for IL-8 by ELISA.

SEQ ID NO: 3 (μg/ml)	IL-8 (pg/ml)
0	10.6
10	17.2
50	123.7

[00134] Table 44: Induction of MCP-1 by Peptide, SEQ ID NO: 1 in mouse airway. BALB/c mice were anaesthetised with avertin and given intratracheal instillation of peptide or water or no instillation (no treatment). The mice were monitored for 4 hours, anaesthetised and the BAL fluid was isolated and analyzed for MCP-1 and TNF-α concentrations by ELISA. The data shown is the mean of 4 or 5 mice for each condition ± standard error.

Condition	MCP-1 (pg/ml)	TNF-α (pg/ml)
Water	16.5 ± 5	664 ± 107
peptide	111 ± 30	734 ± 210
Avertin	6.5 ± 0.5	393 ± 129

[00135] Table 45: Lack of Significant TNF-α induction by the Cationic Peptides. RAW 264.7 macrophage cells were incubated with indicated peptides (40 μg/ml) for 6 hours. The supernatant was collected and tested for levels of TNF-α by ELISA. The data is presented as the mean of three or more experiments + standard error.

Peptide Treatment	TNF-α (pg/ml)
Media background	56 ± 8
LPS treatment, No peptide	15207 ± 186
SEQ ID NO: 1	274 ± 15
SEQ ID NO: 5	223 ± 45
SEQ ID NO: 6	297 ± 32
SEQ ID NO: 7	270 ± 42
SEQ ID NO: 8	166 ± 23
SEQ ID NO: 9	171 ± 33
SEQ ID NO: 10	288 ± 30
SEQ ID NO: 12	299 ± 65
SEQ ID NO: 13	216 ± 42
SEQ ID NO: 14	226 ± 41
SEQ ID NO: 15	346 ± 41
SEQ ID NO: 16	341 ± 68
SEQ ID NO: 17	249 ± 49
SEQ ID NO: 19	397 ± 86
SEQ ID NO: 20	285 ± 56
SEQ ID NO: 21	263 ± 8
SEQ ID NO: 22	195 ± 42
SEQ ID NO: 23	254 ± 58
SEQ ID NO: 24	231 ± 32
SEQ ID NO: 26	281 ± 34
SEQ ID NO: 27	203 ± 42

Peptide Treatment	TNF-α (pg/ml)
SEQ ID NO: 28	192 ± 26
SEQ ID NO: 29	242 ± 40
SEQ ID NO: 31	307 ± 71
SEQ ID NO: 33	196 ± 42
SEQ ID NO: 34	204 ± 51
SEQ ID NO: 35	274 ± 76
SEQ ID NO: 37	323 ± 41
SEQ ID NO: 38	199 ± 38
SEQ ID NO: 43	947 ± 197
SEQ ID NO: 44	441 ± 145
SEQ ID NO: 45	398 ± 90
SEQ ID NO: 48	253 ± 33
SEQ ID NO: 49	324 ± 38
SEQ ID NO: 50	311 ± 144
SEQ ID NO: 53	263 ± 40
SEQ ID NO: 54	346 ± 86

EXAMPLE 6

CATIONIC PEPTIDES INCREASE SURFACE EXPRESSION OF CHEMOKINE RECEPTORS

[00136] To analyze cell surface expression of IL-8RB, CXCR-4, CCR2, and LFA-1, RAW macrophage cells were stained with 10 μg/ml of the appropriate primary antibody (Santa Cruz Biotechnology) followed by FITC-conjugated goat anti-rabbit IgG [IL-8RB and CXCR-4 (Jackson ImmunoResearch Laboratories, West Grove, PA)] or FITC-conjugated donkey anti-goat IgG (Santa Cruz). The cells were analyzed using a FACscan, counting 10,000 events and gating on forward and side scatter to exclude cell debris.

[00137] The polynucleotide array data suggested that some peptides up-regulate the expression of the chemokine receptors IL-8RB, CXCR-4 and CCR2 by 10, 4 and 1.4 fold above unstimulated cells respectively. To confirm the polynucleotide array data,

the surface expression was examined by flow cytometry of these receptors on RAW cells stimulated with peptide for 4 hr. When 50 μg/ml of peptide was incubated with RAW cells for 4 hr, IL-8RB was upregulated an average of 2.4-fold above unstimulated cells, CXCR-4 was up-regulated an average of 1.6-fold above unstimulated cells and CCR2 was up-regulated 1.8-fold above unstimulated cells (Table 46). As a control CEMA was demonstrated to cause similar up-regulation. Bac2A was the only peptide to show significant up-regulation of LFA-1 (3.8 fold higher than control cells).

[00138] Table 46: Increased surface expression of CXCR-4, IL-8RB and CCR2 in response to peptides. RAW macrophage cells were stimulated with peptide for 4 hr. The cells were washed and stained with the appropriate primary and FITC-labeled secondary antibodies. The data shown represents the average (fold change of RAW cells stimulated with peptide from media) ± standard error.

Peptide	Concentration (μg/ml)	Fold Increase in Protein Expression		
		IL-8RB	CXCR-4	CCR2
SEQ ID NO: 1	10	1.0	1.0	1.0
SEQ ID NO:1	50	1.3 ± 0.05	1.3 ± 0.03	1.3 ± 0.03
SEQ ID NO:1	100	2.4 ± 0.6	1.6 ± 0.23	1.8 ± 0.15
SEQ ID NO: 3	100	2.0 ± 0.6	Not Done	4.5
CEMA	50	1.6 ± 0.1	1.5 ± 0.2	1.5 ± 0.15
	100	3.6 ± 0.8	Not Done	4.7 ± 1.1

EXAMPLE 7

PHOSPHORYLATION OF MAP KINASES BY CATIONIC PEPTIDES

[00139] The cells were seeded at $2.5x10^5$ - 5 x 10^5 cells/ml and left overnight. They were washed once in media, serum starved in the morning (serum free media - 4hrs). The media was removed and replaced with PBS, then sat at 37°C for 15 minutes and then brought to room temp for 15 minutes. Peptide was added (concentrations 0.1ug/ml - 50ug/ml) or H_2O and incubated 10 min. The PBS was very quickly removed and replaced with ice-cold radioimmunoprecipitation (RIPA) buffer with inhibitors (NaF, B-glycerophosphate, MOL, Vanadate, PMSF, Leupeptin Aprotinin). The plates were shaken on ice for 10-15 min or until the cells were lysed and the lysates collected. The procedure for THP-1 cells was slightly different; more cells ($2x10^6$) were used. They were serum starved overnight, and to stop the reaction 1ml of ice-cold PBS was added then they sat on ice 5-10 min, were spun down then resuspended in RIPA. Protein concentrations were determined using a protein assay (Pierce, Rockford, IL.). Cell lysates (20 µg of protein) were separated by SDS-PAGE and transferred to nitrocellulose filters. The filters were blocked for 1 h with 10 mM Tris-HCl, pH 7.5, 150 mM NaCl (TBS)/5% skim milk powder and then incubated overnight in the cold with primary antibody in TBS/0.05% Tween 20. After washing for 30 min with TBS/0.05% Tween 20, the filters were incubated for 1 h at room temperature with 1 µg/ml secondary antibody in TBS. The filters were washed for 30 min with TBS/0.05% Tween 20 and then incubated 1 h at room temperature with horseradish peroxidase-conjugated sheep anti-mouse IgG (1:10,000 in TBS/0.05% Tween 20). After washing the filters for 30 min with TBS/0.1% Tween 20, immunoreactive bands were visualized by enhanced chemiluminescence (ECL) detection. For experiments with peripheral blood mononuclear cells: The peripheral blood (50-100ml) was collected from all subjects. Mononuclear cells were isolated from the peripheral blood by density gradient centrifugation on Ficoll-Hypaque. Interphase cells (mononuclear cells) were recovered, washed and then resuspended in recommended primary medium for cell culture (RPMI-1640) with 10% fetal calf serum (FCS) and 1% L-glutamine. Cells were added to 6 well culture plates at $4x10^6$ cells/well and were allowed to adhere at 37° C in 5% CO_2 atmosphere for 1 hour. The

supernatant medium and non-adherent cells were washed off and the appropriate media with peptide was added. The freshly harvested cells were consistently >99% viable as assessed by their ability to exclude trypan blue. After stimulation with peptide, lysates were collected by lysing the cells in RIPA buffer in the presence of various phosphatase- and kinase-inhibitors. Protein content was analyzed and approximately 30 μg of each sample was loaded in a 12% SDS-PAGE gel. The gels were blotted onto nitrocellulose, blocked for 1 hour with 5% skim milk powder in Tris buffered saline (TBS) with 1% Triton X 100. Phosphorylation was detected with phosphorylation-specific antibodies.

[00140] The results of peptide-induced phosphorylation are summarized in Table 46. SEQ ID NO: 2 was found to cause dose dependent phosphorylation of p38 and ERK1/2 in the mouse macrophage RAW cell line and the HBE cells. SEQ ID NO: 3 caused phosphorylation of MAP kinases in THP-1 human monocyte cell line and phosphorylation of ERK1/2 in the mouse RAW cell line.

[00141] Table 47: Phosphorylation of MAP kinases in response to peptides.

Cell Line	Peptide	MAP kinase phosphorylated	
		p38	ERK1/2
RAW 264.7	SEQ ID NO: 3	-	+
	SEQ ID NO: 2	+	+
HBE	SEQ ID NO: 3		+
	SEQ ID NO: 2	+	+
THP-1	SEQ ID NO: 3	+	+
	SEQ ID NO: 2		

[00142] **Table 48:** Peptide Phosphorylation of MAP kinases in human blood monocytes. SEQ ID NO: 1 at 50 μg/ml) was used to promote phosphorylation.

p38 phosphorylation		ERK1/2 phosphorylation	
15 minutes	60 minutes	15 minutes	60 minutes
+	-	+	+

EXAMPLE 8

CATIONIC PEPTIDES PROTECT AGAINST BACTERIAL INFECTION BY ENHANCING THE IMMUNE RESPONSE

[00143] BALB/c mice were given 1x 10^5 Salmonella and cationic peptide (200 μg) by intraperitoneal injection. The mice were monitored for 24 hours at which point they were euthanized, the spleen removed, homogenized and resuspended in PBS and plated on Luria Broth agar plates with Kanamycin (50 μg/ml). The plates were incubated overnight at 37°C and counted for viable bacteria (Table 49 and 50). CD-1 mice were given 1 x 10^8 *S. aureus* in 5 % porcine mucin and cationic peptide (200 μg) by intraperitoneal injection (Table 51). The mice were monitored for 3 days at which point they were euthanized, blood removed and plated for viable counts. CD-1 male mice were given 5.8 x 10^6 CFU EHEC bacteria and cationic peptide (200 μg) by intraperitoneal (IP) injection and monitored for 3 days (Table 52). In each of these animal models a subset of the peptides demonstrated protection against infections. The most protective peptides in the *Salmonella* model demonstrated an ability to induce a common subset of genes in epithelial cells (Table 53) when comparing the protection assay results in Tables 50 and 51 to the gene expression results in Tables 31-37. This clearly indicates that there is a pattern of gene expression that is consistent with the ability of a peptide to demonstrate protection. Many of the cationic peptides were shown not to be directly antimicrobial as tested by the Minimum Inhibitory Concentration (MIC) assay (Table 54). This demonstrates that the ability of peptides to protect against infection relies on the ability of the peptide to stimulate host innate immunity rather than on direct antimicrobial activity.

[00144] Table 49: Effect of Cationic Peptides on Salmonella Infection in BALB/c mice. The BALB/c mice were injected IP with Salmonella and Peptide, and 24 h later the animals were euthanized, the spleen removed, homogenized, diluted in PBS and plate counts were done to determine bacteria viability.

Peptide Treatment	Viable Bacteria in the Spleen (CFU/ml)	Statistical Significance (p value)
Control	$2.70 \pm 0.84 \times 10^5$	
SEQ ID NO: 1	$1.50 \pm 0.26 \times 10^5$	0.12
SEQ ID NO: 6	$2.57 \pm 0.72 \times 10^4$	0.03
SEQ ID NO: 13	$3.80 \pm 0.97 \times 10^4$	0.04
SEQ ID NO: 17	$4.79 \pm 1.27 \times 10^4$	0.04
SEQ ID NO: 27	$1.01 \pm 0.26 \times 10^5$	0.06

[00145] Table 50: Effect of Cationic Peptides on *Salmonella* Infection in BALB/c mice. The BALB/c mice were injected intraperitoneally with *Salmonella* and Peptide, and 24 h later the animals were euthanized, the spleen removed, homogenized, diluted in PBS and plate counts were done to determine bacteria viability.

Peptide Treatment	Viable Bacteria in the Spleen (CFU/ml)
Control	$1.88 \pm 0.16 \times 10^4$
SEQ ID NO: 48	$1.98 \pm 0.18 \times 10^4$
SEQ ID NO: 26	$7.1 \pm 1.37 \times 10^4$
SEQ ID NO: 30	$5.79 \pm 0.43 \times 10^3$
SEQ ID NO: 37	$1.57 \pm 0.44 \times 10^4$
SEQ ID NO: 5	$2.75 \pm 0.59 \times 10^4$
SEQ ID NO: 7	$5.4 \pm 0.28 \times 10^3$

SEQ ID NO: 9	$1.23 \pm 0.87 \times 10^4$
SEQ ID NO: 14	$2.11 \pm 0.23 \times 10^3$
SEQ ID NO: 20	$2.78 \pm 0.22 \times 10^4$
SEQ ID NO: 23	$6.16 \pm 0.32 \times 10^4$

[00146] Table 51. Effect of Cationic Peptides in a Murine *S. aureus* infection model. CD-1 mice were given 1×10^8 bacteria in 5 % porcine mucin via intraperitoneal (IP) injection. Cationic peptide (200 μg) was given via a separate IP injection. The mice were monitored for 3 days at which point they were euthanized, blood removed and plated for viable counts. The following peptides were not effective in controlling *S. aureus* infection: SEQ ID NO: 48, SEQ ID NO: 26

Treatment	CFU/ml (blood)	# Mice Survived (3 days)/ Total mice in group
No Peptide	$7.61 \pm 1.7 \times 10^3$	6 / 8
SEQ ID NO: 1	0	4 / 4
SEQ ID NO: 27	$2.25 \pm 0.1 \times 10^2$	3 / 4
SEQ ID NO: 30	$1.29 \pm 0.04 \times 10^2$	4 / 4
SEQ ID NO: 37	$9.65 \pm 0.41 \times 10^2$	4 / 4
SEQ ID NO: 5	$3.28 \pm 1.7 \times 10^3$	4 / 4
SEQ ID NO: 6	$1.98 \pm 0.05 \times 10^2$	3 / 4
SEQ ID NO: 7	$3.8 \pm 0.24 \times 10^3$	4 / 4
SEQ ID NO: 9	$2.97 \pm 0.25 \times 10^2$	4 / 4
SEQ ID NO: 13	$4.83 \pm 0.92 \times 10^3$	3 / 4
SEQ ID NO: 17	$9.6 \pm 0.41 \times 10^2$	4 / 4
SEQ ID NO: 20	$3.41 \pm 1.6 \times 10^3$	4 / 4
SEQ ID NO: 23	$4.39 \pm 2.0 \times 10^3$	4 / 4

[00147] Table 52 Effect of Peptide in a Murine EHEC infection model. CD-1 male mice (5 weeks old) were given 5.8×10^6 CFU EHEC bacteria via intraperitoneal

(IP) injection. Cationic peptide (200 μg) was given via a separate IP injection. The mice were monitored for 3 days.

Treatment	Peptide	Survival (%)
control	none	25
SEQ ID NO: 23	200μg	100

[00148] Table 53. Up-regulation of patterns of gene expression in A549 epithelial cells induced by peptides that are active *in vivo*. The peptides SEQ ID NO: 30, SEQ ID NO: 7 and SEQ ID NO: 13 at concentrations of 50 μg/ml were each shown to increase the expression of a pattern of genes after 4 h treatment. Peptide was incubated with the human A549 epithelial cells for 4 h and the RNA was isolated, converted into labelled cDNA probes and hybridised to Human Operon arrays (PRHU04). The intensity of polynucleotides in control, unstimulated cells are shown in the second columns for labelling of cDNA (average of Cy3 and Cy5). The Fold Up regulation column refers to the intensity of polynucleotide expression in peptide-simulated cells divided by the intensity of unstimulated cells. The SEQ ID NO: 37 peptide was included as a negative control that was not active in the murine infection models.

Target (Accession number)	Unstimulated Cell Intensity	Fold Up regulation of Gene Expression relative to Untreated Cells			
		SEQ ID NO: 30	SEQ ID NO: 7	SEQ ID NO: 13	SEQ ID NO: 37
Zinc finger protein (AF061261)	13	2.6	9.4	9.4	1.0
Cell cycle gene (S70622)	1.62	8.5	3.2	3.2	0.7
IL-10 Receptor (U00672)	0.2	2.6	9	4.3	0.5

Transferase (AF038664)	0.09	12.3	9.7	9.7	0.1
Homeobox protein (AC004774)	0.38	3.2	2.5	2.5	1.7
Forkhead protein (AF042832)	0.17	14.1	3.5	3.5	0.9
Unknown (AL096803)	0.12	4.8	4.3	4.3	0.6
KIAA0284 Protein (AB006622)	0.47	3.4	2.1	2.1	1.3
Hypothetical Protein (AL022393)	0.12	4.4	4.0	4.0	0.4
Receptor (AF112461)	0.16	2.4	10.0	10.0	1.9
Hypothetical Protein (AK002104)	0.51	4.7	2.6	2.6	1.0
Protein (AL050261)	0.26	3.3	2.8	2.8	1.0
Polypeptide (AF105424)	0.26	2.5	5.3	5.3	1.0
SPR1 protein (AB031480)	0.73	3.0	2.7	2.7	1.3
Dehydrogenase (D17793)	4.38	2.3	2.2	2.2	0.9
Transferase (M63509)	0.55	2.7	2.1	2.1	1.0
Peroxisome factor (AB013818)	0.37	3.4	2.9	2.9	1.4

[00149] Table 54: Most cationic peptides studied here and especially the cationic peptides effective in infection models are not significantly antimicrobial. A dilution series of peptide was incubated with the indicated bacteria overnight in a 96-well plate. The lowest concentration of peptide that killed the bacteria was used as the MIC. The symbol > indicates the MIC is too large to measure. An MIC of 8 μg/ml or less was considered clinically meaningful activity. Abbreviations: *E.coli*, *Escherichia*

coli; *S.aureus*, *Staphylococcus aureus*; *P.aerug*, *Pseudomonas aeruginosa*; *S.Typhim*, *Salmonella enteritidis ssp. typhimurium*; *C. rhod*, *Citobacter rhodensis*; EHEC, Enterohaemorrhagic *E.coli*.

Peptide	MIC (μg/ml)					
	E. coli	*S.aureus*	*P. aerug.*	*S.typhim.*	*C. rhod.*	EHEC
Polymyxin	0.25	16	0.25	0.5	0.25	0.5
Gentamicin	0.25	0.25	0.25	0.25	0.25	0.5
SEQ ID NO: 1	32	>	96	64	8	4
SEQ ID NO: 5	128	>	>	>	64	64
SEQ ID NO: 6	128	>	>	128	64	64
SEQ ID NO: 7	>	>	>	>	>	>
SEQ ID NO: 8	>	>	>	>	>	>
SEQ ID NO: 9	>	>	>	>	>	>
SEQ ID NO: 10	>	>	>	>	>	64
SEQ ID NO: 12	>	>	>	>	>	>
SEQ ID NO: 13	>	>	>	>	>	>
SEQ ID NO: 14	>	>	>	>	>	>
SEQ ID NO: 15	128	>	>	>	128	64
SEQ ID NO: 16	>	>	>	>	>	>
SEQ ID NO: 17	>	>	>	>	>	>
SEQ ID NO: 19	8	16	16	64	4	4
SEQ ID NO: 2	4	16	32	16	64	
SEQ ID NO: 20	8	8	8	8	16	8
SEQ ID NO: 21	64	64	96	64	32	32
SEQ ID NO: 22	8	12	24	8	4	4
SEQ ID NO: 23	4	8	8	16	4	4
SEQ ID NO: 24	16	16	4	16	16	4
SEQ ID NO: 26	0.5	32	64	2	2	0.5
SEQ ID NO: 27	8	64	64	16	2	4
SEQ ID NO: 28	>	>	>	64	64	128

Peptide	MIC (μg/ml)					
	E. coli	*S.aureus*	*P. aerug.*	*S.typhim.*	*C. rhod.*	EHEC
SEQ ID NO: 29	2	>	>	16	32	4
SEQ ID NO: 30	16	>	128	16	16	4
SEQ ID NO: 31	>	>	128	>	>	64
SEQ ID NO: 33	16	32	>	16	64	8
SEQ ID NO: 34	8	>	>	32	64	8
SEQ ID NO: 35	4	128	64	8	8	4
SEQ ID NO: 36	32	>	>	32	32	16
SEQ ID NO: 37	>	>	>	>	>	>
SEQ ID NO: 38	0.5	32	64	4	8	4
SEQ ID NO: 40	4	32	8	4	4	2
SEQ ID NO: 41	4	64	8	8	2	2
SEQ ID NO: 42	1.5	64	4	2	2	1
SEQ ID NO: 43	8	128	16	16	8	4
SEQ ID NO: 44	8	>	128	128	64	64
SEQ ID NO: 45	8	>	128	128	16	16
SEQ ID NO: 47	4	>	16	16	4	4
SEQ ID NO: 48	16	>	128	16	1	2
SEQ ID NO: 49	4	>	16	8	4	4
SEQ ID NO: 50	8	>	16	16	16	8
SEQ ID NO: 51	4	>	8	32	4	8
SEQ ID NO: 52	8	>	32	8	2	2
SEQ ID NO: 53	4	>	8	8	16	8
SEQ ID NO: 54	64	>	16	64	16	32

EXAMPLE 9

USE OF POLYNUCLEOTIDES INDUCED BY BACTERIAL SIGNALLING MOLECULES IN DIAGNOSTIC/SCREENING

[00150] *S. typhimurium* LPS and *E. coli* 0111:B4 LPS were purchased from Sigma Chemical Co. (St. Louis, MO). LTA (Sigma) from *S. aureus*, was resuspended in endotoxin free water (Sigma). The Limulus amoebocyte lysate assay (Sigma) was performed on LTA preparations to confirm that lots were not significantly contaminated by endotoxin (i.e. <1 ng/ml, a concentration that did not cause significant cytokine production in the RAW cell assay). The CpG oligodeoxynucleotides were synthesized with an Applied Biosystems Inc., Model 392 DNA/RNA Synthesizer, Mississauga, ON., then purified and resuspended in endotoxin-free water (Sigma). The following sequences were used CpG: 5'-TCATGACGTTCCTGACGTT-3' (SEQ ID NO: 57) and nonCpG: 5'-TTCAGGACTTTCCTCAGGTT-3' (SEQ ID NO: 58). The nonCpG oligo was tested for its ability to stimulate production of cytokines and was found to cause no significant production of TNF-α or IL-6 and therefore was considered as a negative control. RNA was isolated from RAW 264.7 cells that had been incubated for 4h with medium alone, 100 ng/ml *S. typhimurium* LPS, 1 μg/ml *S. aureus* LTA, or 1 μM CpG (concentrations that led to optimal induction of tumor necrosis factor (TNF-α) in RAW cells). The RNA was used to polynucleotiderate cDNA probes that were hybridized to Clontech Atlas polynucleotide array filters, as described above. The hybridization of the cDNA probes to each immobilized DNA was visualized by autoradiography and quantified using a phosphorimager. Results from at least 2 to 3 independent experiments are summarized in Tables 55-59. It was found that LPS treatment of RAW 264.7 cells resulted in increased expression of more than 60 polynucleotides including polynucleotides encoding inflammatory proteins such as IL-1β, inducible nitric oxide synthase (iNOS), MIP-1α, MIP-1β, MIP-2α, CD40, and a variety of transcription factors. When the changes in polynucleotide expression induced by LPS, LTA, and CpG DNA were compared, it was found that all three of these bacterial products increased the expression of pro-inflammatory polynucleotides such as iNOS, MIP-1α, MIP-2α, IL-1β, IL-15, TNFR1 and NF-κB to a similar extent

(Table 57). Table 57 describes 19 polynucleotides that were up-regulated by the bacterial products to similar extents in that their stimulation ratios differed by less than 1.5 fold between the three bacterial products. There were also several polynucleotides that were down-regulated by LPS, LTA and CpG to a similar extent. It was also found that there were a number of polynucleotides that were differentially regulated in response to the three bacterial products (Table 58), which includes many of these polynucleotides that differed in expression levels by more than 1.5 fold between one or more bacterial products). LTA treatment differentially influenced expression of the largest subset of polynucleotides compared to LPS or CpG, including hyperstimulation of expression of Jun-D, Jun-B, Elk-1 and cyclins G2 and A1. There were only a few polynucleotides whose expression was altered more by LPS or CpG treatment. Polynucleotides that had preferentially increased expression due to LPS treatment compared to LTA or CpG treatment included the cAMP response element DNA-binding protein 1 (CRE-BPI), interferon inducible protein 1 and CACCC Box-binding protein BKLF. Polynucleotides that had preferentially increased expression after CpG treatment compared to LPS or LTA treatment included leukemia inhibitory factor (LIF) and protease nexin 1 (PN-1). These results indicate that although LPS, LTA, and CpG DNA stimulate largely overlapping polynucleotide expression responses, they also exhibit differential abilities to regulate certain subsets of polynucleotides.

[00151] The other polynucleotide arrays used are the Human Operon arrays (identification number for the genome is PRHU04-S1), which consist of about 14,000 human oligos spotted in duplicate. Probes were prepared from 5 μg of total RNA and labeled with Cy3 or Cy5 labeled dUTP. In these experiments, A549 epithelial cells were plated in 100 mm tissue culture dishes at 2.5 x 10^6 cells/dish, incubated overnight and then stimulated with 100 ng/ml *E. coli* O111:B4 LPS for 4 h. Total RNA was isolated using RNAqueous (Ambion). DNA contamination was removed with DNA-free kit (Ambion). The probes prepared from total RNA were purified and hybridized to printed glass slides overnight at 42ºC and washed. After washing, the image was captured using a Perkin Elmer array scanner. The image processing software (Imapolynucleotide 5.0, Marina Del Rey, CA) determines the spot mean intensity, median intensities, and background intensities. An "in house" program was

used to remove background. The program calculates the bottom 10 % intensity for each subgrid and subtracts this for each grid. Analysis was performed with Polynucleotidespring software (Redwood City, CA). The intensities for each spot were normalized by taking the median spot intensity value from the population of spot values within a slide and comparing this value to the values of all slides in the experiment. The relative changes seen with cells treated with LPS compared to control cells can be found in the Tables below. A number of previously unreported changes that would be useful in diagnosing infection are described in Table 60.

[00152] To confirm and assess the functional significance of these changes, the levels of selected mRNAs and proteins were assessed and quantified by densitometry. Northern blots using a CD14, vimentin, and tristetraprolin-specific probe confirmed similar expression after stimulation with all 3 bacterial products (Table 60). Similarly measurement of the enzymatic activity of nitric oxide synthetase, iNOS, using Griess reagent to assess levels of the inflammatory mediator NO, demonstrated comparable levels of NO produced after 24 h, consistent with the similar up-regulation of iNOS expression (Table 59). Western blot analysis confirmed the preferential stimulation of leukaemia inhibitory factor (LIF, a member of the IL-6 family of cytokines) by CpG (Table 59). Other confirmatory experiments demonstrated that LPS up-regulated the expression of TNF-α and IL-6 as assessed by ELISA, and the up-regulated expression of MIP-2α, and IL-1β mRNA and down-regulation of DP-1 and cyclin D mRNA as assessed by Northern blot analysis. The analysis was expanded to a more clinically relevant *ex vivo* system, by examining the ability of the bacterial elements to stimulate pro-inflammatory cytokine production in whole human blood. It was found that *E. coli* LPS, *S. typhimurium* LPS, and *S. aureus* LTA all stimulated similar amounts of serum TNF-α, and IL-1β. CpG also stimulated production of these cytokines, albeit to much lower levels, confirming in part the cell line data.

[00153] Table 55: Polynucleotides Up-regulated by *E. coli* O111:B4 LPS in A549 Epithelial Cells. *E. coli* O111:B4 LPS (100 ng/ml) increased the expression of many polynucleotides in A549 cells as studied by polynucleotide microarrays. LPS was incubated with the A549 cells for 4 h and the RNA was isolated. 5 μg total RNA was used to make Cy3/Cy5 labelled cDNA probes and hybridised onto Human

Operon arrays (PRHU04). The intensity of unstimulated cells is shown in the second column of Table 55. The "Ratio: LPS/control" column refers to the intensity of polynucleotide expression in LPS simulated cells divided by in the intensity of unstimulated cells.

Accession Number	**Gene**	**Control: Media only Intensity**	**Ratio: LPS/control**
D87451	ring finger protein 10	715.8	183.7
AF061261	C3H-type zinc finger protein	565.9	36.7
D17793	aldo-keto reductase family 1, member C3	220.1	35.9
M14630	prothymosin, alpha	168.2	31.3
AL049975	Unknown	145.6	62.3
L04510	ADP-ribosylation factor domain protein 1, 64kD	139.9	213.6
U10991	G2 protein	101.7	170.3
U39067	eukaryotic translation initiation factor 3, subunit 2	61.0	15.9
X03342	ribosomal protein L32	52.6	10.5
NM_004850	Rho-associated, coiled-coil containing protein kinase 2	48.1	11.8
AK000942	Unknown	46.9	8.4
AB040057	serine/threonine protein kinase MASK	42.1	44.3
AB020719	KIAA0912 protein	41.8	9.4
AB007856	FEM-1-like death receptor binding protein	41.2	15.7
J02783	procollagen-proline, 2-oxoglutarate 4-dioxygenase	36.1	14.1
AL137376	Unknown	32.5	17.3
AL137730	Unknown	29.4	11.9

Accession Number	Gene	Control: Media only Intensity	Ratio: LPS/control
D25328	phosphofructokinase, platelet	27.3	8.5
AF047470	malate dehydrogenase 2, NAD	25.2	8.2
M86752	stress-induced-phosphoprotein 1	22.9	5.9
M90696	cathepsin S	19.6	6.8
AK001143	Unknown	19.1	6.4
AF038406	NADH dehydrogenase	17.7	71.5
AK000315	hypothetical protein FLJ20308	17.3	17.4
M54915	pim-1 oncogene	16.0	11.4
D29011	proteasome subunit, beta type, 5	15.3	41.1
AK000237	membrane protein of cholinergic synaptic vesicles	15.1	9.4
AL034348	Unknown	15.1	15.8
AL161991	Unknown	14.2	8.1
AL049250	Unknown	12.7	5.6
AL050361	PTD017 protein	12.6	13.0
U74324	RAB interacting factor	12.3	5.2
M22538	NADH dehydrogenase	12.3	7.6
D87076	KIAA0239 protein	11.6	6.5
NM_006327	translocase of inner mitochondrial membrane 23 (yeast) homolog	11.5	10.0
AK001083	Unknown	11.1	8.6
AJ001403	mucin 5, subtype B, tracheobronchial	10.8	53.4

Accession Number	Gene	Control: Media only Intensity	Ratio: LPS/control
M64788	RAP1, GTPase activating protein 1	10.7	7.6
X06614	retinoic acid receptor, alpha	10.7	5.5
U85611	calcium and integring binding protein	10.3	8.1
U23942	cytochrome P450, 51	10.1	10.2
AL031983	Unknown	9.7	302.8
NM_007171	protein-O-mannosyltransferase 1	9.5	6.5
AK000403	hypothetical protein FLJ20396	9.5	66.6
NM_002950	ribophorin I	9.3	35.7
L05515	cAMP response element-binding protein CRE-BPa	8.9	6.2
X83368	phosphoinositide-3-kinase, catalytic, gamma polypeptide	8.7	27.1
M30269	nidogen (enactin)	8.7	5.5
M91083	chromosome 11 open reading frame 13	8.2	6.6
D29833	salivary proline-rich protein	7.7	5.8
AB024536	immunoglobulin superfamily containing leucine-rich repeat	7.6	8.0
U39400	chromosome 11 open reading frame 4	7.4	7.3
AF028789	unc119 (C.elegans) homolog	7.4	27.0
NM_003144	signal sequence receptor, alpha (translocon-associated protein alpha)	7.3	5.9

Accession Number	Gene	Control: Media only Intensity	Ratio: LPS/control
X52195	arachidonate 5-lipoxygenase-activating protein	7.3	13.1
U43895	human growth factor-regulated tyrosine kinase substrate	6.9	6.9
L25876	cyclin-dependent kinase inhibitor 3	6.7	10.3
L04490	NADH dehydrogenase	6.6	11.1
Z18948	S100 calcium-binding protein	6.3	11.0
D10522	myristoylated alanine-rich protein kinase C substrate	6.1	5.8
NM_014442	sialic acid binding Ig-like lectin 8	6.1	7.6
U81375	solute carrier family 29	6.0	6.4
AF041410	malignancy-associated protein	5.9	5.3
U24077	killer cell immunoglobulin-like receptor	5.8	14.4
AL137614	hypothetical protein	4.8	6.8
NM_002406	mannosyl (alpha-1,3-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase	4.7	5.3
AB002348	KIAA0350 protein	4.7	7.6
AF165217	tropomodulin 4 (muscle)	4.6	12.3
Z14093	branched chain keto acid dehydrogenase E1, alpha polypeptide	4.6	5.4
U82671	caltractin	3.8	44.5

Accession Number	Gene	Control: Media only Intensity	Ratio: LPS/control
AL050136	Unknown	3.6	5.0
NM_005135	solute carrier family 12	3.6	5.0
AK001961	hypothetical protein FLJ11099	3.6	5.9
AL034410	Unknown	3.2	21.3
S74728	antiquitin 1	3.1	9.2
AL049714	ribosomal protein L34 pseudogene 2	3.0	19.5
NM_014075	PRO0593 protein	2.9	11.5
AF189279	phospholipase A2, group IIE	2.8	37.8
J03925	integrin, alpha M	2.7	9.9
NM_012177	F-box protein Fbx5	2.6	26.2
NM_004519	potassium voltage-gated channel, KQT-like subfamily, member 3	2.6	21.1
M28825	CD1A antigen, a polypeptide	2.6	16.8
X16940	actin, gamma 2, smooth muscle, enteric	2.4	11.8
X03066	major histocompatibility complex, class II, DO beta	2.2	36.5
AK001237	hypothetical protein FLJ10375	2.1	18.4
AB028971	KIAA1048 protein	2.0	9.4
AL137665	Unknown	2.0	7.3

[00154] Table 56: Polynucleotides Down-regulated by *E. coli* O111:B4 LPS in A549 Epithelial Cells. *E. coli* O111:B4 LPS (100 ng/ml) decreased the expression of many polynucleotides in A549 cells as studied by polynucleotide microarrays. LPS

was incubated with the A549 cells for 4 h and the RNA was isolated. 5 μg total RNA was used to make Cy3/Cy5 labeled cDNA probes and hybridized onto Human Operon arrays (PRHU04). The intensity of unstimulated cells is shown in the second column of the Table. The "Ratio: LPS/control" column refers to the intensity of polynucleotide expression in LPS simulated cells divided by in the intensity of unstimulated cells.

Accession Number	Gene	Control: Media only Intensity	Ratio: LPS/control
NM_017433	myosin IIIA	167.8	0.03
X60484	H4 histone family member E	36.2	0.04
X60483	H4 histone family member D	36.9	0.05
AF151079	hypothetical protein	602.8	0.05
M96843	inhibitor of DNA binding 2, dominant negative helix-loop-helix protein	30.7	0.05
S79854	deiodinase, iodothyronine, type III	39.4	0.06
AB018266	matrin 3	15.7	0.08
M33374	NADH dehydrogenase	107.8	0.09
AF005220	Homo sapiens mRNA for NUP98-HOXD13 fusion protein, partial cds	105.2	0.09
Z80783	H2B histone family, member L	20.5	0.10
Z46261	H3 histone family, member A	9.7	0.12
Z80780	H2B histone family, member H	35.3	0.12
U33931	erythrocyte membrane protein band 7.2 (stomatin)	18.9	0.13
M60750	H2B histone family, member A	35.8	0.14
Z83738	H2B histone family, member E	19.3	0.15
Y14690	collagen, type V, alpha 2	7.5	0.15
M30938	X-ray repair complementing defective repair in Chinese hamster cells 5	11.3	0.16
L36055	eukaryotic translation initiation factor 4E	182.5	0.16

Accession Number	Gene	Control: Media only Intensity	Ratio: LPS/control
	binding protein 1		
Z80779	H2B histone family, member G	54.3	0.16
AF226869	5(3)-deoxyribonucleotidase; RB-associated KRAB repressor	7.1	0.18
D50924	KIAA0134 gene product	91.0	0.18
AL133415	vimentin	78.1	0.19
AL050179	tropomyosin 1 (alpha)	41.6	0.19
AJ005579	RD element	5.4	0.19
M80899	AHNAK nucleoprotein	11.6	0.19
NM_004873	BCL2-associated athanogene 5	6.2	0.19
X57138	H2A histone family, member N	58.3	0.20
AF081281	lysophospholipase I	7.2	0.22
U96759	von Hippel-Lindau binding protein 1	6.6	0.22
U85977	Human ribosomal protein L12 pseudogene, partial cds	342.6	0.22
D13315	glyoxalase I	7.5	0.22
AC003007	Unknown	218.2	0.22
AB032980	RU2S	246.6	0.22
U40282	integrin-linked kinase	10.1	0.22
U81984	endothelial PAS domain protein 1	4.7	0.23
X91788	chloride channel, nucleotide-sensitive, 1A	9.6	0.23
AF018081	collagen, type XVIII, alpha 1	6.9	0.24
L31881	nuclear factor I/X (CCAAT-binding transcription factor)	13.6	0.24
X61123	B-cell translocation gene 1, anti-proliferative	5.3	0.24
L32976	mitogen-activated protein kinase kinase	6.3	0.24

Accession Number	Gene	Control: Media only Intensity	Ratio: LPS/control
	kinase 11		
M27749	immunoglobulin lambda-like polypeptide 3	5.5	0.24
X57128	H3 histone family, member C	9.0	0.25
X80907	phosphoinositide-3-kinase, regulatory subunit, polypeptide 2	5.8	0.25
Z34282	H.sapiens (MAR11) MUC5AC mRNA for mucin (partial)	100.6	0.26
X00089	H2A histone family, member M	4.7	0.26
AL035252	CD39-like 2	4.6	0.26
X95289	PERB11 family member in MHC class I region	27.5	0.26
AJ001340	U3 snoRNP-associated 55-kDa protein	4.0	0.26
NM_014161	HSPC071 protein	10.6	0.27
U60873	Unknown	6.4	0.27
X91247	thioredoxin reductase 1	84.4	0.27
AK001284	hypothetical protein FLJ10422	4.2	0.27
U90840	synovial sarcoma, X breakpoint 3	6.6	0.27
X53777	ribosomal protein L17	39.9	0.27
AL035067	Unknown	10.0	0.28
AL117665	DKFZP586M1824 protein	3.9	0.28
L14561	ATPase, Ca++ transporting, plasma membrane 1	5.3	0.28
L19779	H2A histone family, member O	30.6	0.28
AL049782	Unknown	285.3	0.28
X00734	tubulin, beta, 5	39.7	0.29
AK001761	retinoic acid induced 3	23.7	0.29
U72661	ninjurin 1	4.4	0.29

Accession Number	Gene	Control: Media only Intensity	Ratio: LPS/control
S48220	deiodinase, iodothyronine, type I	1,296.1	0.29
AF025304	EphB2	4.5	0.30
S82198	chymotrypsin C	4.1	0.30
Z80782	H2B histone family, member K	31.9	0.30
X68194	synaptophysin-like protein	7.9	0.30
AB028869	Unknown	4.2	0.30
AK000761	Unknown	4.3	0.30

[00155] Table 57: Polynucleotides expressed to similar extents after stimulation by the bacterial products LPS, LTA, and CpG DNA. Bacterial products (100 ng/ml *S. typhimurium* LPS, 1μg/ml *S. aureus* LTA or 1 μM CpG) were shown to potently induce the expression of several polynucleotides. Peptide was incubated with the RAW cells for 4 h and the RNA was isolated, converted into labeled cDNA probes and hybridized to Atlas arrays. The intensity of control, unstimulated cells is shown in the second column. The "Ratio LPS/LTA/CpG: Control" column refers to the intensity of polynucleotide expression in bacterial product-simulated cells divided by the intensity of unstimulated cells.

Accession number	Control Unstim. Intensity	Ratio LPS: Control	Ratio LTA: Control	Ratio CpG: Control	Protein/polynucleotide
M15131	20	82	80	55	IL-1β
M57422	20	77	64	90	tristetraprolin
X53798	20	73	77	78	MIP-2α
M35590	188	50	48	58	MIP-1β
L28095	20	49	57	50	ICE

Accession number	Control Unstim. Intensity	Ratio LPS: Control	Ratio LTA: Control	Ratio CpG: Control	Protein/polynucleotide
M87039	20	37	38	45	iNOS
X57413	20	34	40	28	TGFβ
X15842	20	20	21	15	c-rel proto-oncopolynucleotide
X12531	489	19	20	26	MIP-1α
U14332	20	14	15	12	IL-15
M59378	580	10	13	11	TNFR1
U37522	151	6	6	6	TRAIL
M57999	172	3.8	3.5	3.4	NF-κB
U36277	402	3.2	3.5	2.7	I-κB (alpha subunit)
X76850	194	3	3.8	2.5	MAPKAP-2
U06924	858	2.4	3	3.2	Stat 1
X14951	592	2	2	2	CD18
X60671	543	1.9	2.4	2.8	NF-2
M34510	5970	1.6	2	1.4	CD14
X51438	2702	1.3	2.2	2.0	vimentin
X68932	4455	0.5	0.7	0.5	c-Fms
Z21848	352	0.5	0.6	0.6	DNA polymerase
X70472	614	0.4	0.6	0.5	B-myb

[00156] Table 58: Polynucleotides that were differentially regulated by the bacterial products LPS, LTA, and CpG DNA. Bacterial products (100 ng/ml *S. typhimurium* LPS, 1μg/ml *S. aureus* LTA or 1 μM CpG) were shown to potently induce the expression of several polynucleotides. Peptide was incubated with the RAW cells for 4 h and the RNA was isolated, converted into labeled cDNA probes and hybridized to Atlas arrays. The intensity of control, unstimulated cells is shown in the second column. The "Ratio LPS/LTA/CpG: Control" column refers to the

intensity of polynucleotide expression in bacterial product-simulated cells divided by the intensity of unstimulated cells.

Accession number	Unstim. Control Intensity	Ratio LPS: Control	Ratio LTA: Control	Ratio CpG: Control	Protein/polynucleotide
X72307	20	1.0	23	1.0	hepatocyte growth factor
L38847	20	1.0	21	1.0	hepatoma transmembrane kinase ligand
L34169	393	0.3	3	0.5	thrombopoietin
J04113	289	1	4	3	Nur77
Z50013	20	7	21	5	H-ras proto-oncopolynucleotide
X84311	20	4	12	2	Cyclin A1
U95826	20	5	14	2	Cyclin G2
X87257	123	2	4	1	Elk-1
J05205	20	18	39	20	Jun-D
J03236	20	11	19	14	Jun-B
M83649	20	71	80	42	Fas 1 receptor
M83312	20	69	91	57	CD40L receptor
X52264	20	17	23	9	ICAM-1
M13945	573	2	3	2	Pim-1
U60530	193	2	3	3	Mad related protein
D10329	570	2	3	2	CD7
X06381	20	55	59	102	Leukemia inhibitory factor (LIF)
X70296	20	6.9	13	22	Protease nexin 1 (PN-1)
U36340	20	38	7	7	CACCC Box- binding protein BKLF
S76657	20	11	6	7	CRE-BPI
U19119	272	10	4	4	interferon inducible protein 1

[00157] Table 59: Confirmation of Table 57 and 58 Array Data. a) Total RNA was isolated from unstimulated RAW macrophage cells and cells treated for 4 hr with 100 ng/ml *S. typhimurium* LPS, 1 μg/ml *S. aureus* LTA, 1 μM CpG DNA or media alone and Northern blots were performed the membrane was probed for GAPDH, CD14, vimentin, and tristetraprolin as described previously [Scott et al]. The hybridization intensities of the Northern blots were compared to GAPDH to look for inconsistencies in loading. These experiments were repeated at least three times and the data shown is the average relative levels of each condition compared to media (as measured by densitometry) ± standard error.

b) RAW 264.7 cells were stimulated with 100 ng/ml *S. typhimurium* LPS, 1 μg/ml *S. aureus* LTA, 1 μM CpG DNA or media alone for 24 hours. Protein lysates were prepared, run on SDS PAGE gels and western blots were performed to detect LIF (R&D Systems). These experiments were repeated at least three times and the data shown is the relative levels of LIF compared to media (as measured by densitometry) ± standard error.

c) Supernatant was collected from RAW macrophage cells treated with 100 ng/ml *S. typhimurium* LPS, 1 μg/ml *S. aureus* LTA, 1 μM CpG DNA, or media alone for 24 hours and tested for the amount of NO formed in the supernatant as estimated from the accumulation of the stable NO metabolite nitrite with the Griess reagent as described previously [Scott, et al]. The data shown is the average of three experiments ± standard error.

Product	Relative levels			
	Untreated	LPS	LTA	CpG
CD14[a]	1.0	2.2 ± 0.4	1.8 ± 0.2	1.5 ± 0.3
Vimentin[a]	1.0	1.2 ± 0.07	1.5 ± 0.05	1.3 ± 0.07
Tristetraprolin[a]	1.0	5.5 ± 0.5	5.5 ± 1.5	9.5 ± 1.5
LIF[b]	1.0	2.8 ± 1.2	2.7 ± 0.6	5.1 ± 1.6
NO[c]	8 ± 1.5	47 ± 2.5	20 ± 3	21 ± 1.5

[00158] Table 60. Pattern of Gene expression in A549 Human Epithelial cells up-regulated by bacterial signalling molecules (LPS). *E. coli* O111:B4 LPS (100 ng/ml) increased the expression of many polynucleotides in A549 cells as studied by polynucleotide microarrays. LPS was incubated with the A549 cells for 4 h and the RNA was isolated. 5 μg total RNA was used to make Cy3/Cy5 labelled cDNA probes and hybridised onto Human Operon arrays (PRHU04). The examples of polynucleotide expression changes in LPS simulated cells represent a greater than 2-fold intensity level change of LPS treated cells from untreated cells.

Accession Number	Gene
AL050337	interferon gamma receptor 1
U05875	interferon gamma receptor 2
NM_002310	leukemia inhibitory factor receptor
U92971	coagulation factor II (thrombin) receptor-like 2
Z29575	tumor necrosis factor receptor superfamily member 17
L31584	Chemokine receptor 7
J03925	cAMP response element-binding protein
M64788	RAP1, GTPase activating protein
NM_004850	Rho-associated kinase 2
D87451	ring finger protein 10
AL049975	Unknown
U39067	eukaryotic translation initiation factor 3, subunit 2
AK000942	Unknown
AB040057	serine/threonine protein kinase MASK
AB020719	KIAA0912 protein
AB007856	FEM-1-like death receptor binding protein
AL137376	Unknown
AL137730	Unknown
M90696	cathepsin S

AK001143	Unknown
AF038406	NADH dehydrogenase
AK000315	hypothetical protein FLJ20308
M54915	pim-1 oncogene
D29011	proteasome subunit, beta type, 5
AL034348	Unknown
D87076	KIAA0239 protein
AJ001403	mucin 5, subtype B, tracheobronchial
J03925	integrin, alpha M

EXAMPLE 10

ALTERING SIGNALING TO PROTECT AGAINST BACTERIAL INFECTIONS

[00159] The *Salmonella* Typhimurium strain SL1344 was obtained from the American Type Culture Collection (ATCC; Manassas, VA) and grown in Luria-Bertani (LB) broth. For macrophage infections, 10 ml LB in a 125 mL flask was inoculated from a frozen glycerol stock and cultured overnight with shaking at 37°C to stationary phase. RAW 264.7 cells ($1x10^5$ cells/well) were seeded in 24 well plates. Bacteria were diluted in culture medium to give a nominal multiplicity of infection (MOI) of approximately 100, bacteria were centrifuged onto the monolayer at 1000 rpm for 10 minutes to synchronize infection, and the infection was allowed to proceed for 20 min in a 37°C, 5% CO_2 incubator. Cells were washed 3 times with PBS to remove extracellular bacteria and then incubated in DMEM + 10% FBS containing 100 μg/ml gentamicin (Sigma, St. Louis, MO) to kill any remaining extracellular bacteria and prevent re-infection. After 2 h, the gentamicin concentration was lowered to 10 μg/ml and maintained throughout the assay. Cells were pretreated with inhibitors for 30 min prior to infection at the following concentrations: 50 μM PD 98059 (Calbiochem), 50 μM U 0126 (Promega), 2 mM diphenyliodonium (DPI), 250 μM acetovanillone (apocynin, Aldrich), 1 mM ascorbic acid (Sigma), 30 mM N-acetyl cysteine (Sigma), and 2 mM N^G-L-monomethyl arginine (L-NMMA,

Molecular Probes) or 2 mM N^G-D-monomethyl arginine (D-NMMA, Molecular Probes). Fresh inhibitors were added immediately after infection, at 2 h, and 6-8 h post-infection to ensure potency. Control cells were treated with equivalent volumes of dimethylsulfoxide (DMSO) per mL of media. Intracellular survival/replication of S. Typhimurium SL1344 was determined using the gentamicin-resistance assay, as previously described. Briefly, cells were washed twice with PBS to remove gentamicin, lysed with 1% Triton X-100/0.1% SDS in PBS at 2 h and 24 h post-infection, and numbers of intracellular bacteria calculated from colony counts on LB agar plates. Under these infection conditions, macrophages contained an average of 1 bacterium per cell as assessed by standard plate counts, which permitted analysis of macrophages at 24 h post-infection. Bacterial filiamentation is related to bacterial stress. NADPH oxidase and iNOS can be activated by MEK/ERK signaling. The results (Table 61) clearly demonstrate that the alteration of cell signaling is a method whereby intracellular Salmonella infections can be resolved. Thus since bacteria to up-regulate multiple genes in human cells, this strategy of blocking signaling represents a general method of therapy against infection.

[00160] Table 61: Effect of the Signaling Molecule MEK on Intracellular Bacteria in IFN-γ-primed RAW cells.

Treatment[a]	**Effect**[b]
0	None
MEK inhibitor U 0126	Decrease bacterial filamentation (bacterial stress)[c] Increase in the number of intracellular S. Typhimurium
MEK inhibitor PD 98059	Decrease bacterial filamentation (bacterial stress)[c] Increase in the number of intracellular S. Typhimurium

Treatment[a]	Effect[b]
NADPH oxidase inhibitor[d]	Decrease bacterial filamentation (bacterial stress)[c] Increase in the number of intracellular S. Typhimurium

EXAMPLE 11

ANTI-VIRAL ACTIVITY

[00161] SDF-1, a C-X-C chemokine is a natural ligand for HIV-1 coreceptor-CXCR4. The chemokine receptors CXCR4 and CCR5 are considered to be potential targets for the inhibition of HIV-1 replication. The crystal structure of SDF-1 exhibits antiparallel β-sheets and a positively charged surface, features that are critical in binding to the negatively charged extracellular loops of CXCR4. These findings suggest that chemokine derivatives, small-size CXCR4 antagonists, or agonists mimicking the structure or ionic property of chemokines may be useful agents for the treatment of X4 HIV-1 infection. It was found that the cationic peptides inhibited SDF-1 induced T-cell migration suggesting that the peptides may act as CXCR4 antagonists. The migration assays were performed as follows. Human Jurkat T cells were resuspended to 5 x 10^6 / ml in chemotaxis medium (RPMI 1640 / 10mM Hepes / 0.5 % BSA). Migration assays were performed in 24 well plates using 5 μm polycarbonate Transwell inserts (Costar). Briefly, peptide or controls were diluted in chemotaxis medium and placed in the lower chamber while 0.1 ml cells (5 x 10^6 / ml) was added to the upper chamber. After 3 hr at 37°C, the number of cells that had migrated into the lower chamber was determined using flow cytometry. The medium from the lower chamber was passed through a FACscan for 30 seconds, gating on forward and side scatter to exclude cell debris. The number of live cells was compared to a "100 % migration control" in which 5 x 10^5 /ml cells had been pipetted directly into the lower chamber and then counted on the FACscan for 30 seconds. The results demonstrate that the addition of peptide results in an inhibition of the migration of Human Jurkat T-cells (Table 62) probably by influencing CXCR4 expression (Tables 63 and 64).

[00162] **Table 62:** Peptide inhibits the migration of human Jurkat-T cells:

	Migration (%)			
Experiment	**Positive control**	**SDF-1 (100 ng/ml)**	**SDF-1 + SEQ ID 1 (50 μg/ml)**	**Negative control**
1	100 %	32 %	0 %	<0.01 %
2	100 %	40 %	0 %	0 %

[00163] **Table 63**: Corresponding polynucleotide array data to Table 56:

Polynucleotide / Protein	**Polynucleotide Function**	**Unstimulated Intensity**	**Ratio peptide: Unstimulated**	**Accession Number**
CXCR-4	Chemokine receptor	36	4	D87747

[00164] **Table 64:** Corresponding FACs data to Tables 62 and 63:

Peptide	**Concentration (μg/ml)**	**Fold Increase in Protein Expression CXCR-4**
SEQ ID NO: 1	10	No change
SEQ ID NO:1	50	1.3 ± 0.03
SEQ ID NO:1	100	1.6 ± 0.23
SEQ ID NO: 3	100	1.5 ± 0.2

[00165] 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.

WHAT IS CLAIMED IS:

1. A method of identifying a polynucleotide or pattern of polynucleotides regulated by one or more sepsis or inflammatory inducing agents and inhibited by a cationic peptide comprising contacting the polynucleotide or polynucleotides with one or more sepsis or inflammatory inducing agents, contacting the polynucleotide or polynucleotides with a cationic peptide either simultaneously or immediately thereafter, and determining a change in expression, wherein a change is indicative of a polynucleotide or pattern of polynucleotides that is regulated by a sepsis or inflammatory inducing agent and reduced by a cationic peptide.

2. The method of claim 1, wherein the sepsis or inflammatory inducing agent is LPS, LTA or CpG DNA, bacterial components or whole cells, or related agents.

3. The method of claim 1, comprising determining the level of expression of the polynucleotide prior to and following contacting with the sepsis or inflammatory inducing agent.

4. A polynucleotide or polynucleotide pattern identified by the method of claim 1.

5. A polynucleotide of claim 3, wherein the polynucleotide encodes a polypeptide involved in an inflammatory or septic response.

6. A method of identifying an agent that blocks sepsis or inflammation comprising combining a polynucleotide of claim 5 with an agent, wherein expression of the polynucleotide in the presence of the agent is modulated as compared with expression in the absence of the agent and wherein the modulation in expression affects the inflammatory or septic response.

7. The method of claim 6, wherein the effect is inhibition of the inflammatory or septic response.

8. An agent identified by the method of claim 6.

9. The agent of claim 8, wherein the agent is a peptide, peptidomimetic, chemical compound, nucleic acid molecule or a polypeptide.

10. The agent of claim 8, wherein the peptide is selected from SEQ ID NO:4-54.

11. A method of identifying a pattern of polynucleotide expression for inhibition of an inflammatory or septic response comprising:

contacting cells with LPS, LTA, CpG DNA and/or intact bacteria or bacterial components in the presence or absence of a cationic peptide;

detecting a pattern of polynucleotide expression for the cells in the presence and absence of the peptide, wherein the pattern in the presence of the peptide represents inhibition of an inflammatory or septic response.

12. The method of claim 11, further comprising contacting cells with one or more compounds suspected of inhibiting an inflammatory or septic response and identifying a compound that provides a pattern of polynucleotide expression similar to a pattern obtained with a cationic peptide that inhibits an inflammatory or septic response.

13. A compound identified by the method of claim 11.

14. A method of identifying an agent that enhances innate immunity comprising:

contacting a polynucleotide or polynucleotides that encode a polypeptide involved in innate immunity, with an agent of interest, wherein expression of the polynucleotide in the presence of the agent is modulated as compared with expression of the polynucleotide in the absence of the agent and wherein the modulated expression results in enhancement of innate immunity.

15. The method of claim 14, wherein the agent does not stimulate a septic reaction.

16. The method of claim 14, wherein the agent inhibits the inflammatory or septic response.

17. The method of claim 14, wherein the agent blocks the inflammatory or septic response.

18. The method as in any of claims 16 or 17, wherein the agent increases the expression of an anti-inflammatory encoding polynucleotide.

19. The method of claim 18, wherein the anti-inflammatory gene is selected from a subset that includes IL-1 R antagonist homolog 1 (AI167887), IL-10 R beta (AA486393), IL-10 R alpha (U00672), TNF Receptor member 1B (AA150416), TNF receptor member 5 (H98636), TNF receptor member 11b (AA194983), IK cytokine down-regulator of HLA II (R39227), TGFB inducible early growth response 2 (AI473938), CD2 (AA927710), glucocorticoid-related polynucleotides (AK000892), or IL-10 (M5762720.

20. The method of claim 19, wherein the agent inhibits the expression of TNF-alpha.

21. The method of claim 19, wherein the agent inhibits the expression of interleukins.

22. The method of claim 23, wherein the interleukin is IL-8.

23. The method of claim 16, wherein the agent is a peptide.

24. The method of claim 23, wherein the peptide is selected from SEQ ID NO:4-54.

25. An agent identified by the method of claim 14.

26. An agent of claim 25, wherein the agent is a peptide, peptidomimetic, chemical compound, or a nucleic acid molecule.

27. A method of identifying a pattern of polynucleotide expression for identification of a compound that selectively enhances innate immunity comprising:

detecting a pattern of polynucleotide expression for cells contacted in the presence and absence of a cationic peptide, wherein the pattern in the presence of the peptide represents stimulation of innate immunity;

detecting a pattern of polynucleotide expression for cells contacted in the presence of a test compound, wherein a pattern with the test compound that is similar to the pattern observed in the presence of the cationic peptide, is indicative of a compound that enhances innate immunity.

28. A compound identified by the method of claim 27.

29. The method of claim 27, wherein the compound does not stimulate a septic reaction.

30. The method of claim 27, wherein the polynucleotide expression pattern includes expression of pro-inflammatory polynucleotides.

31. The method of claim 30, wherein the pro-inflammatory polynucleotides include ring finger protein 10 (D87451), serine/threonine protein kinase MASK (AB040057), KIAA0912 protein (AB020719), KIAA0239 protein (D87076), RAP1, GTPase activating protein 1 (M64788), FEM-1-like death receptor binding protein (AB007856), cathepsin S (M90696), hypothetical protein FLJ20308 (AK000315), pim-1 oncogene (M54915), proteasome subunit beta type 5 (D29011), KIAA0239 protein (D87076), mucin 5 subtype B tracheobronchial (AJ001403), cAMP response element-binding protein CREBPa, integrin alpha M (J03925), Rho-associated kinase 2 (NM_004850), PTD017 protein (AL050361) unknown genes (AK001143, AK034348, AL049250, AL16199, AL031983), retinoic acid receptor (X06614), G protein-coupled receptors (Z94155, X81892, U52219, U22491, AF015257, U66579) chemokine (C-C motif) receptor 7 (L31584), tumor necrosis factor receptor superfamily member 17 (Z29575), interferon gamma receptor 2 (U05875), cytokine receptor-like factor 1 (AF059293), class I cytokine receptor (AF053004), coagulation factor II (thrombin) receptor-like 2 (U92971), leukemia inhibitory factor receptor (NM_002310), interferon gamma receptor 1 (AL050337) or any combination thereof.

32. The method of claim 27, wherein the expression pattern includes expression of polynucleotides encoding chemokines.

33. The method of claim 27, wherein the expression pattern includes expression of cell differentiation factors.

34. The method of claim 27, wherein the polynucleotide expression pattern includes expression of cell surface receptors.

35. The method of claim 34, wherein the cell surface receptors include chemokine receptors or integrin receptors.

36. A method of identifying an agent that is capable of selectively enhancing innate immunity comprising:

contacting a cell containing a polynucleotide or polynucleotides that encode a polypeptide involved in innate immunity, with an agent of interest, wherein expression of the polynucleotide or polynucleotides in the presence of the agent is modulated as compared with expression in the absence of the agent and wherein the modulated expression results in enhancement of innate immunity.

37. The method of claim 26 in which the pattern of expression is utilized in screening for compounds that enhance innate immunity.

38. A compound of claim 28, wherein the compound stimulates chemokine or chemokine receptor expression.

39. A compound of claim 38, wherein the chemokine or chemokine receptor is CXCR4, CCR5, CCR2, CCR6, MIP-1 alpha, IL-8, MCP-1, MCP-2, MCP-3, MCP-4, or MCP-5.

40. A compound of claim 28, wherein the compound is a peptide, peptidomimetic, chemical compound, or a nucleic acid molecule.

41. A method of identifying an agent that is capable of both suppressing or blocking septic or inflammatory responses and enhancing innate immunity comprising:

contacting a cell containing i) a polynucleotide or polynucleotides that encode a polypeptide capable of suppressing inflammatory or septic responses and ii) a polynucleotide or polynucleotides that encode a polypeptide involved in innate immunity, with an agent of interest, wherein expression of in the presence of the agent is modulated as compared with expression of the polynucleotide or polynucleotides in the absence of the agent and wherein the modulated expression results in suppression of inflammatory or septic responses and enhancement of innate immunity.

42. A method for inferring a state of infection in a mammalian subject from a nucleic acid sample of the subject comprising identifying in the nucleic acid sample a polynucleotide expression pattern exemplified by an increase in polynucleotide expression of at least 2 polynucleotides in Table 55 as compared to a non-infected subject.

43. A method for inferring a state of infection in a mammalian subject from a nucleic acid sample of the subject comprising identifying in the nucleic acid sample a polynucleotide expression pattern exemplified by a decrease in polynucleotide expression of at least 2 polynucleotides in Table 56 as compared to a non-infected subject.

44. A method for inferring a state of infection in a mammalian subject from a nucleic acid sample of the subject comprising identifying in the nucleic acid sample a polynucleotide expression pattern exemplified by a polynucleotide expression of at least 2 polynucleotides in Table 57 as compared to a non-infected subject.

45. The method of any of claims 30, 31 or 32, wherein the state of infection is due to a bacteria, virus, fungus or parasitic agent.

46. The method of any of claims 30, 31 or 32, wherein the state of infection is due to a Gram positive or Gram negative bacteria.

47. A polynucleotide expression pattern of a subject having a state of infection identified by the method of claim 31.

48. A cationic peptide that is an antagonist of CXCR-4.

49. A method of identifying a cationic peptide that is an antagonist of CXCR-4 comprising contacting T cells with SDF-1 in the presence of absence of a test peptide and measuring chemotaxis, wherein a decrease in chemotaxis in the presence of the test peptide is indicative of a peptide that is an antagonist of CXCR-4.

50. An isolated cationic peptide comprising the general formula $X_1X_2X_3IX_4PX_4IPX_5X_2X_1$ (SEQ ID NO: 4), wherein X_1 is one or two of R, L or K, X_2 is one of C, S or A, X_3 is one of R or P, X_4 is one of A or V and X_5 is one of V or W.

51. The cationic peptide of claim 38, wherein the peptide is selected from the group consisting of: LLCRIVPVIPWCK (SEQ ID NO: 5), LRCPIAPVIPVCKK (SEQ ID NO: 6), KSRIVPAIPVSLL (SEQ ID NO: 7), KKSPIAPAIPWSR (SEQ ID NO: 8), RRARIVPAIPVARR (SEQ ID NO: 9) and LSRIAPAIPWAKL (SEQ ID NO: 10).

52. The peptide of claim 38, wherein the peptide has anti-inflammatory activity.

53. The peptide of claim 38, wherein the peptide has anti-sepsis activity.

54. An isolated cationic peptide comprising the general formula $X_1LX_2X_3KX_4X_2X_5X_3PX_3X_1$ (SEQ ID NO: 11), wherein X_1 is one or two of D, E, S, T or N, X2 is one or two of P, G or D, X_3 is one of G, A, V, L, I or Y, X_4 is one of R, K or H and X_5 is one of S, T, C, M or R.

55. The cationic peptide of claim 42, wherein the peptide is selected from the group consisting of: DLPAKRGSAPGST (SEQ ID NO: 12), SELPGLKHPCVPGS (SEQ ID NO: 13), TTLGPVKRDSIPGE (SEQ ID NO: 14), SLPIKHDRLPATS (SEQ ID NO: 15), ELPLKRGRVPVE (SEQ ID NO: 16) and NLPDLKKPRVPATS (SEQ ID NO: 17).

56. The peptide of claim 42, wherein the peptide has anti-inflammatory activity.

57. The peptide of claim 42, wherein the peptide has anti-sepsis activity.

58. An isolated cationic peptide comprising the general formula $X_1X_2X_3X_4WX_4WX_4X_5K$ (SEQ ID NO: 18), wherein X_1 is one to four chosen from A, P or R, X_2 is one or two aromatic amino acids (F, Y and W), X_3 is one of P or K, X_4 is one, two or none chosen from A, P, Y or W and X_5 is one to three chosen from R or P.

59. The cationic peptide of claim 46, wherein the peptide is selected from the group consisting of: RPRYPWWPWWPYRPRK (SEQ ID NO: 19), RRAWWKAWWARRK (SEQ ID NO: 20), RAPYWPWAWARPRK (SEQ ID NO: 21), RPAWKYWWPWPWPRRK (SEQ ID NO: 22), RAAFKWAWAWWRRK (SEQ ID NO: 23) and RRRWKWAWPRRK (SEQ ID NO: 24).

60. The peptide of claim 46, wherein the peptide has anti-inflammatory activity.

61. The peptide of claim 46, wherein the peptide has anti-sepsis activity.

62. An isolated cationic peptide comprising the general formula $X_1X_2X_3X_4X_1VX_3X_4RGX_4X_3X_4X_1X_3X_1$ (SEQ ID NO: 25) wherein X_1 is one or two of R or K, X_2 is a polar or charged amino acid (S, T, M, N, Q, D, E, K, R and H), X_3 is C, S, M, D or A and X_4 is F, I, V, M or R.

63. The cationic peptide of claim 50, wherein the peptide is selected from the group consisting of: RRMCIKVCVRGVCRRKCRK (SEQ ID NO: 26), KRSCFKVSMRGVSRRRCK (SEQ ID NO: 27), KKDAIKKVDIRGMDMRRAR (SEQ ID NO: 28), RKMVKVDVRGIMIRKDRR (SEQ ID NO: 29), KQCVKVAMRGMALRRCK (SEQ ID NO: 30) and RREAIRRVAMRGRDMKRMRR (SEQ ID NO: 31).

64. The peptide of claim 50, wherein the peptide has anti-inflammatory activity.

65. The peptide of claim 50, wherein the peptide has anti-sepsis activity.

66. An isolated cationic peptide comprising the general formula $X_1X_2X_3X_4X_1VX_5X_4RGX_4X_5X_4X_1X_3X_1$ (SEQ ID NO: 32), wherein X_1 is one or two

of R or K, X_2 is a polar or charged amino acid (S, T, M, N, Q, D, E, K, R and H), X_3 is one of C, S, M, D or A, X_4 is one of F, I, V, M or R and X_5 is one of A, I, S, M, D or R.

67. The cationic peptide of claim 54, wherein the peptide is selected from the group consisting of: RTCVKRVAMRGIIRKRCR (SEQ ID NO: 33), KKQMMKRVDVRGISVKRKR (SEQ ID NO: 34), KESIKVIIRGMMVRMKK (SEQ ID NO: 35), RRDCRRVMVRGIDIKAK (SEQ ID NO: 36), KRTAIKKVSRRGMSVKARR (SEQ ID NO: 37) and RHCIRRVSMRGIIMRRCK (SEQ ID NO: 38).

68. The peptide of claim 54, wherein the peptide has anti-inflammatory activity.

69. The peptide of claim 54, wherein the peptide has anti-sepsis activity.

70. An isolated cationic peptide comprising the general formula $KX_1KX_2FX_2KMLMX_2ALKKX_3$ (SEQ ID NO: 39), wherein X_1 is a polar amino acid (C, S, T, M, N and Q); X_2 is one of A, L, S or K and X_3 is 1-17 amino acids chosen from G, A, V, L, I, P, F, S, T, K and H.

71. The cationic peptide of claim 58, wherein the peptide is selected from the group consisting of: KCKLFKKMLMLALKKVLTTGLPALKLTK (SEQ ID NO: 40), KSKSFLKMLMKALKKVLTTGLPALIS (SEQ ID NO: 41), KTKKFAKMLMMALKKVVSTAKPLAILS (SEQ ID NO: 42), KMKSFAKMLMLALKKVLKVLTTALTLKAGLPS (SEQ ID NO: 43), KNKAFAKMLMKALKKVTTAAKPLTG (SEQ ID NO: 44) and KQKLFAKMLMSALKKKTLVTTPLAGK (SEQ ID NO: 45).

72. The peptide of claim 58, wherein the peptide has anti-inflammatory activity.

73. The peptide of claim 58, wherein the peptide has anti-sepsis activity.

74. An isolated cationic peptide comprising the general formula $KWKX_2X_1X_1X_2X_2X_1X_2X_2X_1X_1X_2X_2IFHTALKPISS$ (SEQ ID NO: 46), wherein X_1 is a hydrophobic amino acid and X_2 is a hydrophilic amino acid.

75. The cationic peptide of claim 62, wherein the peptide is selected from the group consisting of: KWKSFLRTFKSPVRTIFHTALKPISS (SEQ ID NO: 47), KWKSYAHTIMSPVRLIFHTALKPISS (SEQ ID NO: 48), KWKRGAHRFMKFLSTIFHTALKPISS (SEQ ID NO: 49), KWKKWAHSPRKVLTRIFHTALKPISS (SEQ ID NO: 50), KWKSLVMMFKKPARRIFHTALKPISS (SEQ ID NO: 51) and KWKHALMKAHMLWHMIFHTALKPISS (SEQ ID NO: 52).

76. The peptide of claim 62, wherein the peptide has anti-inflammatory activity.

77. The peptide of claim 62, wherein the peptide has anti-sepsis activity.

78. An isolated cationic peptide comprising the sequence KWKSFLRTFKSPVRTVFHTALKPISS (SEQ ID NO: 53).

79. An isolated cationic peptide comprising the sequence KWKSYAHTIMSPVRLVFHTALKPISS (SEQ ID NO: 54).

80. The method of claim 28, wherein the agent is a Zinc finger protein (AF061261); Cell cycle gene (S70622); IL-10 Receptor U00672); Transferase (AF038664); Homeobox protein (AC004774); Forkhead protein (AF042832); Unknown (AL096803); KIAA0284 Protein (AB006622); Hypothetical Protein (AL022393); Receptor (AF112461); Hypothetical Protein (AK002104); Protein (AL050261); Polypeptide (AF105424); SPR1 protein (AB031480); Dehydrogenase (D17793); Transferase (M63509); and Peroxisome factor (AB013818).

81. The polynucleotide expression pattern of a subject having a state of infection identified by claim 56 wherein the genes upregulated are Accession number D87451 - ring finger protein 10; Accession number AL049975, Unknown; Accession number U39067, eukaryotic translation initiation factor 3 subunit 2; Accession number AK000942, Unknown; Accession number AB040057, serine/threonine protein kinase MASK; Accession number AB020719, KIAA0912 protein; Accession number AB007856, FEM-1-like death receptor binding protein; Accession number AL137376, Unknown; Accession number AL137730, Unknown; Accession number M90696, cathepsin S; Accession number AK001143, Unknown; Accession number

AF038406, NADH dehydrogenase; Accession number AK000315, hypothetical protein FLJ20308; Accession number M54915, pim-1 oncogene; Accession number D29011, proteasome subunit beta type 5; Accession number AL034348, Unknown; Accession number D87076, KIAA0239 protein; Accession number AJ001403, tracheobronchial mucin 5 subtype B; Accession number J03925, integrin alpha M, Rho-associated kinase 2 (NM_004850), PTD017 protein (AL050361) unknown genes (AK001143, AK034348, AL049250, AL16199, AL031983), retinoic acid receptor (X06614), G protein-coupled receptors (Z94155, X81892, U52219, U22491, AF015257, U66579) chemokine (C-C motif) receptor 7 (L31584), tumor necrosis factor receptor superfamily member 17 (Z29575), interferon gamma receptor 2 (U05875), cytokine receptor-like factor 1 (AF059293), class I cytokine receptor (AF053004), coagulation factor II (thrombin) receptor-like 2 (U92971), leukemia inhibitory factor receptor (NM_002310), interferon gamma receptor 1 (AL050337), or any combination thereof.

82. The method of claim 32, wherein the chemokines include CXCR4, CXCR1, CXCR2, CCR2, CCR4, CCR5, CCR6, MIP-1 alpha, MDC, MIP-3 alpha, MCP-1, MCP-2, MCP-3, MCP-4, MCP-5, and RANTES.

83. The method of claim 33, wherein the cell differentiation factors includeTGFβ inducible early growth response 2 (AI473938), zinc finger proteins (AF061261, U00115, X78924), and transcription factors (U31556, AL137681, X68560).

84. A compound of claim 38, wherein the compound modifies kinase activity.

85. A compound of claim 84, wherein the kinase is selected from MAP kinase kinase 3 (D87116), MAP kinase kinase 6 (H07920), MAP kinase kinase 5 (W69649), MAP kinase 7 (H39192), MAP kinase 12 (AI936909), MAP kinase-activated protein kinase 3 (W68281), or MAP kinase kinase 1 (L11284).

86. A compound of claim 21, wherein the compound decreases proteasome subunit expression.

87. A compound of claim 86, wherein the proteasome subunit includes polynucleotides with accession numbers D11094, L02426, D00763, AB009398, AF054185, M34079, M34079, or AL031177.

88. An isolated cationic peptide that reduces polynucleotide expression of SDF-1 receptor.