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(57) **Abrégé/Abstract:**

The present invention relates to retroviral constructs that encode novel monoclonal antibodies, novel fusion proteins, and chimeric monoclonal antibodies and to methods of using and producing the same. In particular, the present invention relates to methods of producing a fusion protein comprising a microorganism targeting molecule (e.g., immunoglobulin or innate immune system receptor molecule) and a biocide (e.g., bactericidal enzymes) in transgenic animals (e.g., bovines) and in cell cultures. The present invention also relates to therapeutic and prophylactic methods of using a fusion protein comprising a microorganism targeting molecule and a biocide in health care (e.g., human and veterinary), agriculture (e.g., animal and plant production), and food processing (e.g., beef carcass processing). The present invention also relates to methods of using a fusion protein comprising a microorganism targeting molecule and a biocide in various diagnostic applications in number of diverse fields such as agriculture, medicine, and national defense.



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(54) Title: TARGETED BIOCIDES

(57) Abstract: The present invention relates to retroviral constructs that encode novel monoclonal antibodies, novel fusion proteins, and chimeric monoclonal antibodies and to methods of using and producing the same. In particular, the present invention relates to methods of producing a fusion protein comprising a microorganism targeting molecule (e.g., immunoglobulin or innate immune system receptor molecule) and a biocide (e.g., bactericidal enzymes) in transgenic animals (e.g., bovines) and in cell cultures. The present invention also relates to therapeutic and prophylactic methods of using a fusion protein comprising a microorganism targeting molecule and a biocide in health care (e.g., human and veterinary), agriculture (e.g., animal and plant production), and food processing (e.g., beef carcass processing). The present invention also relates to methods of using a fusion protein comprising a microorganism targeting molecule and a biocide in various diagnostic applications in number of diverse fields such as agriculture, medicine, and national defense.



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TARGETED BIOCIDES

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FIELD OF THE INVENTION

The present invention relates to retroviral constructs that encode novel monoclonal antibodies, novel fusion proteins, and chimeric monoclonal antibodies and to methods of using and producing the same. In particular, the present invention relates to methods of
10 producing a fusion protein comprising a microorganism targeting molecule (*e.g.*, immunoglobulin or innate immune system receptor molecule) and a biocide (*e.g.*, bactericidal enzyme) in transgenic animals (*e.g.*, bovines) and in cell cultures. The present invention also relates to therapeutic and prophylactic methods of using a fusion protein comprising a microorganism targeting molecule and a biocide in health care (*e.g.*, human
15 and veterinary), agriculture (*e.g.*, animal and plant production), and food processing (*e.g.*, beef carcass processing). The present invention also relates to methods of using a fusion protein comprising a microorganism targeting molecule and a biocide in various diagnostic applications in number of diverse fields such as agriculture, medicine, and national defense.

20 BACKGROUND OF THE INVENTION

The majority of people in the industrialized world have access to an abundance of inexpensive processed food products. The safety, quality, and wholesomeness of these products are usually unquestioned. The availability of inexpensive food products is largely a result of advances in farm mechanization and improved industries of scale in food
25 processing and distribution operations. The mechanization of the family farm has not come without certain drawbacks however.

One of the drawbacks of large-scale food processing operations, and of meat processing in particular, is the occasional contamination (*e.g.*, bacterial, fungal, *etc.*) and subsequent distribution of large quantities of contaminated products sometimes with dire consequences.
30 Food safety researchers have determined that the introduction of even a few contaminated carcasses into the production lines of large scale food processing operations is often enough

to contaminate entire batches of product. The meat packing industry is particularly susceptible to carcass contamination during dehiding, evisceration, splitting, chilling, and fabrication. Further contamination of previously uncontaminated meat products may occur during grinding, processing, and transport. This type of contamination has lead to several major meat product recalls, including the recall of 24 million pounds of ground beef by the Hudson Beef Co. in 1997, and more recently, the recall of 19 million pounds of beef and related products by the ConAgra Beef Company in July 2002. (See, Recall Release, FSIS-RC-055-2002). The economic impact of food safety and spoilage is very large. USDA ERS estimates that the leading six bacterial food borne pathogens cause \$2.9-6.7 billion in medical costs and lost productivity annually in the US (Buzby et al., Bacterial Foodborne Disease: Medical Costs and Productivity Losses. 1996. Food and Consumer Economics Division, Economic Research Service U.S. Department of Agriculture. Agricultural Economic Report 741)

Many meat product recalls are the result of contamination by the bacterium *Escherichia coli* O157:H7. This bacterium is commonly isolated from the gastrointestinal tract and feces of cattle. Direct contact with cattle can be a source of human infection. However, the principal route of transmission to humans is through fecal contamination of carcasses at slaughter. (J. Tuttle *et al.*, Epidemiol Infect., 122:185-192 [1999]). Every year in the United States the O157:H7 bacterium causes about 70,000 cases of hemorrhagic diarrhea and renal disease. Children, the elderly, and the immunocompromised are most susceptible to foodborne illness caused by *Escherichia coli* O157:H7. Virulent strains of *Escherichia coli* are not the only foodborne pathogens of concern.

Listeria monocytogenes has emerged as another dangerous, but relatively uncommon foodborne pathogen. Despite being an uncommon source of illness, *L. monocytogenes* is ubiquitous in agricultural and food processing environments and can cause serious human and animal infections. The infection caused by *L. monocytogenes* is commonly called Listeriosis. Listeriosis occurs in sporadic and epidemic forms throughout the world. (See e.g., B. Lorber, Clin. Infect. Dis., 24(1):1-9 [1997]; J.M. Farber *et al.*, Microbiol. Rev., 55:476-511 [1991]; and W.F. Schlech, Clin. Infect. Dis., 31:770-775 [2000]). A multistate outbreak of Listeriosis has been reported in the United States. (Morb. Mortal. Wkly. Report, 49(50):1129-1130 [2000] *erratum* in Morb. Mortal. Wkly. Report, 50(6):101

[2001]). Since May 2000, 29 illnesses caused by a strain of *Listeria monocytogenes* have been identified in 10 states: New York (15 cases); Georgia (3 cases); Connecticut, Ohio, and Michigan (2 cases each); and California, Pennsylvania, Tennessee, Utah, and Wisconsin (1 case each).

5 Listeriosis, in its most severe form, is an invasive disease that affects immunocompromised patients and has the highest case-fatality rate of any foodborne illnesses. (B.G. Gellin *et al.*, Amer. J. Epidemiol., 133:392-401 [1991]; D.B. Louria *et al.*, Ann. NY Acad. Sci., 174:545-551 [1970]; J. McLauchlin, Epidemiol. Infect., 104:191-201 [1990]; V. Goulet and P. Marchetti, Scand. J. Infect. Dis., 28:367-374 [1996]; and C.J. Bula
10 *et al.*, Clin. Infect Dis., 20:66-72 [1995]). In immunocompetent persons, it can also cause severe disease as well as outbreaks of benign febrile gastroenteritis. (P. Aureli *et al.*, New Engl. J. Med., 342:1236-41 [2000]). Another form of human disease is perinatal infection, which is associated with a high rate of fetal loss (including full-term stillbirths) and serious neonatal disease (J. McLauchlin, Epidemiol. Infect., 104:181-190 [1990]).

15 Most, perhaps all, of listeriosis in humans occurs after consumption of contaminated food (e.g., meat and cheese) products. (A. Schuchat *et al.*, J. Amer. Med. Assoc., 267:2041-2045 [1992]). While uncommon, Listeriosis causes about half the foodborne disease fatalities in the US each year. Additionally, many mild cases of listeriosis and inapparent *Listeria* infections go unreported. For those susceptible to listeriosis, ingestion of even
20 small doses of *L. monocytogenes* is often sufficient for infection. About 2,500 cases of listeriosis are reported in the US each year, of these about 20% or 500 cases are fatal.

In 1989, the USDA FSIS implemented a testing program for *L. monocytogenes* in cooked meat products and adopted a zero tolerance position for *L. monocytogenes* contamination in ready to eat products. Guidelines promulgated by the American
25 Association of Meat Processors for current Good Manufacturing Practices for Ready to Eat meat products address the need for environmental monitoring for *Listeria* as a component of HACCP programs. The ecology of *L. monocytogenes* and its increasing prevalence and/or detection in food preparation establishments has lead to major recalls of processed meat products. In October 2002 the USDA issued a recall notice, which when further expanded,
30 constituted the largest meat product recall on record for 28 million pounds of processed turkey products (See, USDA FSIS Recall Notification Report 090-2002 EXP Recall from

Pilgrims Pride Corp dba Wampler Foods Inc. 11-4-2002). The recall was in response to detection of *L. monocytogenes* at multiple points in the facilities and equipment used to process the recalled turkey.

5 A number of approaches have been tried to increase the safety and wholesomeness of the nation's meat and agricultural products. For example, some approaches have focused on the exposing food products to one or more types of pathogen destroying processes, including ionizing radiation or ultra high temperatures and pressures. (See e.g., US 5,891,490; 6,013,918; 6,086,936; and 6,165,526 etc.). US 6,165,526 is representative of these approaches. This patent describes an UV radiation and ultra high temperature method
10 for sterilizing food products.

A number of other approaches have focused on providing mixtures of chemicals (e.g., acids, surfactants, emulsifying agents, and organic phosphates) that inactivate bacteria and bacterial spores in food products. (See e.g., US 5,550,145; and 5,618,840). US 5,618,840, for instance, describes an antibacterial oil-in-water emulsion for inhibiting the
15 growth of *Helicobacter pylori*.

The various compositions and methods previously described for food sterilization have certain advantages and certain other disadvantages. One disadvantage is that the manufacture and addition of large quantities of artificial chemicals to food products can be costly and logistically difficult. Moreover, the current chemical food sterilization agents are
20 indiscriminate and are thus inappropriate for addition into food products such as cheese and yogurt that require the beneficial action of certain bacteria for their production. The addition of artificial chemical compounds to food products or subjecting the products to irradiation or temperature and pressure extremes can also produce unpleasant organoleptic qualities. Another disadvantage is the public's generally negative perception of food
25 irradiation and the addition of chemical additives.

Still other efforts have been directed to producing food washes to remove residual surface impurities such as waxes and pesticides sometimes acquired during food product production, processing, and transporting. For instance, US 6,367,488 describes a chemical wash for fruits and vegetables made from surfactants, such as oleate, and alcohol
30 ethoxylates, and neutralized phosphoric acid. While these washes are useful for removing

surface contaminates and surface bacteria from solid food products, these compositions are inappropriate for sterilizing homogenized food products such as ground beef.

While each of these above-mentioned compositions and methods has particular advantages and disadvantages, the need still exists for compositions and methods that
5 reduce the amount of pathogenic bacteria shed by feedlot animals (*e.g.*, bovines, porcines, and the like), that induce immunity in feedlot animals to pathogens, and for edible compositions that safely destroy harmful foodborne pathogens.

A further major economic problem confronting the food processing industry is that of bacterial spoilage. In particular, dairy and processed meat products are susceptible to
10 bacterial spoilage by organisms such as the Lactic acid bacteria (*e.g.*, *Lactobacillus etc.*) (Kraft AA. Health hazards vs. food spoilage. Boca Raton, FL.: CRC Press, Inc., 1992). These organisms are widely distributed in nature, and can easily out-compete other bacteria under low oxygen tension and low pH conditions that are common in processed dairy and meat foods (Stamer. Lactic acid bacteria. In: Defigueiredo MP and Splittstoesser DF eds.
15 Westport, CT: AVI Publishing, 1976). Over 20% of the fruit and vegetable products harvested for human consumption are believed to be lost to post-harvest microbial spoilage (Jay, J. Modern Food Microbiology 4th ed Van Norstand Reinhold New York, 1992).

SUMMARY OF THE INVENTION

20 The present invention relates to retroviral constructs that encode novel monoclonal antibodies, novel fusion proteins, and chimeric monoclonal antibodies and to methods of using and producing the same. In particular, the present invention relates to methods of producing a fusion protein comprising a microorganism targeting molecule (*e.g.*, immunoglobulin or innate immune system receptor molecule) and a biocide (*e.g.*,
25 bactericidal enzyme) in transgenic animals (*e.g.*, bovines) and in cell cultures. The present invention also relates to therapeutic and prophylactic methods of using a fusion protein comprising a microorganism targeting molecule and a biocide in health care (*e.g.*, human and veterinary), agriculture (*e.g.*, animal and plant production), and food processing (*e.g.*, beef carcass processing). The present invention also relates to methods of using a fusion
30 protein comprising a microorganism targeting molecule and a biocide in various diagnostic applications in number of diverse fields such as agriculture, medicine, and national defense.

In some embodiments, the present invention provides a composition comprising a recombinant fusion protein, wherein the protein comprises a microorganism targeting molecule joined to at least a portion of a protein biocide molecule, wherein the microorganism targeting molecule binds to a pathogenic or spoilage microorganism. In some embodiments, the microorganism targeting molecule binds to pathogen associated molecular patterns. In some embodiments, the microorganism targeting molecule comprises CD14, lipopolysaccharide binding protein, surfactant protein D, or Mannan binding lectin. In some embodiments, the microorganism targeting molecule comprises at least a portion of an immunoglobulin molecule. In some embodiments, the microorganism targeting molecule binds to a bacteria, a parasite, a protozoan, an apicomplexan protozoan (e.g., coccidian, cryptosporidian, toxoplasman, malarian or trypanosomatid protozoans), a fungus, a virus, or a foodborne or waterborne pathogen (e.g., *E coli spp.*, *Listeria monocytogenes*, *Salmonella spp*, *Staphylococcus*, *Clostridium botulinum*, *Clostridium perfringens* or *Cryptosporidium parvum*) or a food spoilage organism (e.g., *Lactobacillus spp*, *Leuconostoc spp*, *Pediococcus spp*, and *Streptococcus spp*). In some embodiments, the immunoglobulin molecule is derived from a monoclonal antibody. In some embodiments, the monoclonal antibody is dimeric. In other embodiments, the monoclonal antibody is trimeric. In yet other embodiments, the monoclonal antibody is tetrameric. In still further embodiments, the monoclonal antibody is pentameric. In certain embodiments, the monoclonal antibody is hexameric. In some embodiments, the monoclonal antibody comprises IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgA_{sec}, IgD, or IgE. In some embodiments, the protein biocide molecule is attached to a J chain of the monoclonal antibody. In some embodiments, the microorganism targeting molecule and the at least a portion of a protein biocide molecule are joined by a poly amino acid linker molecule from about 2 to 500, preferably from about 5 to 100, and even more preferably from about 10 to 30 amino acids long. In some embodiments, the poly amino acid linker molecule consists of amino acids selected from the group consisting of Gly, Ser, Asn, Thr, Pro, and Ala. In some embodiments, the amino acid linker comprises a sequence of amino acid residues having the formula:



wherein $n \geq 1$,

wherein $x \geq 1$, and

wherein $y \geq 1$. In some embodiments, $n = 1$, wherein $x = 4$, and wherein $y \geq 1$. In other embodiments, $y = 1, 2, 3, 4, 5, 6, 7$, or 8 . In some embodiments, the protein biocide comprises at least an active portion of an enzyme. In some embodiments, the enzyme comprises lysozyme, phospholipase A2, lactoferrin, lactoperoxidase, bacterial permeability
5 increasing protein, lysostaphin, or aprotinin.

The present invention also provides a composition comprising: a recombinant fusion protein, wherein the protein comprises a microorganism targeting molecule joined to at least a portion of a protein biocide molecule, wherein the microorganism targeting molecule binds to a bacteria, and wherein the microorganism targeting molecule is an
10 innate immune system receptor, and wherein the fusion protein neutralizes the bacteria; and a buffer. In some embodiments, the composition is for the treatment of a non-human object suspected of being contaminated with a bacteria, wherein the composition is for application to the non-human object under conditions such that the recombinant fusion protein neutralizes the bacteria. In further embodiments, the composition is for
15 the treatment of an animal carcass or part thereof suspected of being contaminated or infected with a bacteria, wherein the composition is for application to the animal carcass or part thereof under conditions such that the recombinant fusion protein neutralizes the bacteria. In additional embodiments, the composition is for the treatment of a food product suspected of being contaminated with a bacteria, wherein the composition is for
20 application to the food product under conditions such that the recombinant fusion protein neutralizes the bacteria. In other embodiments, the composition is for the treatment of a subject suspected of being contaminated or infected with a bacteria, wherein the composition is for application to the subject under conditions such that the recombinant fusion protein neutralizes the bacteria. In some embodiments, the composition is for the
25 treatment of a plant or a part of a plant suspected of being infected or contaminated by a bacteria, wherein the composition is for application to the plant or plant part under conditions such that the recombinant fusion protein neutralizes the bacteria.

The present invention further provides a retroviral construct comprising a nucleic acid sequence encoding a microorganism targeting molecule linked to a portion of a biocide
30 molecule. In some embodiments the microorganism targeting molecule is a receptor active in the innate immune system (e.g., CD14, lipopolysaccharide binding protein, mannan binding lectin, surfactant binding protein, toll receptor) or a recombinant analog thereof.

The present invention also provides a vector construct comprising a nucleic acid sequence encoding the recombinant fusion protein of the present invention.

The present invention additionally provides a method of treating an object, comprising: providing a recombinant fusion protein, wherein the protein comprises a microorganism targeting molecule joined to at least a portion of a biocide molecule, wherein the microorganism targeting molecule binds to a pathogen or spoilage organism; and an object suspected of being contaminated with a pathogen or spoilage organism; and applying the recombinant fusion protein to the object under conditions such that the recombinant fusion protein neutralizes the pathogen or spoilage organism suspected of contaminating the surface. In some embodiments, the object is food processing equipment, military equipment (e.g., tanks), personal protective gear, medical devices, building structures (e.g., heating and ventilation equipment, walls, wall cavities, plumbing systems) surfaces in a household, or household equipment (e.g., appliances, sinks, toilet bowls, bathroom tiles, bath tubs, or showers).

The present invention also provides a non-therapeutic method of treating a non-human object, comprising: a) providing i) a recombinant fusion protein, wherein the protein comprises a microorganism targeting molecule joined to at least a portion of a protein biocide molecule, wherein the microorganism targeting molecule binds to a bacteria, and wherein the microorganism targeting molecule is an innate immune system receptor; and ii) a non-human object suspected of being contaminated with a bacteria; and b) applying the recombinant fusion protein to the non-human object under conditions such that the recombinant fusion protein neutralizes the bacteria suspected of contaminating the non-human object.

The present invention also provides a method of treating an animal carcass or part thereof comprising: providing a recombinant fusion protein comprising microorganism targeting molecule joined to at least a portion of a biocide molecule, wherein the microorganism targeting molecule binds to a foodborne pathogen; and an animal carcass suspected of being contaminated with a pathogen; and applying the recombinant fusion protein to the animal carcass or part thereof under conditions such that the recombinant fusion protein neutralizes the pathogen suspected of contaminating the animal carcass. In some embodiments, the animal carcass comprises a bovine carcass or part thereof. In other embodiments, the animal carcass comprises a porcine carcass or part thereof. In still further

embodiments, the animal carcass comprises an avian carcass or part thereof. In yet other embodiments, the animal carcass comprises an aquatic animal carcass or part thereof.

The present invention also provides a method of treating an animal carcass or part thereof comprising: a) providing i) a recombinant fusion protein comprising a
5 microorganism targeting molecule joined to at least a portion of a protein biocide molecule, wherein the microorganism targeting molecule binds to a bacteria, and wherein the microorganism targeting molecule is an innate immune system receptor; and ii) an animal carcass suspected of being contaminated or infected with a bacteria; and b) applying the recombinant fusion protein to the animal carcass or part thereof under
10 conditions such that the recombinant fusion protein neutralizes the bacteria suspected of contaminating or infecting the animal carcass.

In other embodiments, the present invention provides a method of treating a food product (e.g., a meat product, a processed meat, a dairy product, wine, beer, animal feed or a processing component thereof) comprising: providing a recombinant fusion protein
15 comprising microorganism targeting molecule joined to at least a portion of a biocide molecule, wherein the microbial targeting molecule binds to a foodborne pathogen or a spoilage organism; and a food product suspected of being contaminated with a pathogen or spoilage organism; and applying the recombinant fusion protein to the food product under conditions such that the recombinant fusion protein neutralizes the pathogen or spoilage
20 organism suspected of contaminating the food product.

The present invention also provides a method of treating a food product comprising: a) providing i) a recombinant fusion protein comprising a microorganism targeting molecule joined to at least a portion of a protein biocide molecule, wherein the microorganism targeting molecule binds to a bacteria, and wherein the microorganism
25 targeting molecule is an innate immune system receptor; and ii) a food product suspected of being contaminated with a bacteria; and b) applying the recombinant fusion protein to the food product under conditions such that the recombinant fusion protein neutralizes the bacteria suspected of contaminating the food product.

In still further embodiments, the present invention provides a method of treating a subject comprising: providing a recombinant fusion protein comprising a microorganism targeting molecule joined to at least a portion of a biocide molecule, wherein the microorganism targeting molecule binds to a pathogen; and a subject suspected of being contaminated or infected with a pathogen; applying the recombinant fusion protein to the subject under conditions such that the recombinant fusion protein neutralizes the pathogen suspected of contaminating or infecting the subject. In some embodiments, the subject is a mammal (*e.g.*, a ruminant (*e.g.*, bovine) or a human. In other embodiments, the subject is an avian species. In still further embodiments, the subject is a plant. In some embodiments, the subject is contaminated with or infected with an antibiotic resistance organism or an artificially engineered organism (*e.g.*, a bioterrorism agent). In certain embodiments, the subject is deceased.

The present invention also provides a use of a recombinant fusion protein for the treatment of a subject suspected of being contaminated or infected with a bacteria, wherein the recombinant fusion protein comprises a microorganism targeting molecule joined to at least a portion of a protein biocide molecule, wherein the microorganism targeting molecule binds to the bacteria, and wherein the microorganism targeting molecule is an innate immune system receptor; and wherein the recombinant fusion protein is for application to the subject under conditions such that the recombinant fusion protein neutralizes the bacteria.

The present invention also provides a method of supplementing a food ration comprising: providing a recombinant fusion protein comprising a microorganism targeting molecule joined to at least a portion of a biocide molecule by a poly amino acid linker molecule, wherein the immunoglobulin binds to a foodborne pathogen; and a food ration; and supplementing the food ration with the recombinant fusion protein.

The present invention also provides a method of supplementing a food ration comprising: a) providing i) a recombinant fusion protein comprising a microorganism targeting molecule joined to at least a portion of a protein biocide molecule, wherein the microorganism targeting molecule binds to a bacteria, and wherein the microorganism targeting molecule is an innate immune system receptor, and wherein the fusion protein neutralizes the bacteria; and ii) a food ration; and b) supplementing the food ration with the recombinant fusion protein.

The present invention further provides a food comprising a recombinant fusion protein comprising a microorganism targeting molecule joined to at least a portion of a biocide molecule, wherein the immunoglobulin binds to a foodborne pathogen and at least one foodstuff. In some embodiments, the food is for humans. In other embodiments, the food is for a farm animal or a companion animal (e.g. a horse, dog, or cat).

The present invention also provides a food comprising: a recombinant fusion protein comprising a microorganism targeting molecule joined to at least a portion of a protein biocide molecule, wherein the microorganism targeting molecule binds to a bacteria, and wherein the microorganism targeting molecule is an innate immune system receptor, and wherein the fusion protein neutralizes the bacteria; and at least one foodstuff.

In other embodiments, the present invention provides a composition comprising a milk protein and a recombinant fusion protein wherein the protein comprises a microorganism targeting molecule joined to at least a portion of a protein biocide molecule, wherein the immunoglobulin binds to an infectious microorganism.

The present invention also provides a composition comprising: a milk protein; and a recombinant fusion protein wherein the recombinant fusion protein comprises a microorganism targeting molecule joined to at least a portion of a protein biocide molecule, wherein the microorganism targeting molecule binds to a bacteria, and wherein the microorganism targeting molecule is an innate immune system receptor, and wherein the fusion protein neutralizes the bacteria.

In still further embodiments, the present invention provides a method of treating a plant or a part of a plant, comprising: providing a recombinant fusion protein wherein the protein comprises a microorganism targeting molecule joined to at least a portion of a protein biocide molecule, wherein the microorganism targeting molecule binds to a microorganism; and a plant or plant part (e.g., a seed, a growing plant, a fruit, a vegetable, a root, a stem, a leaf, an agricultural crop plant, a horticultural plant or an ornamental plant) suspected of being infected or contaminated by a microorganism; and applying the recombinant fusion protein to the plant or plant part under conditions such that the recombinant fusion protein neutralizes the microorganism. In some embodiments, the microorganism is an agricultural bioterrorism agent.

The present invention also provides a method of treating a plant or a part of a plant, comprising: a) providing i) a recombinant fusion protein, wherein the protein comprises a microorganism targeting molecule joined to at least a portion of a protein biocide molecule, wherein the microorganism targeting molecule binds to a bacteria, and
5 wherein the microorganism targeting molecule is an innate immune system receptor; and ii) a plant suspected of being infected or contaminated by a bacteria; and b) applying the recombinant fusion protein to the plant or plant part under conditions such that the recombinant fusion protein neutralizes the bacteria.

In yet other embodiments, the present invention provides a transgenic organism (e.g.,
10 an animal, a plant, or a microorganism) comprising a nucleic acid sequence encoding a microorganism targeting molecule linked to at least a portion of a protein biocide molecule.

DESCRIPTION OF THE FIGURES

Fig. 1 shows one contemplated retrovector construct embodiment of the present
15 invention.

Figures 2A-2D show various contemplated retrovector elements used for production in mammalian cell culture of certain biocide fusions. Figure 2A shows a full size antibody with biocide linked to the N-terminus of the heavy chain. Figure 2B shows a full size antibody with biocide linked to the C-terminus of the heavy chain. Figure 2C shows a
20 single chain antibody with biocide linked to the N-terminus of the light chain. Figure 2D shows a single chain antibody with biocide linked to the C-terminus of the heavy chain. In Figures 2A-2D abbreviations used are as follows: LTR, long terminal repeat; EPR, extended packaging region; neo, neomycin selection marker; sCMV, simian cytomegalovirus; SP,

signal peptide; X, biocide; L, (G4S)³⁻⁴ linker; HC, antibody heavy chain; IRES1, internal ribosome entry site from encephalomyocarditis virus; LC, antibody light chain; and RESE (RNA stabilization element).

5 Figure 3 shows PLA2 neutralization of *C. parvum* in one embodiment of the present invention.

Figure 4A shows retrovector elements used for mammalian cell culture production of recombinant 3E2 IgM antibody as a hexamer. Figure 4B shows retrovector gene construct used for GPEX production of recombinant 3E2 IgM antibody as a pentamer with J-chain. Figure 4C shows C a retrovector construct used for transgenic production of recombinant 10 3E2 IgM antibody as a hexamer. Figure 4D, shows a retrovector gene construct used for transgenic production of recombinant 3E2 IgM antibody as a pentamer with J-chain

Figures 5A-5D show retrovector elements used for mammalian cell culture production of biocide fusion proteins in certain embodiments of the present invention. Figure 5A shows a full size antibody with biocide linked to the N-terminus of the heavy 15 chain. Figure 5B shows a full size antibody with biocide linked to the C-terminus of the heavy chain. Figure 5C shows a single chain antibody with biocide linked to the N-terminus of the light chain. Figure 5D shows a single chain antibody with biocide linked to the C-terminus of the heavy chain.

Figure 6 shows the components of constructs of some embodiments of the present 20 invention featuring a (Gly₄Ser)₃ linker.

Figure 7 shows the components of constructs of some embodiments of the present invention that contain an immunoglobulin and a biocide.

Figure 8 shows an exemplary Human CD14-PLA2 construct of the present invention (SEQ ID NO:97).

25 Figure 9 shows an exemplary Human LBP-PLA2 construct of the present invention (SEQ ID NO:98).

Figure 10 shows an exemplary Human MBL-PLA2 construct of the present invention (SEQ ID NO:99).

30 Figure 11 shows an exemplary Human SP-D-PLA2 construct of the present invention (SEQ ID NO:100).

Figure 12 shows an exemplary Mouse IgM-PLA2 construct of the present invention (SEQ ID NO:101).

DEFINITIONS

5 To facilitate an understanding of the present invention, a number of terms and phrases are defined below:

As used herein, the terms "biocide" or "biocides" refer to at least a portion of a naturally occurring or synthetic molecule (*e.g.*, peptides) that directly kills or promotes the death and/or attenuation of (*e.g.*, prevents growth and/or replication) of biological targets
10 (*e.g.*, bacteria, parasites, yeast, viruses, fungi, protozoans and the like). Examples of biocides include, but are not limited to, bactericides, viricides, fungicides, parasiticides, and the like.

As used herein, the terms "protein biocide" and "protein biocides" refer to at least a portion of a naturally occurring or synthetic peptide molecule that directly kills or promotes
15 the death and/or attenuation of (*e.g.*, prevents growth and/or replication) of biological targets (*e.g.*, bacteria, parasites, yeast, viruses, fungi, protozoans and the like). Examples of biocides include, but are not limited to, bactericides, viricides, fungicides, parasiticides, and the like.

As used herein, the term "neutralization," "pathogen neutralization," "and spoilage
20 organism neutralization" refer to destruction or inactivation (*e.g.*, loss of virulence) of a "pathogen" or "spoilage organism" (*e.g.*, bacterium, parasite, virus, fungus, mold, prion, and the like) thus preventing the pathogen's or spoilage organism's ability to initiate a disease state in a subject or cause degradation of a food product.

As used herein, the term "spoilage organism" refers to microorganisms (*e.g.*, bacteria
25 or fungi), which cause degradation of the nutritional or organoleptic quality of food and reduces its economic value and shelf life. Exemplary food spoilage microorganisms include, but are not limited to, *Zygosaccharomyces bailii*, *Aspergillus niger*, *Saccharomyces cerevisiae*, *Lactobacillus plantarum*, *Streptococcus faecalis*, and *Leuconostoc mesenteroides*.

30 As used herein, the term "microorganism targeting molecule" refers to any molecule (*e.g.*, protein) that interacts with a microorganism. In preferred embodiments, the

microorganism targeting molecule specifically interacts with microorganisms at the exclusion of non- microorganism host cells. Preferred microorganism targeting molecules interact with broad classes of microorganism (*e.g.*, all bacteria or all gram positive or negative bacteria). However, the present invention also contemplates microorganism targeting molecules that interact with a specific species or sub-species of microorganism. In some preferred embodiments, microorganism targeting molecules interact with "Pathogen Associated Molecular Patterns (PAMPS)". In some embodiments, microorganism targeting molecules are recognition molecules that are known to interact with or bind to PAMPS (*e.g.*, including, but not limited to, as CD14, lipopolysaccharide binding protein (LBP), surfactant protein D (SP-D), and Mannan binding lectin (MBL)). In other embodiments, microorganism targeting molecules are antibodies (*e.g.*, monoclonal antibodies directed towards PAMPS or monoclonal antibodies directed to specific organisms or serotype specific epitopes).

As used herein the term "biofilm" refers to an aggregation of microorganisms (*e.g.*, bacteria) surrounded by an extracellular matrix or slime adherent on a surface *in vivo* or *ex vivo*, wherein the microorganisms adopt altered metabolic states.

As used herein, the term "host cell" refers to any eukaryotic cell (*e.g.*, mammalian cells, avian cells, amphibian cells, plant cells, fish cells, insect cells, yeast cells, and bacteria cells, and the like), whether located *in vitro* or *in vivo* (*e.g.*, in a transgenic organism).

As used herein, the term "cell culture" refers to any *in vitro* culture of cells. Included within this term are continuous cell lines (*e.g.*, with an immortal phenotype), primary cell cultures, finite cell lines (*e.g.*, non-transformed cells), and any other cell population maintained *in vitro*, including oocytes and embryos.

As used herein, the term "vector" refers to any genetic element, such as a plasmid, phage, transposon, cosmid, chromosome, retrovirus, virion, *etc.*, which is capable of replication when associated with the proper control elements and which can transfer gene sequences between cells. Thus, the term includes cloning and expression vehicles, as well as viral vectors.

As used herein, the term "multiplicity of infection" or "MOI" refers to the ratio of integrating vectors: host cells used during transfection or infection of host cells. For example, if 1,000,000 vectors are used to transfect 100,000 host cells, the multiplicity of

infection is 10. The use of this term is not limited to events involving infection, but instead encompasses introduction of a vector into a host by methods such as lipofection, microinjection, calcium phosphate precipitation, and electroporation.

As used herein, the term "genome" refers to the genetic material (*e.g.*, chromosomes) of an organism or a host cell.

The term "nucleotide sequence of interest" refers to any nucleotide sequence (*e.g.*, RNA or DNA), the manipulation of which may be deemed desirable for any reason (*e.g.*, treat disease, confer improved qualities, *etc.*), by one of ordinary skill in the art. Such nucleotide sequences include, but are not limited to, coding sequences, or portions thereof, of structural genes (*e.g.*, reporter genes, selection marker genes, oncogenes, drug resistance genes, growth factors, *etc.*), and non-coding regulatory sequences that do not encode an mRNA or protein product (*e.g.*, promoter sequence, polyadenylation sequence, termination sequence, enhancer sequence, *etc.*).

The term "gene" refers to a nucleic acid (*e.g.*, DNA or RNA) sequence that comprises coding sequences necessary for the production of a polypeptide or precursor (*e.g.*, proinsulin). The polypeptide can be encoded by a full length coding sequence or by any portion of the coding sequence so long as the desired activity or functional properties (*e.g.*, enzymatic activity, ligand binding, signal transduction, *etc.*) of the full-length or fragment are retained. The term also encompasses the coding region of a structural gene and includes sequences located adjacent to the coding region on both the 5' and 3' ends for a distance of about 1 kb or more on either end such that the gene corresponds to the length of the full-length mRNA. The sequences that are located 5' of the coding region and which are present on the mRNA are referred to as 5' untranslated sequences. The sequences that are located 3' or downstream of the coding region and which are present on the mRNA are referred to as 3' untranslated sequences. The term "gene" encompasses both cDNA and genomic forms of a gene. A genomic form or clone of a gene contains the coding region interrupted with non-coding sequences termed "introns" or "intervening regions" or "intervening sequences." Introns are segments of a gene that are transcribed into nuclear RNA (hnRNA); introns may contain regulatory elements such as enhancers. Introns are removed or "spliced out" from the nuclear or primary transcript; introns therefore are absent in the messenger RNA

(mRNA) transcript. The mRNA functions during translation to specify the sequence or order of amino acids in a nascent polypeptide.

DNA molecules (*e.g.*, genes) are said to have "5' ends" and "3' ends" because mononucleotides are reacted to make oligonucleotides in a manner such that the 5' phosphate of one mononucleotide pentose ring is attached to the 3' oxygen of its neighbor in one direction via a phosphodiester linkage. Therefore, an end of an oligonucleotide is referred to as the "5' end" if its 5' phosphate is not linked to the 3' oxygen of a mononucleotide pentose ring. An end of an oligonucleotide is referred to as the "3' end" if its 3' oxygen is not linked to a 5' phosphate of another mononucleotide pentose ring. As used herein, a nucleic acid sequence, even if internal to a larger oligonucleotide, also may be said to have 5' and 3' ends. In either a linear or circular DNA molecule, discrete elements are referred to as being "upstream" or 5' of the "downstream" or 3' elements. This terminology reflects the fact that transcription proceeds in a 5' to 3' fashion along the DNA strand. The promoter and enhancer elements which direct the transcription of a linked gene are generally located 5' or upstream of the coding region. However, enhancer elements can exert their effect even when located 3' of the promoter element and the coding region. Transcription termination and polyadenylation signals are located 3' or downstream of the coding region.

As used herein, the term "exogenous gene" refers to a gene that is not naturally present in a host organism or cell, or is artificially introduced into a host organism or cell.

As used herein, the term "transgene" means a nucleic acid sequence (*e.g.*, encoding one or more fusion protein polypeptides), which is introduced into the genome of a transgenic organism. A transgene can include one or more transcriptional regulatory sequences and other nucleic acid, such as introns, that may be necessary for optimal expression and secretion of a nucleic acid encoding the fusion protein. A transgene can include an enhancer sequence. A fusion protein sequence can be operatively linked to a tissue specific promoter, *e.g.*, mammary gland specific promoter sequence that results in the secretion of the protein in the milk of a transgenic mammal, a urine specific promoter, or an egg specific promoter.

As used herein, the term "transgenic cell" refers to a cell containing a transgene.

A "transgenic organism," as used herein, refers to a transgenic animal or plant.

As used herein, a "transgenic animal" is a non-human animal in which one or more, and preferably essentially all, of the cells of the animal contain a transgene introduced by way of human intervention, such as by transgenic techniques known in the art. The transgene can be introduced into the cell, directly or indirectly by introduction into a precursor of the cell, by way of deliberate genetic manipulation, such as by microinjection or by infection with a recombinant virus.

Mammals are defined herein as all animals, excluding humans, which have mammary glands and produce milk.

As used herein, a "dairy animal" refers to a milk producing non-human mammal that is larger than a laboratory rodent (*e.g.*, a mouse). In preferred embodiments, the dairy animals produce large volumes of milk and have long lactating periods (*e.g.*, cows or goats).

As used herein, the term "plant" refers to either a whole plant, a plant part, a plant cell, or a group of plant cells, including plants that are actively growing (*e.g.* in soil) and those that have been harvested. The class of plants used in methods of the invention is generally as broad as the class of higher plants amenable to transformation techniques, including both monocotyledonous and dicotyledonous plants. It includes plants of a variety of ploidy levels, including polyploid, diploid and haploid.

As used herein, a "transgenic plant" is a plant, preferably a multi-celled or higher plant, in which one or more, and preferably essentially all, of the cells of the plant contain a transgene introduced by way of human intervention, such as by transgenic techniques known in the art.

As used herein, the term "gene expression" refers to the process of converting genetic information encoded in a gene into RNA (*e.g.*, mRNA, rRNA, tRNA, or snRNA) through "transcription" of the gene (*i.e.*, via the enzymatic action of an RNA polymerase), and for protein encoding genes, into protein through "translation" of mRNA. Gene expression can be regulated at many stages in the process. "Up-regulation" or "activation" refers to regulation that increases the production of gene expression products (*i.e.*, RNA or protein), while "down-regulation" or "repression" refers to regulation that decrease production. Molecules (*e.g.*, transcription factors) that are involved in up-regulation or down-regulation are often called "activators" and "repressors," respectively.

As used herein, the term "protein of interest" refers to a protein encoded by a nucleic acid of interest.

As used herein, the term "native" (or wild type) when used in reference to a protein refers to proteins encoded by partially homologous nucleic acids so that the amino acid sequence of the proteins varies. As used herein, the term "variant" encompasses proteins encoded by homologous genes having both conservative and nonconservative amino acid substitutions that do not result in a change in protein function, as well as proteins encoded by homologous genes having amino acid substitutions that cause decreased (*e.g.*, null mutations) protein function or increased protein function.

The term "isolated" when used in relation to a nucleic acid, as in "an isolated oligonucleotide" refers to a nucleic acid sequence that is identified and separated from at least one contaminant nucleic acid with which it is ordinarily associated in its natural source. Isolated nucleic acids are nucleic acids present in a form or setting that is different from that in which they are found in nature. In contrast, non-isolated nucleic acids are nucleic acids such as DNA and RNA that are found in the state in which they exist in nature.

As used herein, the terms "nucleic acid molecule encoding," "DNA sequence encoding," "DNA encoding," "RNA sequence encoding," and "RNA encoding" refer to the order or sequence of deoxyribonucleotides or ribonucleotides along a strand of deoxyribonucleic acid or ribonucleic acid. The order of these deoxyribonucleotides or ribonucleotides determines the order of amino acids along the polypeptide (protein) chain translated from the mRNA. The DNA or RNA sequence thus codes for the amino acid sequence.

As used herein, the terms "complementary" or "complementarity" are used in reference to polynucleotides (*i.e.*, a sequence of nucleotides) related by the base-pairing rules. For example, for the sequence "A-G-T," is complementary to the sequence "T-C-A." Complementarity may be "partial," in which only some of the nucleic acids' bases are matched according to the base pairing rules. Or, there may be "complete" or "total" complementarity between the nucleic acids. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands. This is of particular importance in amplification reactions, as well as detection methods that depend upon binding between nucleic acids.

The terms "homology" and "percent identity" when used in relation to nucleic acids refers to a degree of complementarity. There may be partial homology (*i.e.*, partial identity) or complete homology (*i.e.*, complete identity). A partially complementary sequence is one that at least partially inhibits a completely complementary sequence from hybridizing to a target nucleic acid sequence and is referred to using the functional term "substantially homologous." The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization assay (Southern or Northern blot, solution hybridization and the like) under conditions of low stringency. A substantially homologous sequence or probe (*i.e.*, an oligonucleotide which is capable of hybridizing to another oligonucleotide of interest) will compete for and inhibit the binding (*i.e.*, the hybridization) of a completely homologous sequence to a target sequence under conditions of low stringency. This is not to say that conditions of low stringency are such that non-specific binding is permitted; low stringency conditions require that the binding of two sequences to one another be a specific (*i.e.*, selective) interaction. The absence of non-specific binding may be tested by the use of a second target which lacks even a partial degree of complementarity (*e.g.*, less than about 30% identity); in the absence of non-specific binding the probe will not hybridize to the second non-complementary target.

The art knows well that numerous equivalent conditions may be employed to comprise low stringency conditions; factors such as the length and nature (DNA, RNA, base composition) of the probe and nature of the target (DNA, RNA, base composition, present in solution or immobilized, *etc.*) and the concentration of the salts and other components (*e.g.*, the presence or absence of formamide, dextran sulfate, polyethylene glycol) are considered and the hybridization solution may be varied to generate conditions of low stringency hybridization different from, but equivalent to, the above listed conditions. In addition, the art knows conditions that promote hybridization under conditions of high stringency (*e.g.*, increasing the temperature of the hybridization and/or wash steps, the use of formamide in the hybridization solution, *etc.*).

When used in reference to a double-stranded nucleic acid sequence such as a cDNA or genomic clone, the term "substantially homologous" refers to any probe that can hybridize to either or both strands of the double-stranded nucleic acid sequence under conditions of low stringency as described above.

When used in reference to a single-stranded nucleic acid sequence, the term "substantially homologous" refers to any probe that can hybridize (*i.e.*, it is the complement of) the single-stranded nucleic acid sequence under conditions of low stringency as described above.

5 As used herein, the term "hybridization" is used in reference to the pairing of complementary nucleic acids. Hybridization and the strength of hybridization (*i.e.*, the strength of the association between the nucleic acids) is impacted by such factors as the degree of complementary between the nucleic acids, stringency of the conditions involved, the T_m of the formed hybrid, and the G:C ratio within the nucleic acids. A single molecule
10 that contains pairing of complementary nucleic acids within its structure is said to be "self-hybridized."

As used herein, the term " T_m " is used in reference to the "melting temperature" of a nucleic acid. The melting temperature is the temperature at which a population of double-stranded nucleic acid molecules becomes half dissociated into single strands. The equation
15 for calculating the T_m of nucleic acids is well known in the art. As indicated by standard references, a simple estimate of the T_m value may be calculated by the equation: $T_m = 81.5 + 0.41(\% G + C)$, when a nucleic acid is in aqueous solution at 1 M NaCl (*See e.g.*, Anderson and Young, Quantitative Filter Hybridization, in *Nucleic Acid Hybridization* [1985]). Other references include more sophisticated computations that take structural as
20 well as sequence characteristics into account for the calculation of T_m .

As used herein the term "stringency" is used in reference to the conditions of temperature, ionic strength, and the presence of other compounds such as organic solvents, under which nucleic acid hybridizations are conducted. With "high stringency" conditions, nucleic acid base pairing will occur only between nucleic acid fragments that have a high
25 frequency of complementary base sequences. Thus, conditions of "weak" or "low" stringency are often required with nucleic acids that are derived from organisms that are genetically diverse, as the frequency of complementary sequences is usually less.

"High stringency conditions" when used in reference to nucleic acid hybridization comprise conditions equivalent to binding or hybridization at 42°C in a solution consisting
30 of 5X SSPE (43.8 g/l NaCl, 6.9 g/l NaH_2PO_4 H_2O and 1.85 g/l EDTA, pH adjusted to 7.4

with NaOH), 0.5% SDS, 5X Denhardt's reagent and 100 µg/ml denatured salmon sperm DNA followed by washing in a solution comprising 0.1X SSPE, 1.0% SDS at 42°C when a probe of about 500 nucleotides in length is employed.

"Medium stringency conditions" when used in reference to nucleic acid hybridization comprise conditions equivalent to binding or hybridization at 42°C in a solution consisting of 5X SSPE (43.8 g/l NaCl, 6.9 g/l NaH₂PO₄ H₂O and 1.85 g/l EDTA, pH adjusted to 7.4 with NaOH), 0.5% SDS, 5X Denhardt's reagent and 100 µg/ml denatured salmon sperm DNA followed by washing in a solution comprising 1.0X SSPE, 1.0% SDS at 42°C when a probe of about 500 nucleotides in length is employed.

"Low stringency conditions" comprise conditions equivalent to binding or hybridization at 42°C in a solution consisting of 5X SSPE (43.8 g/l NaCl, 6.9 g/l NaH₂PO₄ H₂O and 1.85 g/l EDTA, pH adjusted to 7.4 with NaOH), 0.1% SDS, 5X Denhardt's reagent [50X Denhardt's contains per 500 ml: 5 g Ficoll™ (Type 400, Pharmacia), 5 g BSA (Fraction V; Sigma)] and 100 µg/ml denatured salmon sperm DNA followed by washing in a solution comprising 5X SSPE, 0.1% SDS at 42°C when a probe of about 500 nucleotides in length is employed.

A gene may produce multiple RNA species that are generated by differential splicing of the primary RNA transcript. cDNAs that are splice variants of the same gene will contain regions of sequence identity or complete homology (representing the presence of the same exon or portion of the same exon on both cDNAs) and regions of complete non-identity (for example, representing the presence of exon "A" on cDNA 1 wherein cDNA 2 contains exon "B" instead). Because the two cDNAs contain regions of sequence identity they will both hybridize to a probe derived from the entire gene or portions of the gene containing sequences found on both cDNAs; the two splice variants are therefore substantially homologous to such a probe and to each other.

The terms "in operable combination," "in operable order," and "operably linked" as used herein refer to the linkage of nucleic acid sequences in such a manner that a nucleic acid molecule capable of directing the transcription of a given gene and/or the synthesis of a desired protein molecule is produced. The term also refers to the linkage of amino acid sequences in such a manner so that a functional protein is produced.

As used herein, the term "selectable marker" refers to a gene that encodes an enzymatic activity that confers the ability to grow in medium lacking what would otherwise be an essential nutrient (*e.g.*, the *HIS3* gene in yeast cells); in addition, a selectable marker may confer resistance to an antibiotic or drug upon the cell in which the selectable marker is expressed. Selectable markers may be "dominant"; a dominant selectable marker encodes an enzymatic activity that can be detected in any eukaryotic cell line. Examples of dominant selectable markers include, but are not limited to, the bacterial aminoglycoside 3' phosphotransferase gene (also referred to as the *neo* gene) that confers resistance to the drug G418 in mammalian cells, the bacterial hygromycin G phosphotransferase (*hyg*) gene that confers resistance to the antibiotic hygromycin and the bacterial xanthine-guanine phosphoribosyl transferase gene (also referred to as the *gpt* gene) that confers the ability to grow in the presence of mycophenolic acid. Other selectable markers are not dominant in that their use must be in conjunction with a cell line that lacks the relevant enzyme activity. Examples of non-dominant selectable markers include the thymidine kinase (*tk*) gene that is used in conjunction with *tk*⁻ cell lines, the CAD gene which is used in conjunction with CAD-deficient cells and the mammalian hypoxanthine-guanine phosphoribosyl transferase (*hprt*) gene which is used in conjunction with *hprt*⁻ cell lines. A review of the use of selectable markers in mammalian cell lines is provided in Sambrook, J. *et al.*, *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, New York (1989) pp.16.9-16.15.

As used herein, the term "reporter gene" refers to a gene encoding a protein that may be assayed. Examples of reporter genes include, but are not limited to, luciferase (*See, e.g.*, deWet *et al.*, *Mol. Cell. Biol.* 7:725 [1987] and US Pat Nos., 6,074,859; 5,976,796; 5,674,713; and 5,618,682), green fluorescent protein (*e.g.*, GenBank™ Accession Number U43284; a number of GFP variants are commercially available from CLONTECH Laboratories, Palo Alto, CA), chloramphenicol acetyltransferase, β -galactosidase, alkaline phosphatase, and horseradish peroxidase.

As used herein, the term "regulatory element" refers to a genetic element that controls some aspect of the expression of nucleic acid sequences. For example, a promoter is a regulatory element that facilitates the initiation of transcription of an operably linked

coding region. Other regulatory elements are splicing signals, polyadenylation signals, termination signals, RNA export elements, internal ribosome entry sites, *etc.* (defined *infra*).

Transcriptional control signals in eukaryotes comprise "promoter" and "enhancer" elements. Promoters and enhancers consist of short arrays of DNA sequences that interact specifically with cellular proteins involved in transcription (Maniatis *et al.*, Science 236:1237 [1987]). Promoter and enhancer elements have been isolated from a variety of eukaryotic sources including genes in yeast, insect and mammalian cells, and viruses (analogous control elements, *i.e.*, promoters, are also found in prokaryotes). The selection of a particular promoter and enhancer depends on what cell type is to be used to express the protein of interest. Some eukaryotic promoters and enhancers have a broad host range while others are functional in a limited subset of cell types (for review See *e.g.*, Voss *et al.*, Trends Biochem. Sci., 11:287 [1986]; and Maniatis *et al.*, *supra*). For example, the SV40 early gene enhancer is very active in a wide variety of cell types from many mammalian species and has been widely used for the expression of proteins in mammalian cells (Dijkema *et al.*, EMBO J. 4:761 [1985]). Two other examples of promoter/enhancer elements active in a broad range of mammalian cell types are those from the human elongation factor 1 α gene (Uetsuki *et al.*, J. Biol. Chem., 264:5791 [1989]; Kim *et al.*, Gene 91:217 [1990]; and Mizushima and Nagata, Nuc. Acids. Res., 18:5322 [1990]) and the long terminal repeats of the Rous sarcoma virus (Gorman *et al.*, Proc. Natl. Acad. Sci. USA 79:6777 [1982]) and the human cytomegalovirus (Boshart *et al.*, Cell 41:521 [1985]). In preferred embodiments, inducible retroviral promoters are utilized.

As used herein, the term "promoter/enhancer" denotes a segment of DNA which contains sequences capable of providing both promoter and enhancer functions (*i.e.*, the functions provided by a promoter element and an enhancer element, see above for a discussion of these functions). For example, the long terminal repeats of retroviruses contain both promoter and enhancer functions. The enhancer/promoter may be "endogenous" or "exogenous" or "heterologous." An "endogenous" enhancer/promoter is one that is naturally linked with a given gene in the genome. An "exogenous" or "heterologous" enhancer/promoter is one that is placed in juxtaposition to a gene by means of genetic manipulation (*i.e.*, molecular biological techniques such as cloning and

recombination) such that transcription of that gene is directed by the linked enhancer/promoter.

Regulatory elements may be tissue specific or cell specific. The term "tissue specific" as it applies to a regulatory element refers to a regulatory element that is capable of directing selective expression of a nucleotide sequence of interest to a specific type of tissue (e.g., mammary gland) in the relative absence of expression of the same nucleotide sequence(s) of interest in a different type of tissue (e.g., liver).

Tissue specificity of a regulatory element may be evaluated by, for example, operably linking a reporter gene to a promoter sequence (which is not tissue-specific) and to the regulatory element to generate a reporter construct, introducing the reporter construct into the genome of an animal such that the reporter construct is integrated into every tissue of the resulting transgenic animal, and detecting the expression of the reporter gene (e.g., detecting mRNA, protein, or the activity of a protein encoded by the reporter gene) in different tissues of the transgenic animal. The detection of a greater level of expression of the reporter gene in one or more tissues relative to the level of expression of the reporter gene in other tissues shows that the regulatory element is "specific" for the tissues in which greater levels of expression are detected. Thus, the term "tissue-specific" (e.g., liver-specific) as used herein is a relative term that does not require absolute specificity of expression. In other words, the term "tissue-specific" does not require that one tissue have extremely high levels of expression and another tissue have no expression. It is sufficient that expression is greater in one tissue than another. By contrast, "strict" or "absolute" tissue-specific expression is meant to indicate expression in a single tissue type (e.g., liver) with no detectable expression in other tissues.

The term "cell type specific" as applied to a regulatory element refers to a regulatory element which is capable of directing selective expression of a nucleotide sequence of interest in a specific type of cell in the relative absence of expression of the same nucleotide sequence of interest in a different type of cell within the same tissue (e.g., cells infected with retrovirus, and more particularly, cells infected with BLV or HTLV). The term "cell type specific" when applied to a regulatory element also means a regulatory element capable of promoting selective expression of a nucleotide sequence of interest in a region within a single tissue.

The cell type specificity of a regulatory element may be assessed using methods well known in the art (*e.g.*, immunohistochemical staining and/or Northern blot analysis).

Briefly, for immunohistochemical staining, tissue sections are embedded in paraffin, and paraffin sections are reacted with a primary antibody specific for the polypeptide product

5 encoded by the nucleotide sequence of interest whose expression is regulated by the regulatory element. A labeled (*e.g.*, peroxidase conjugated) secondary antibody specific for the primary antibody is allowed to bind to the sectioned tissue and specific binding detected (*e.g.*, with avidin/biotin) by microscopy. Briefly, for Northern blot analysis, RNA is

isolated from cells and electrophoresed on agarose gels to fractionate the RNA according to

10 size followed by transfer of the RNA from the gel to a solid support (*e.g.*, nitrocellulose or a nylon membrane). The immobilized RNA is then probed with a labeled oligo-

deoxyribonucleotide probe or DNA probe to detect RNA species complementary to the probe used. Northern blots are a standard tool of molecular biologists.

The term "promoter," "promoter element," or "promoter sequence" as used herein, 15 refers to a DNA sequence which when ligated to a nucleotide sequence of interest is capable of controlling the transcription of the nucleotide sequence of interest into mRNA. A promoter is typically, though not necessarily, located 5' (*i.e.*, upstream) of a nucleotide sequence of interest whose transcription into mRNA it controls, and provides a site for specific binding by RNA polymerase and other transcription factors for initiation of 20 transcription.

Promoters may be constitutive or regulatable. The term "constitutive" when made in reference to a promoter means that the promoter is capable of directing transcription of an operably linked nucleic acid sequence in the absence of a stimulus (*e.g.*, heat shock, chemicals, *etc.*). In contrast, a "regulatable" promoter is one that is capable of directing a 25 level of transcription of an operably linked nucleic acid sequence in the presence of a stimulus (*e.g.*, heat shock, chemicals, *etc.*), which is different from the level of transcription of the operably linked nucleic acid sequence in the absence of the stimulus.

The presence of "splicing signals" on an expression vector often results in higher levels of expression of the recombinant transcript. Splicing signals mediate the removal of 30 introns from the primary RNA transcript and consist of a splice donor and acceptor site (Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor

Laboratory Press, New York [1989], pp. 16.7-16.8). A commonly used splice donor and acceptor site is the splice junction from the 16S RNA of SV40.

Efficient expression of recombinant DNA sequences in eukaryotic cells requires expression of signals directing the efficient termination and polyadenylation of the resulting transcript. Transcription termination signals are generally found downstream of the polyadenylation signal and are a few hundred nucleotides in length. The term "poly A site" or "poly A sequence" as used herein denotes a DNA sequence that directs both the termination and polyadenylation of the nascent RNA transcript. Efficient polyadenylation of the recombinant transcript is desirable as transcripts lacking a poly A tail are unstable and are rapidly degraded. The poly A signal utilized in an expression vector may be "heterologous" or "endogenous." An endogenous poly A signal is one that is found naturally at the 3' end of the coding region of a given gene in the genome. A heterologous poly A signal is one that is isolated from one gene and placed 3' of another gene. A commonly used heterologous poly A signal is the SV40 poly A signal. The SV40 poly A signal is contained on a 237 bp BamHI/BclI restriction fragment and directs both termination and polyadenylation (Sambrook, supra, at 16.6-16.7).

Eukaryotic expression vectors may also contain "viral replicons" or "viral origins of replication." Viral replicons are viral DNA sequences that allow for the extrachromosomal replication of a vector in a host cell expressing the appropriate replication factors. Vectors that contain either the SV40 or polyoma virus origin of replication replicate to high "copy number" (up to 10⁴ copies/cell) in cells that express the appropriate viral T antigen. Vectors that contain the replicons from bovine papillomavirus or Epstein-Barr virus replicate extrachromosomally at "low copy number" (~100 copies/cell). However, it is not intended that expression vectors be limited to any particular viral origin of replication.

As used herein, the term "long terminal repeat" or "LTR" refers to transcriptional control elements located in or isolated from the U3 region 5' and 3' of a retroviral genome. As is known in the art, long terminal repeats may be used as control elements in retroviral vectors, or isolated from the retroviral genome and used to control expression from other types of vectors.

As used herein, the terms "RNA export element" or "Pre-mRNA Processing Enhancer (PPE)" refer to 3' and 5' cis-acting post-transcriptional regulatory elements that

enhance export of RNA from the nucleus. "PPE" elements include, but are not limited to Mertz sequences (described in US 5,914,267 and 5,686,120)

and woodchuck mRNA processing enhancer (WPRE; WO 99/14310).

5 As used herein, the term "polycistronic" refers to an mRNA encoding more than one polypeptide chain (*See, e.g.*, WO 93/03143, WO 88/05486, and European Pat. No. 117058).

Likewise, the term "arranged in polycistronic sequence" refers to the arrangement of genes encoding two different polypeptide chains in a single mRNA.

10 As used herein, the term "internal ribosome entry site" or "IRES" refers to a sequence located between polycistronic genes that permits the production of the expression product originating from the second gene by internal initiation of the translation of the dicistronic mRNA. Examples of internal ribosome entry sites include, but are not limited to, those derived from foot and mouth disease virus (FDV), encephalomyocarditis virus, poliovirus and RDV (Scheper *et al.*, Biochem. 76: 801-809 [1994]; Meyer *et al.*, J. Virol. 15 69: 2819-2824 [1995]; Jang *et al.*, 1988, J. Virol. 62: 2636-2643 [1998]; Haller *et al.*, J. Virol. 66: 5075-5086 [1995]). Vectors incorporating IRESs may be assembled as is known in the art. For example, a retroviral vector containing a polycistronic sequence may contain the following elements in operable association: nucleotide polylinker, gene of interest, an internal ribosome entry site and a mammalian selectable marker or another gene of interest. 20 The polycistronic cassette is situated within the retroviral vector between the 5' LTR and the 3' LTR at a position such that transcription from the 5' LTR promoter transcribes the polycistronic message cassette. The transcription of the polycistronic message cassette may also be driven by an internal promoter (*e.g.*, cytomegalovirus promoter) or an inducible promoter (*e.g.*, the inducible promoters of the present invention), which may be preferable 25 depending on the use. The polycistronic message cassette can further comprise a cDNA or genomic DNA (gDNA) sequence operatively associated within the polylinker. Any mammalian selectable marker can be utilized as the polycistronic message cassette mammalian selectable marker. Such mammalian selectable markers are well known to those of skill in the art and can include, but are not limited to, kanamycin/G418, hygromycin B or mycophenolic acid resistance markers. 30

As used herein, the term "retrovirus" refers to a retroviral particle which is capable of entering a cell (*i.e.*, the particle contains a membrane-associated protein such as an envelope protein or a viral G glycoprotein which can bind to the host cell surface and facilitate entry of the viral particle into the cytoplasm of the host cell) and integrating the retroviral genome (as a double-stranded provirus) into the genome of the host cell.

As used herein, the term "retroviral vector" refers to a retrovirus that has been modified to express a gene of interest. Retroviral vectors can be used to transfer genes efficiently into host cells by exploiting the viral infectious process. Foreign or heterologous genes cloned (*i.e.*, inserted using molecular biological techniques) into the retroviral genome can be delivered efficiently to host cells that are susceptible to infection by the retrovirus. Through well-known genetic manipulations, the replicative capacity of the retroviral genome can be destroyed. The resulting replication-defective vectors can be used to introduce new genetic material to a cell but they are unable to replicate. A helper virus or packaging cell line can be used to permit vector particle assembly and egress from the cell. Such retroviral vectors comprise a replication-deficient retroviral genome containing a nucleic acid sequence encoding at least one gene of interest (*i.e.*, a polycistronic nucleic acid sequence can encode more than one gene of interest), a 5' retroviral long terminal repeat (5' LTR); and a 3' retroviral long terminal repeat (3' LTR).

The term "pseudotyped retroviral vector" refers to a retroviral vector containing a heterologous membrane protein. The term "membrane-associated protein" refers to a protein (*e.g.*, a viral envelope glycoprotein or the G proteins of viruses in the Rhabdoviridae family such as VSV, Piry, Chandipura and Mokola), which is associated with the membrane surrounding a viral particle; these membrane-associated proteins mediate the entry of the viral particle into the host cell. The membrane associated protein may bind to specific cell surface protein receptors, as is the case for retroviral envelope proteins or the membrane-associated protein may interact with a phospholipid component of the plasma membrane of the host cell, as is the case for the G proteins derived from members of the Rhabdoviridae family.

A "subject" is an animal such as vertebrate, preferably a mammal, more preferably a human or a bovine. Mammals, however, are understood to include, but are not limited to, murines, simians, humans, bovines, cervids, equines, porcines, canines, felines *etc.*).

An "effective amount" is an amount sufficient to effect beneficial or desired results. An effective amount can be administered in one or more administrations,

"Co-administration" refers to administration of more than one agent or therapy to a subject. Co-administration may be concurrent or, alternatively, the chemical compounds described herein may be administered in advance of or following the administration of the other agent(s). One skilled in the art can readily determine the appropriate dosage for co-administration. When co-administered with another therapeutic agent, both the agents may be used at lower dosages. Thus, co-administration is especially desirable where the claimed compounds are used to lower the requisite dosage of known toxic agents.

As used herein, the term "toxic" refers to any detrimental or harmful effects on a cell or tissue.

A "pharmaceutical composition" is intended to include the combination of an active agent with a carrier, inert or active, making the composition suitable for diagnostic or therapeutic use *in vivo*, *in vivo* or *ex vivo*.

As used herein, the term "pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, and an emulsion, such as an oil/water or water/oil emulsion, and various types of wetting agents. The compositions also can include stabilizers and preservatives. For examples of carriers, stabilizers and adjuvants see Martin, Remington's Pharmaceutical Sciences, 15th Ed., Mack Publ. Co., Easton, PA (1975).

"Pharmaceutically acceptable salt" as used herein, relates to any pharmaceutically acceptable salt (acid or base) of a compound of the present invention, which, upon administration to a recipient, is capable of providing a compound of this invention or an active metabolite or residue thereof. As is known to those of skill in the art, "salts" of the compounds of the present invention may be derived from inorganic or organic acids and bases. Examples of acids include hydrochloric, hydrobromic, sulfuric, nitric, perchloric, fumaric, maleic, phosphoric, glycolic, lactic, salicylic, succinic, toluene-p-sulfonic, tartaric, acetic, citric, methanesulfonic, ethanesulfonic, formic, benzoic, malonic, naphthalene-2-sulfonic and benzenesulfonic acid. Other acids, such as oxalic, while not in themselves pharmaceutically acceptable, may be employed in the preparation of salts useful as

intermediates in obtaining the compounds of the invention and their pharmaceutically acceptable acid.

As used herein, the term "nutraceutical," refers to a food substance or part of a food, which includes a fusion protein. Nutraceuticals can provide medical or health benefits, including the prevention, treatment, or cure of a disorder. The transgenic protein will often be present in the nutraceutical at concentration of at least 100 µg/kg, more preferably at least 1 mg/kg, most preferably at least 10 mg/kg. A nutraceutical can include the milk of a transgenic animal.

As used herein, the term "purified" or "to purify" refers to the removal of undesired components from a sample. As used herein, the term "substantially purified" refers to molecules, either nucleic or amino acid sequences, that are removed from their natural environment, isolated or separated, and are at least 60% free, preferably 75% free, and most preferably 90% free from other components with which they are naturally associated. An "isolated polynucleotide" is therefore a substantially purified polynucleotide.

The terms "bacteria" and "bacterium" refer to all prokaryotic organisms, including those within all of the phyla in the Kingdom Procaryotae. It is intended that the term encompass all microorganisms considered to be bacteria including Mycoplasma, Chlamydia, Actinomyces, Streptomyces, and Rickettsia. All forms of bacteria are included within this definition including cocci, bacilli, spirochetes, spheroplasts, protoplasts, *etc.* Also included within this term are prokaryotic organisms that are gram negative or gram positive. "Gram negative" and "gram positive" refer to staining patterns with the Gram-staining process that is well known in the art. (*See e.g.*, Finegold and Martin, Diagnostic Microbiology, 6th Ed., CV Mosby St. Louis, *pp.* 13-15 [1982]). "Gram positive bacteria" are bacteria that retain the primary dye used in the Gram stain, causing the stained cells to appear dark blue to purple under the microscope. "Gram negative bacteria" do not retain the primary dye used in the Gram stain, but are stained by the counterstain. Thus, gram negative bacteria appear red. In some embodiments, the bacteria are those capable of causing disease (pathogens) and those that cause product degradation or spoilage.

As used herein, the term "antigen binding protein" refers to proteins that bind to a specific antigen. "Antigen binding proteins" include, but are not limited to, immunoglobulins, including polyclonal, monoclonal, chimeric, single chain, and humanized

antibodies, Fab fragments, F(ab')₂ fragments, and Fab expression libraries. Various procedures known in the art are used for the production of polyclonal antibodies. For the production of antibody, various host animals can be immunized by injection with the peptide corresponding to the desired epitope including but not limited to rabbits, mice, rats, sheep, goats, *etc.* In a preferred embodiment, the peptide is conjugated to an immunogenic carrier (*e.g.*, diphtheria toxoid, bovine serum albumin (BSA), or keyhole limpet hemocyanin (KLH)). Various adjuvants are used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (Bacille Calmette-Guerin) and *Corynebacterium parvum*.

For preparation of monoclonal antibodies, any technique that provides for the production of antibody molecules by continuous cell lines in culture may be used (*See e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY). These include, but are not limited to, the hybridoma technique originally developed by Köhler and Milstein (Köhler and Milstein, *Nature*, 256:495-497 [1975]), as well as the trioma technique, the human B-cell hybridoma technique (*See e.g.*, Kozbor *et al.*, *Immunol. Today*, 4:72 [1983]), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole *et al.*, in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96 [1985]). In other embodiments, suitable monoclonal antibodies, including recombinant chimeric monoclonal antibodies and chimeric monoclonal antibody fusion proteins are prepared as described herein.

According to the invention, techniques described for the production of single chain antibodies (US 4,946,778) can be adapted to produce specific single chain antibodies as desired. An additional embodiment of the invention utilizes the techniques known in the art for the construction of Fab expression libraries (Huse *et al.*, *Science*, 246:1275-1281 [1989]) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity.

In some embodiments, monoclonal antibodies are generated using the ABL-MYC method (*See e.g.*, U.S. Patent 5,705,150 and 5,244,656)

(Neoclone, Madison, WI). ABL-MYC is a recombinant retrovirus that constitutively expresses v-abl and c-myc oncogenes. When used to infect antigen-activated splenocytes, this retroviral system rapidly induces antigen-specific plasmacytomas. ABL-MYC targets antigen-stimulated (Ag-stimulated) B-cells for transformation.

Antibody fragments that contain the idiotype (antigen binding region) of the antibody molecule can be generated by known techniques. For example, such fragments include but are not limited to: the F(ab')₂ fragment that can be produced by pepsin digestion of an antibody molecule; the Fab' fragments that can be generated by reducing the disulfide bridges of an F(ab')₂ fragment, and the Fab fragments that can be generated by treating an antibody molecule with papain and a reducing agent.

Genes encoding antigen-binding proteins can be isolated by methods known in the art. In the production of antibodies, screening for the desired antibody can be accomplished by techniques known in the art (e.g., radioimmunoassay, ELISA (enzyme-linked immunosorbant assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitin reactions, immunodiffusion assays, *in situ* immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), Western Blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays, *etc.*), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, *etc.*) *etc.*

GENERAL DESCRIPTION OF THE INVENTION

One embodiment of the present invention provides compositions and methods for treating and/or preventing illnesses in animals caused by pathogens. More particularly, the present invention provides therapeutic and prophylactic compositions directed to combating bacterial, parasitical, and fungal infections in humans and other animals (e.g., feedlot and domestic animals such as cows, chickens, turkeys, pigs, and sheep).

In preferred embodiments, the present invention provides fusion proteins comprising microorganism targeting molecules (e.g., including, but not limited to, monoclonal antibody and innate immune system receptors) directed against bacterial, parasitic, and fungal pathogens and methods of using and creating these molecules. In some of these

embodiments, the antibodies are chimeras (*e.g.*, murine-bovine). The present invention is not limited however to providing fusion proteins or chimeras.

In other embodiments, the present invention provides chimeric monoclonal antibodies directed against foodborne bacterial, protozoan and parasitic pathogens.

5 However, the bacterial pathogens need not be foodborne (*e.g.*, gastrointestinal). For example, additional embodiments are directed to providing therapeutic compositions and methods to combat other bacterial infections via other possible routes of transmission (*e.g.*, respiratory, salivary, fecal-oral, skin-to-skin, bloodborne, genital, urinary, eye-to-eye, zoonotic, *etc.*). Moreover, other aspects of the present invention provide chimeric
10 monoclonal antibodies against viruses, prions, fungal, protozoan and other parasitic and pathogenic sources of illness.

In addition to the compositions and methods discussed above, the present invention further provides chimeric recombinant monoclonal antibody fusion proteins. In some of these embodiments, the fusion proteins comprise one or more portions of an
15 immunoglobulin and a portion of a biocide molecule, such as bactericides, viricides, fungicides, parasiticides, and the like. In preferred embodiments, the present invention provides antibody biocide fusion proteins, wherein the biocide component comprises a bactericidal enzyme such as human lysozyme, phospholipase A2 (groups I, II, V, X, and XII), lactoferrin, lactoperoxidase, and bacterial permeability increasing protein. In
20 additional embodiments, the present provides fusion proteins comprising immune system complement proteins including cytokines such as the interferons (*e.g.*, IFN- α , IFN- β , and IFN- γ) and the tumor necrosis factors (*e.g.*, TNF- α , and TNF- β) and defensins. In preferred embodiments, the antibody portion of these fusion proteins binds specifically to a foodborne bacterial pathogen (*e.g.*, *E. coli* O157:H7, *Listeria monocytogenes*,
25 *Campylobacter jejuni*, and the like).

The present invention also provides compositions comprising fusion proteins in an edible carrier such as whey protein. Preferred methods of using these compositions include, but are not limited to, food additives for human and animal (*e.g.*, bovines) consumption, carcass decontaminating compounds used during processing and finishing feedlot animal
30 (*e.g.*, bovine) carcasses and poultry, as well as pharmaceutical compositions for both human

and veterinary medicine. The present invention is not limited to the uses specifically recited herein.

In some embodiments, suitable food additive formulations of the present compositions include, but are not limited to, compositions directly applied to food products
5 such as processed meat slices and dairy products in the form of sprays, powders, injected solutions, coatings, gels, rinses, dips, films (*e.g.*, bonded), extrusions, among other known formulations.

Likewise, the present invention further provides compositions (*e.g.*, rinses, sprays, and the like) for sanitizing food-processing, medical, military or household equipment. For
10 example, some preferred embodiments of the present invention provide compositions for disinfecting meat-processing equipment. In this regard, the present invention contemplates that a number of food (*e.g.*, meat) processors will benefit from using the compositions and methods of the present invention in their operations. In this regard, the present invention contemplates providing compositions to the entire range of meat processing operations from
15 the largest commercial slaughterhouses to individual consumers.

Those skilled in the art will appreciate that the compositions disclosed herein can be readily formulated to include additional compounds common in the pharmaceutical arts such as, excipients, extenders, preservatives, and bulking agents depending on the intended use of a composition. Furthermore, ingestible formulations of these compositions may also
20 comprise any material approved by the United States Department of Agriculture (USDA) for incorporation into food products such as substances that are generally recognized as safe (GRAS) including, food additives, flavorings, colorings, vitamins, minerals, and phytonutrients. The term phytonutrients as used herein, refers to organic compounds isolated from plants having biological effects including, but not limited to, compounds from
25 the following classes of molecules: isoflavonoids, oligomeric proanthcyanidins, indol-3-carbinol, sulforaphane, fibrous ligands, plant phytosterols, ferulic acid, anthocyanocides, triterpenes, omega 3/6 fatty acids, polyacetylene, quinones, terpenes, catechins, gallates, and quercetin.

In still further embodiments, the fusion proteins of the present invention are purified
30 from the lactations of transgenic non-human mammals such as, cows, pigs, sheep, and goats. In particularly preferred embodiments, the transgenic animal is a cow.

Consequently, the present invention further provides novel genetic constructs and methods of producing transgenic animals that express the compositions of the present invention in their lactation. The present invention also provides methods of inducing transgenic animals (*e.g.*, bovines) to lactate upon maturation.

5 The present invention also provides methods of stably transfecting cell lines (*e.g.*, mammalian, plant, insect, and amphibian) with encoding the fusion proteins disclosed herein. In preferred embodiments, the constructs of the present invention allow complex multicistronic gene constructs to be stably inserted into cells (*e.g.*, mammalian, bacteria, fungal cells, plant, *etc.*). The production of fusion proteins in mammalian cell lines (or in
10 transgenic mammals) allows for their proper assembly and processing. Another method suitable for use in some embodiments of the present invention is protein production in mammalian tissue culture bioreactors.

 Monoclonal antibodies are typically produced in mammalian cells to ensure correct processing, however mammalian tissue culture bioreactors are often expensive to operate
15 thus placing products beyond mass applications. The ability to manufacture monoclonals in the milk of transgenic animals (*e.g.*, bovines) is contemplated to expand the scope of monoclonal antibodies typically from individual medicine to applications for large populations. Production of the disclosed compositions in the milk of transgenic mammals (*e.g.*, bovines) provides large quantities for economical distribution to food safety and
20 processing operations. For instance, in preferred embodiments, the present invention contemplates that at reasonable expression levels of about one gram per liter of milk, a herd of 100 transgenic cows will produce about a metric ton of recombinant protein per year. This enables production of recombinant monoclonals at 100 fold less cost than in cell culture bioreactors. Accordingly, in preferred embodiments the present invention provides
25 methods of creating transgenic bovines that produce the compositions of the present invention in their lactation. The present invention also provides methods of isolating and purifying the compositions of the present invention from the lactation of milk producing herd animals (*e.g.*, cows, sheep, and goats and the like).

 In still further embodiments, the present invention provides fusion protein enriched
30 colostrum, or colostrum like products, for use as milk substitutes and nutritional supplements for nursing mammals and in particular for nursing feedlot animals. In

preferred embodiments, these compositions comprise the microorganism targeting molecule fusion proteins of the present invention. The present invention also contemplates that introducing these compositions to nursing feedlot animals will reduce the colonization of the animal's gastrointestinal tract by pathogenic organisms such as *E. coli* O157:H7 and
5 *Listeria monocytogenes* and *Cryptosporidium parvum*. Furthermore, the compositions may be added to feeds to control diseases such as coccidiosis, which are common in both cattle and chicken feeding operations. In particular, providing a fusion protein enriched milk replacer or colostrum supplement reduces the load of *E. coli* O157:H7 in the gastrointestinal tract of the neonate and specifically places the targeted pathogenic organisms at a
10 competitive disadvantage in relation to normal gastrointestinal flora. The present invention further contemplates inducing a protective immune response in animals fed the preset fusion protein enriched colostrums and colostrum-like compositions. Accordingly, additional preferred embodiments of the present invention are directed to inducing an immune response in animals feed the present compositions.

15 The present invention provides compositions and methods directed against foodborne pathogens such as, but not limited to, *E. coli* O157:H7, *Listeria monocytogenes*, *Campylobacter jejuni*, *Clostridium botulinum*, *Clostridium perfringens*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus pneumoniae*, *Staphylococcus saprophyticus*, *Staphylococcus mutans*, *Shigella dysenteriae*, *Salmonella typhi*, *Salmonella*
20 *paratyphi*, *Salmonella enteritidis*, *Cryptosporidium parvum*, fungi, and the like. The present invention further provides composition and methods directed against food spoilage organisms such as, but not limited to, bacteria (e.g., *Lactobacillus*, *Leuconostoc*, *Pediococcus*, and *Streptococcus* and fungi (e.g., *Monilia*, *Trichoderma*, *Crinipellis*, *Moniliophthora*, *Phytophthora*, *Botrytis*, and *Fusarium*).

25 The present invention also provides compositions and methods directed against protozoans, particularly, apicomplexan protozoans including, but not limited to coccidian, cryptosporidian, toxoplasman, malarian and trypanosomatid protozoans.

In preferred embodiments, the compositions of the present invention comprise a targeting molecule, for example an immunoglobulin subunit (or portion thereof), a biocide
30 molecule (or portion thereof) such as, a bactericidal enzyme, (e.g., lysozyme), and a linker that connects the targeting molecule and the biocide molecule. In other preferred

embodiments, the compositions further comprise a signaling molecule or sequence that predictably directs the composition to an intracellular or extracellular location.

In certain embodiments, the present invention provides broad spectrum antimicrobials. Broad spectrum antimicrobials find use as a preventative tool where the identity of possible food contaminants is unknown, and new organisms can emerge as serious threats. Broad spectrum antimicrobials are also well suited for use in medicine and biodefense in confronting an infection of unknown etiology.

Broad spectrum antimicrobials take advantage of the innate immune system, which provides an important front line defense through receptors on specialized cells (e.g., macrophages, neutrophils) that are capable of binding the vast majority of microbes to which these body surfaces are exposed. In some embodiments, recognition molecules such as CD14, lipopolysaccharide binding protein (LBP), surfactant protein D (SP-D), Toll receptors, and Mannan binding lectin (MBL) that recognize and bind to Pathogen Associated Molecular Patterns (PAMPs) common to many organisms are used as the targeting portion of fusion proteins of the present invention.

In other embodiments, broadly reactive monoclonal antibodies that bind to PAMPs are used as the targeting portion of the fusion proteins of the present invention. In preferred embodiments, biocidal enzymes are delivered in high concentrations to the surface of bacteria by expressing the two components, a microorganism targeting molecule and a biocidal payload as a fusion protein.

In still further embodiments, IgM (e.g., for increased avidity of binding to repetitive PAMPs) and secretory IgA (e.g., for greater stability in harsh environments) are used and instead of attaching the biocide directly to the targeting molecule, it is attached to the J chain that is used to assemble both pentameric IgM and dimeric IgA. By using components of the innate immune system the present invention provides antimicrobials that function effectively ex vivo (e.g., in food safety settings), as well as in vivo (e.g., in clinical medicine and veterinary medicine), which can confront a broad range of bacteria through the broad affinity of the innate recognition. Furthermore, because the recognition targets bacterial features that are essential to bacterial invasion and attachment, resistance is very unlikely to occur. The present invention thus provides a novel class of antimicrobials that find use in a variety of settings.

I. Immunoglobulins

Immunoglobulins (antibodies) are proteins generated by the immune system to provide a specific molecule capable of complexing with an invading molecule commonly referred to as an antigen. Natural antibodies have two identical antigen-binding sites, both of which are specific to a particular antigen. The antibody molecule recognizes the antigen by complexing its antigen-binding sites with areas of the antigen termed epitopes. The epitopes fit into the conformational architecture of the antigen-binding sites of the antibody, enabling the antibody to bind to the antigen.

The immunoglobulin molecule is composed of two identical heavy and two identical light polypeptide chains, held together by interchain disulfide bonds. Each individual light and heavy chain folds into regions of about 110 amino acids, assuming a conserved three-dimensional conformation. The light chain comprises one variable region (termed V_L) and one constant region (C_L), while the heavy chain comprises one variable region (V_H) and three constant regions (C_{H1} , C_{H2} and C_{H3}). Pairs of regions associate to form discrete structures. In particular, the light and heavy chain variable regions, V_L and V_H , associate to form an " F_V " area that contains the antigen-binding site.

The variable regions of both heavy and light chains show considerable variability in structure and amino acid composition from one antibody molecule to another, whereas the constant regions show little variability. Each antibody recognizes and binds an antigen through the binding site defined by the association of the heavy and light chain, variable regions into an F_V area. The light-chain variable region V_L and the heavy-chain variable region V_H of a particular antibody molecule have specific amino acid sequences that allow the antigen-binding site to assume a conformation that binds to the antigen epitope recognized by that particular antibody.

Within the variable regions are found regions in which the amino acid sequence is extremely variable from one antibody to another. Three of these so-called "hypervariable" regions or "complementarity-determining regions" (CDR's) are found in each of the light and heavy chains. The three CDRs from a light chain and the three CDRs from a corresponding heavy chain form the antigen-binding site.

Cleavage of naturally occurring antibody molecules with the proteolytic enzyme papain generates fragments that retain their antigen-binding site. These fragments, commonly known as Fab's (for Fragment, antigen binding site) are composed of the C_L, V_L, C_H1 and V_H regions of the antibody. In the Fab the light chain and the fragment of the heavy chain are covalently linked by a disulfide linkage.

Monoclonal antibodies against target antigens (*e.g.*, a cell surface protein, such as receptors) are produced by a variety of techniques including conventional monoclonal antibody methodologies such as the somatic cell hybridization techniques of Kohler and Milstein, *Nature*, 256:495 (1975). Although in some embodiments, somatic cell hybridization procedures are preferred, other techniques for producing monoclonal antibodies are contemplated as well (*e.g.*, viral or oncogenic transformation of B lymphocytes).

The preferred animal system for preparing hybridomas is the murine system. Hybridoma production in the mouse is a well-established procedure. Immunization protocols and techniques for isolation of immunized splenocytes for fusion are known in the art. Fusion partners (*e.g.*, murine myeloma cells) and fusion procedures are also known.

Human monoclonal antibodies (mAbs) directed against human proteins can be generated using transgenic mice carrying the complete human immune system rather than the mouse system. Splenocytes from the transgenic mice are immunized with the antigen of interest, which are used to produce hybridomas that secrete human mAbs with specific affinities for epitopes from a human protein. (*See e.g.*, Wood *et al.*, WO 91/00906, Kucherlapati *et al.*, WO 91/10741; Lonberg *et al.*, WO 92/03918; Kay *et al.*, WO 92/03917; N. Lonberg *et al.*, *Nature*, 368:856-859 [1994]; L.L. Green *et al.*, *Nature Genet.*, 7:13-21 [1994]; S.L. Morrison *et al.*, *Proc. Nat. Acad. Sci. USA*, 81:6851-6855 [1994]; Bruggeman *et al.*, *Immunol.*, 7:33-40 [1993]; Tuailon *et al.*, *Proc. Nat. Acad. Sci. USA*, 90:3720-3724 [1993]; and Bruggeman *et al.* *Eur. J. Immunol.*, 21:1323-1326 [1991]).

Monoclonal antibodies can also be generated by other methods known to those skilled in the art of recombinant DNA technology. An alternative method, referred to as the "combinatorial antibody display" method, has been developed to identify and isolate antibody fragments having a particular antigen specificity, and can be utilized to produce

monoclonal antibodies. (*See e.g.*, Sastry *et al.*, Proc. Nat. Acad. Sci. USA, 86:5728 [1989]; Huse *et al.*, Science, 246:1275 [1989]; and Orlandi *et al.*, Proc. Nat. Acad. Sci. USA, 86:3833 [1989]). After immunizing an animal with an immunogen as described above, the antibody repertoire of the resulting B-cell pool is cloned. Methods are generally known for
 5 obtaining the DNA sequence of the variable regions of a diverse population of immunoglobulin molecules by using a mixture of oligomer primers and the PCR. For instance, mixed oligonucleotide primers corresponding to the 5' leader (signal peptide) sequences and/or framework 1 (FR1) sequences, as well as primer to a conserved 3' constant region primer can be used for PCR amplification of the heavy and light chain variable
 10 regions from a number of murine antibodies. (*See e.g.*, Larrick *et al.*, Biotechniques, 11:152-156 [1991]). A similar strategy can also be used to amplify human heavy and light chain variable regions from human antibodies (*See e.g.*, Larrick *et al.*, Methods: Companion to Methods in Enzymology, 2:106-110 [1991]).

In one embodiment, RNA is isolated from B lymphocytes, for example, peripheral
 15 blood cells, bone marrow, or spleen preparations, using standard protocols (*e.g.*, US 4,683,292; Orlandi, *et al.*, Proc. Nat. Acad. Sci. USA, 86:3833-3837 [1989]; Sastry *et al.*, Proc. Nat. Acad. Sci. USA, 86:5728-5732 [1989]; and Huse *et al.*, Science, 246:1275 [1989]). First strand cDNA is synthesized using primers specific for the constant region of the heavy chain(s) and each of the κ and λ light
 20 chains, as well as primers for the signal sequence. Using variable region PCR primers, the variable regions of both heavy and light chains are amplified, each alone or in combination, and ligated into appropriate vectors for further manipulation ingenerating the display packages. Oligonucleotide primers useful in amplification protocols may be unique or degenerate or incorporate inosine at degenerate positions. Restriction endonuclease
 25 recognition sequences may also be incorporated into the primers to allow for the cloning of the amplified fragment into a vector in a predetermined reading frame for expression.

The V-gene library cloned from the immunization-derived antibody repertoire can be expressed by a population of display packages, preferably derived from filamentous phage, to form an antibody display library. Ideally, the display package comprises a system that
 30 allows the sampling of very large variegated antibody display libraries, rapid sorting after each affinity separation round, and easy isolation of the antibody gene from purified display

packages. In addition to commercially available kits for generating phage display libraries, examples of methods and reagents particularly amenable for use in generating a variegated antibody display library can be found in, for example, US 5,223,409; WO 92/18619; WO 91/17271; WO 92/20791; WO 92/15679; WO 93/01288; WO 92/01047; WO 92/09690;

5 WO 90/02809 ; Fuchs *et al.*,

Biol. Technology, 9:1370-1372 [1991]; Hay *et al.*, Hum. Antibod. Hybridomas, 3:81-85 [1992]; Huse *et al.*, Science, 46:1275-1281 [1989]; Hawkins *et al.*, J. Mol. Biol., 226:889-896 [1992]; Clackson *et al.*, Nature, 352:624-628 [1991]; Gram *et al.*, Proc. Nat. Acad. Sci. USA, 89:3576-3580 [1992]; Garrad *et al.*, Bio/Technolog, 2:1373-1377 [1991];

10 Hoogenboom *et al.*, Nuc. Acid Res., 19:4133-4137 [1991]; and Barbas *et al.*, Proc. Nat. Acad. Sci. USA, 88:7978 [1991]. In certain embodiments, the V region domains of heavy and light chains can be expressed on the same polypeptide, joined by a flexible linker to form a single-chain Fv fragment, and the scFV gene subsequently cloned into the desired expression vector or phage genome.

15 As generally described in McCafferty *et al.*, Nature, 348:552-554 (1990), complete V_H and V_L domains of an antibody, joined by a flexible linker (*e.g.*, (Gly₄-Ser)₃) can be used to produce a single chain antibody which can render the display package separable based on antigen affinity. Isolated scFV antibodies immunoreactive with the antigen can subsequently be formulated into a pharmaceutical preparation for use in the subject method.

20 Once displayed on the surface of a display package (*e.g.*, filamentous phage), the antibody library is screened with the target antigen, or peptide fragment thereof, to identify and isolate packages that express an antibody having specificity for the target antigen. Nucleic acid encoding the selected antibody can be recovered from the display package (*e.g.*, from the phage genome) and subcloned into other expression vectors by standard
25 recombinant DNA techniques.

Specific antibody molecules with high affinities for a surface protein can be made according to methods known to those in the art, *e.g.*, methods involving screening of libraries US 5,233,409 and US 5,403,484.

Further, the methods of these libraries can be used in screens to obtain binding
30 determinants that are mimetics of the structural determinants of antibodies.

In particular, the Fv binding surface of a particular antibody molecule interacts with its target ligand according to principles of protein-protein interactions, hence sequence data for V_H and V_L (the latter of which may be of the κ or λ chain type) is the basis for protein engineering techniques known to those with skill in the art. Details of the protein surface
5 that comprises the binding determinants can be obtained from antibody sequence information, by a modeling procedure using previously determined three-dimensional structures from other antibodies obtained from NMR studies or crystallographic data.

In one embodiment, a variegated peptide library is expressed by a population of display packages to form a peptide display library. Ideally, the display package comprises a
10 system that allows the sampling of very large variegated peptide display libraries, rapid sorting after each affinity separation round, and easy isolation of the peptide-encoding gene from purified display packages. Peptide display libraries can be in, *e.g.*, prokaryotic organisms and viruses, which can be amplified quickly, are relatively easy to manipulate, and which allows the creation of large number of clones. Preferred display packages
15 include, for example, vegetative bacterial cells, bacterial spores, and most preferably, bacterial viruses (especially DNA viruses). However, the present invention also contemplates the use of eukaryotic cells, including yeast and their spores, as potential display packages. Phage display libraries are known in the art.

Other techniques include affinity chromatography with an appropriate "receptor,"
20 *e.g.*, a target antigen, followed by identification of the isolated binding agents or ligands by conventional techniques (*e.g.*, mass spectrometry and NMR). Preferably, the soluble receptor is conjugated to a label (*e.g.*, fluorophores, colorimetric enzymes, radioisotopes, or luminescent compounds) that can be detected to indicate ligand binding. Alternatively, immobilized compounds can be selectively released and allowed to diffuse through a
25 membrane to interact with a receptor.

Combinatorial libraries of compounds can also be synthesized with "tags" to encode the identity of each member of the library. (*See e.g.*, W.C. Still *et al.*, WO 94/08051).

In general, this method features the use of inert but readily detectable tags that are attached to the solid support or to the compounds.
30 When an active compound is detected, the identity of the compound is determined by identification of the unique accompanying tag. This tagging method permits the synthesis

of large libraries of compounds that can be identified at very low levels among to total set of all compounds in the library.

The term modified antibody is also intended to include antibodies, such as monoclonal antibodies, chimeric antibodies, and humanized antibodies which have been
 5 modified by, for example, deleting, adding, or substituting portions of the antibody. For example, an antibody can be modified by deleting the hinge region, thus generating a monovalent antibody. Any modification is within the scope of the invention so long as the antibody has at least one antigen binding region specific.

Chimeric mouse-human monoclonal antibodies can be produced by recombinant
 10 DNA techniques known in the art. For example, a gene encoding the Fc constant region of a murine (or other species) monoclonal antibody molecule is digested with restriction enzymes to remove the region encoding the murine Fc, and the equivalent portion of a gene encoding a human Fc constant region is substituted. (*See e.g.*, Robinson *et al.*, PCT/US86/02269; European Patent Application 184,187; European Patent Application
 15 171,496; European Patent Application 173,494; WO 86/01533; US 4,816,567; European Patent Application 125,023;

Better *et al.*, Science, 240:1041-1043 [1988]; Liu *et al.*, Proc. Nat. Acad. Sci. USA, 84:3439-3443 [1987]; Liu *et al.*, J. Immunol., 139:3521-3526 [1987]; Sun *et al.*, Proc. Nat. Acad. Sci. USA, 84:214-218 [1987]; Nishimura *et al.*, Canc. Res., 47:999-1005 [1987];
 20 Wood *et al.*, Nature, 314:446-449 [1985]; and Shaw *et al.*, J. Natl. Cancer Inst., 80:1553-1559 [1988]).

The chimeric antibody can be further humanized by replacing sequences of the Fv variable region that are not directly involved in antigen binding with equivalent sequences from human Fv variable regions. General reviews of humanized chimeric antibodies are
 25 provided by S.L. Morrison, Science, 229:1202-1207 (1985) and by Oi *et al.*, Bio. Techniques, 4:214 (1986). Those methods include isolating, manipulating, and expressing the nucleic acid sequences that encode all or part of immunoglobulin Fv variable regions from at least one of a heavy or light chain. Sources of such nucleic acid are well known to those skilled in the art and, for example, may be obtained from 7E3, an anti-GPIIb/IIIa
 30 antibody producing hybridoma. The recombinant DNA encoding the chimeric antibody, or fragment thereof, can then be cloned into an appropriate expression vector.

Suitable humanized antibodies can alternatively be produced by CDR substitution (e.g., US 5,225,539; Jones *et al.*, Nature, 321:552-525 [1986]; Verhoeyan *et al.*, Science, 239:1534 [1988]; and Beidler *et al.*, J. Immunol., 141:4053 [1988]). All of the CDRs of a particular human antibody may be replaced with at least a portion of a non-human CDR or only some of the CDRs may be replaced with non-human CDRs. It is only necessary to replace the number of CDRs required for binding of the humanized antibody to the Fc receptor.

An antibody can be humanized by any method that is capable of replacing at least a portion of a CDR of a human antibody with a CDR derived from a non-human antibody. The human CDRs may be replaced with non-human CDRs; using oligonucleotide site-directed mutagenesis.

Also within the scope of the invention are chimeric and humanized antibodies in which specific amino acids have been substituted, deleted or added. In particular, preferred humanized antibodies have amino acid substitutions in the framework region, such as to improve binding to the antigen. For example, in a humanized antibody having mouse CDRs, amino acids located in the human framework region can be replaced with the amino acids located at the corresponding positions in the mouse antibody. Such substitutions are known to improve binding of humanized antibodies to the antigen in some instances.

In preferred embodiments, the fusion proteins include a monoclonal antibody subunit (e.g., a human, murine, or bovine), or a fragment thereof, (e.g., an antigen binding fragment thereof). The monoclonal antibody subunit or antigen binding fragment thereof can be a single chain polypeptide, a dimer of a heavy chain and a light chain, a tetramer of two heavy and two light chains, or a pentamer (e.g., IgM). IgM is a pentamer of five monomer units held together by disulfide bonds linking their carboxyl-terminal (C μ 4/C μ 4) domains and C μ 3/C μ 3 domains. The pentameric structure of IgM provides 10 antigen-binding sites, thus serum IgM has a higher valency than other types of antibody isotypes. With its high valency, pentameric IgM is more efficient than other antibody isotypes at binding multidimensional antigens (e.g., viral particles and red blood cells. However, due to its large pentameric structure, IgM does not diffuse well and is usually found in low concentrations in intercellular tissue fluids. The J chain of IgM allows the molecule to bind to receptors on secretory cells, which transport the molecule across epithelial linings to the

external secretions that bathe the mucosal surfaces. In some embodiments, of the present invention take advantage of the low diffusion rate of pentameric IgM to help concentrate the fusion proteins of present invention at a site of interest. In preferred embodiments, monoclonal IgM, and fusion and chimeric proteins thereof, are directed to destroying
5 *Cryptosporidium parvum* and other types of parasitic pathogens.

In some embodiments, an IgA is utilized to make a directed biocide. IgA's are preferably produced using either one, two or three constructs. IgA made by use of two or three retrovector constructs. For example, a retroviral construct can be produced in which the J-chain expression is driven by the long terminal repeat (LTR) promoter, and expression
10 of a heavy chain and light chain separated by an IRES sequence is driven by an internal promoter. In another example, the heavy chain and light chain are provided in one vector and the J chain is provided in another vector. In another example, a third construct expressing the secretory component truncated form from poly IgR is provided.

In still other embodiments, secretion of a directed biocide is enhanced by
15 transfecting a cell producing a directed biocide with a vector (e.g., a retroviral vector) that expressed secretory component. See U.S. Pat. No. 6,300,104; Koteswarra and Morrison, Proc. Natl. Acad. Sci. USA 94:6364-68 (1997).

In some preferred embodiments, the monoclonal antibody is a murine antibody or a fragment thereof. In other preferred embodiments, the monoclonal antibody is a bovine
20 antibody or a fragment thereof. For example, the murine antibody can be produced by a hybridoma that includes a B cell obtained from a transgenic mouse having a genome comprising a heavy chain transgene and a light chain transgene fused to an immortalized cell. The antibodies can be of various isotypes, including, but not limited to: IgG (e.g., IgG1, IgG2, IgG2a, IgG2b, IgG2c, IgG3, IgG4); IgM; IgA1; IgA2; IgA_{sec}; IgD; and IgE. In
25 some preferred embodiments, the antibody is an IgG isotype. In other preferred embodiments, the antibody is an IgM isotype. The antibodies can be full-length (e.g., an IgG1, IgG2, IgG3, or IgG4 antibody) or can include only an antigen-binding portion (e.g., a Fab, F(ab')₂, Fv or a single chain Fv fragment).

In preferred embodiments, the immunoglobulin subunit of the fusion proteins is a
30 recombinant antibody (e.g., a chimeric or a humanized antibody), a subunit, or an antigen

binding fragment thereof (*e.g.*, has a variable region, or at least a complementarity determining region (CDR)).

In preferred embodiments, the immunoglobulin subunit of the fusion protein is monovalent (*e.g.*, includes one pair of heavy and light chains, or antigen binding portions thereof). In other embodiments, the immunoglobulin subunit of the fusion protein is a divalent (*e.g.*, includes two pairs of heavy and light chains, or antigen binding portions thereof). In preferred embodiments, the transgenic fusion proteins include an immunoglobulin heavy chain or a fragment thereof (*e.g.*, an antigen binding fragment thereof).

In still other embodiments, the fusion proteins and/or recombinant antibodies comprise an immunoglobulin having only heavy chains such as the HCAs found in certain *Camelidae* (*e.g.*, camels, dromedaries, and llamas) species, spotted ratfish, and nurse shark. While the present invention is not limited to any particular mechanisms, the present invention contemplates that there are differences between conventional antibodies and HCAs in both the V_H and C_H regions. For instance, as reported by Muyldermans *et al.* and Nguyen *et al.*, the sequences of HCAs variable domains (V_HH) differ significantly from those of conventional antibodies (V_H). (S. Muyldermans *et al.*, Protein, Eng., 7:1129-1135 [1994]; V.K. Nguyen *et al.*, J. Mol. Biol., 275:413-418 [1998]; and V.K. Nguyen *et al.*, Immunogenetics DOI 10.1007/s00251-002-0433-0 [2002]). Additionally, HCAs lack the first domain of the constant region (C_H); the matured V_HH -DJ is directly joined to the hinge region. Separate sets of V and C genes encode conventional antibodies and HCAs, however, conventional antibodies and HCAs have some common D genes and appear to have identical J_H regions. (V.K. Nguyen *et al.*, EMBO J., 19:921-930 [2000]; and V.K. Nguyen *et al.*, Adv. Immunol., 79:261-296 [2001]).

In yet other embodiments, IgM is used as the microorganism targeting molecule. IgMs bind with multiple epitopes, effectively enhancing the avidity of the binding. The genes for both SP-D and MBL of these molecules have been sequenced and both have been produced as recombinant molecules in full or truncated forms (Shrive *et al.*, J Mol Biol 2003; 331:509-23; Arora *et al.*, J Biol Chem 2001; 276:43087-94).

In other embodiments, the microorganism targeting molecules are monoclonal antibodies that target PAMPs. In some embodiments, the monoclonal antibodies utilize the

multimeric structure of IgM and IgA. The repetitive structure of many PAMPs allows antibodies to bind to two identical epitopes on one molecule or on separate molecules (cross-linking) on the bacterial surface. This type of interaction results in an overall higher binding energy per antibody molecule than the engagement of one single arm of the immunoglobulin in the binding; for the antibody to detach, both binding sites would have to be released at once. The avidity is proportionally higher for IgA, which is a dimer, bearing a total of 4 binding sites, or IgM which usually is a pentamer, having 10 binding sites.

Packing plants are harsh environments; secretory IgA is adapted to function in the gastrointestinal tract and its dimeric configuration is supported by a portion of the secretory component that assists its membrane transport. Thus IgM and IgA may offer advantages as targeting molecules over IgG. In certain embodiments, the fact that they are linked by a J chain, and in the case of IgA coexpressed with secretory component, allows for attaching the payload biocide to these auxiliary chains instead of to the Fc portion of the immunoglobulin itself. Accordingly, in some embodiments, the immunoglobulin J-chain is used as a microorganism targeting molecule.

In some embodiments, a system of hybridoma-like antibody preparation, developed by Neoclone (Madison, WI), is used in the production of monoclonal antibodies. Splenocytes from immunized mice are immortalized using a retrovector-mediated introduction of the abl-myc genes. On reintroduction into recipient mice one dominant immortalized B cell clone (plasmacytoma) outgrows all others and produces a monoclonal antibody in the ascitic fluid. The B cell clone can be harvested with the ascitic fluid that contains high concentration of monoclonal antibody. This process can be completed in 8-10 weeks.

II. Innate System Receptors

In some embodiments of the present invention, innate immunity receptors are used as microorganism targeting molecules due to their high affinity of interaction with a multitude of microorganisms. These receptors are all highly conserved structures across species, even across classes (Ezekowitz RAB and Hoffmann JA. Innate Immunity. Totowa, NJ: HUmana Press, 2003). Many of these evolutionarily ancient receptors are found in

Invertebrae (Aderem A and Ulevitch RJ. Nature 2000; 406:782-7). Preferred microorganism targeting molecules are those that exist as soluble molecules in circulation.

In some embodiments, the microorganism targeting molecule is CD14, found on monocytes/macrophages and neutrophils (Haziot *et al.*, J Immunol 1988; 141:547-52). This molecule exists as both a membrane-bound form, with a GPI anchor, and as a soluble form. Both versions of CD14 bind LPS with high affinity. The reported dissociation constant for LPS binding to CD14 is K_D $3-7 \times 10^{-8}$ M (Tobias *et al.*, J Biol Chem 1995; 270:10482-8). Haziot and co-workers have produced recombinant soluble human CD14 and have used it to study the binding to LPS (Haziot *et al.*, J Immunol 1995; 154:6529-32), showing that soluble CD14 binds LPS under various conditions. LPS is one of the main components of the cell wall of Gram-negative bacteria. CD14 also binds to peptidoglycan structures with high affinity ($K_d=25$ nM) (Dziarski, Cell Mol Life Sci 2003; 60:1793-804; Dziarski *et al.*, Chem Immunol 2000; 74:83-107; Dziarski *et al.*, Infect Immun 2000; 68:5254-60.). Peptidoglycan (PGN) structures make up a substantial portion of the cell wall of Gram-negative and Gram-positive bacteria. In addition, CD14 binds to lipoteichoic acid on Gram-positive bacteria, lipoarabinomannan of mycobacteriae, lipoproteins from spirochetes and mycobacteriae, and others (Dziarski, Cell Mol Life Sci 2003; 60:1793-804). CD14 is a highly versatile receptor that interacts with an impressive variety of different bacteria.

In other embodiments, the microorganism targeting molecule is the LPS-binding protein (LBP) (Tobias *et al.*, J Exp Med 1986; 164:777-93). LBP is an acute phase protein that is released into circulation upon a bacterial infection with Gram-negative bacteria or exposure to LPS. LBP can bind circulating or bound LPS, and through this interaction inhibit LPS-related septic shock (Lamping *et al.*, J Clin Invest 1998; 101:2065-71). LBP can inhibit LPS-induced signaling by blocking LPS transfer from CD14 to Toll like receptor 4 (Thompson *et al.*, J Biol Chem 2003; 278:28367-71). In addition, LBP is capable of removing LPS from mCD14 with high efficiency, indicating that LBP binds LPS more strongly than CD14. Separate studies aimed at comparing binding efficiencies between LPS, LBP, and CD14 have confirmed that LBP binds LPS with a roughly ten-fold higher affinity K_D 3.5×10^{-9} M (Tobias *et al.*, J Biol Chem 1995; 270:10482-8) than CD14. LBP has been cloned, sequenced and expressed by various groups (Schumann *et al.*, Science

1990; 249:1429-31; Theofan *et al.*, J Immunol 1994; 152:3623-9; Thompson *et al.*, J Biol Chem 2003; 278:28367-71).

In still further embodiments, the microorganism targeting molecule is a member of the collectins, also called defense collagens (Van De Wetering *et al.*, Eur J Biochem 2004; 5 271:1229-49). Surfactant protein D (SP-D) has been shown to interact with rough and smooth LPS on bacterial surfaces (Clark *et al.*, Microbes Infect 2000; 2:273-8; Lawson and Reid, Immunol Rev 2000; 173:66-78) and therefore can target Gram-negative microorganisms. In other embodiments, mannan-binding lectin (MBL), from this family, which is known to target peptidoglycans (and hence Gram-positive bacteria) (Lu *et al.*, 10 Biochim Biophys Acta 2002; 1572:387-400), is utilized. Collectins assemble into multimers, effectively multiplying the number of binding sites per complex available for interaction with the microorganism's repetitive surface.

In other embodiments the Toll receptor family are used as pathogen targeting molecules; this group of cell bound receptors functions singly or in concert with other innate 15 immune system receptors (Ezekowitz and Hoffmann, Innate Immunity. Totowa, NJ: HUmana Press, 2003; Janeway and Medzhitov. Annu Rev Immunol 2002; 20:197-216; Medzhitov and Janeway, Trends Microbiol 2000; 8:452-6. Toll like receptors (TLRs) comprise a family of cell surface receptors that are related to the *Drosophila* Toll protein, a molecule involved in defense against fungal infection in the fly (Aderem and Ulevitch, 20 Nature, 406:785-787 [2000]). Ten mammalian TLRs have been identified (Aderem and Ulevitch, *Supra*). Two members of the family, TLR2 and TLR4, have been better characterized and shown to mediate the response to multiple bacterial cell-wall components including lipopolysaccharide (LPS), lipopeptides, peptidoglycans (PGN) and lipoteichoic acid (LTA) (Yang *et al.*, Nature, 395:284-288 [1998]; Poltorak *et al.*, Science, 282:2085- 25 2088 [1998]; Aliprantis *et al.*, Science, 285:736-739 [1999]; Chow *et al.*, J. Biol. Chem., 274:10689-10692 [2000]; and Schwandner *et al.*, J. Biol. Chem., 274: 17406-17409 [2000]). Mammalian TLRs have multiple leucine-rich repeats in the ectodomain and an intracellular Toll-IL1 receptor (TIR) domain that mediates a signaling cascade to the nucleus (Aderem and Ulevitch, *Supra*). Stimulation of TLR2 and TLR4 leads to the recruitment of 30 the adaptor molecule MyD88 and the serine kinase IL-1R-associated kinase (IRAK), two

signaling components that together with TRAF-6 mediate activation of NF- κ B (Aderem and Ulevitch, *Supra*).

III. Linkers

5 In preferred embodiments, the transgenic fusion proteins comprise a targeting molecule (*e.g.*, immunoglobulin heavy chain (or fragment thereof) and a light chain or (a fragment thereof)) connected to a biocide molecule by a linker. In preferred embodiments, the targeting molecule is linked via a peptide linker or is directly fused (*e.g.*, covalently bonded) to the biocide molecule. In preferred embodiments, the transgenic fusion proteins
10 assemble into dimeric, trimeric, tetrameric, pentameric, hexameric or higher polymeric complexes.

In preferred embodiments, the present invention provides retroviral constructs that encode in operable configuration an immunoglobulin (or portion thereof), a biocide molecule (or portion thereof), and a linker group that connects the immunoglobulin and the
15 biocide. In some of these embodiments, the linker group comprises one amino acid moiety (*e.g.*, X_n ; wherein X is any amino acid or amino acid derivative; and $n = 1$). In some of these embodiments, the linker group comprises at least one amino acid moiety (*e.g.*, X_n ; wherein X is any amino acid or amino acid derivative; and $n \geq 2$). Similarly, in other embodiments, the linker group comprises two or more repeating amino acids (*e.g.*, $X_n Y_z$;
20 wherein X and Y are any amino acid or amino acid derivative; and $n \geq 1$ and $z \geq 1$). In still further embodiments, the linker group comprises two or more repeating amino acids that form a repeating unit (*e.g.*, $(X_n Y_z)_r$; wherein $r \geq 1$). The present invention is not intended to be limited, however, to the aforementioned linker groups. Those skilled in the art will appreciate that a number of other linker group configurations and compositions find use in
25 certain embodiments of the present invention.

In particularly preferred embodiments, the linker group used has one or more of the following characteristics: 1) sufficient length and flexibility to allow for the rotation of the targeting molecule (*e.g.*, immunoglobulin) and the biocide molecule (*e.g.*, lysozyme) relative to one another; 2) a flexible extended conformation; 3) a propensity for developing
30 ordered secondary or tertiary structures that interact with functional components; 4) nonreactive with the functional components of the construct (*e.g.*, minimal hydrophobic or

charged character to react with the functional protein domains); 5) sufficient resistant to degradation (*e.g.*, digestion by proteases); and 6) allows the fusion protein to form a complex (*e.g.*, a di-, tri-, tetra-, penta-, or higher multimeric complex) while retaining biological (*e.g.*, biocidal) activity. The linker sequence should separate the target molecule and the biocide molecule of the fusion protein by a distance sufficient to ensure that each component properly folds into its secondary and tertiary structures.

In preferred embodiments, the peptide linker is from about 2 to 500, more preferably of from about 50 to 100, and even more preferably, from about 10 to 30 amino acids long. A polypeptide linker sequence of about 20 amino acids provides a suitable separation of functional protein domains, although longer or shorter linker sequences are contemplated. For example, in particularly preferred embodiments, the peptide linker is between 17 to 20 amino acids in length.

The present invention further contemplates peptide linkers comprised of the following amino acids: Gly, Ser, Asn, Thr or Ala. Typical surface amino acids in flexible protein regions include Gly, Ser, and Asn. The present invention contemplates that various amino acid sequence permutations of Gly, Ser, and optionally Asn, provide suitable linker sequences. However, the present invention is not limited to peptide linkers comprised of the aforementioned amino acids. For example, in some embodiments, the peptide linkers comprise further uncharged polar amino acids (*e.g.*, Gln, or Tyr) and/or nonpolar amino acids (*e.g.*, Val, Leu, Ileu, Pro, Phe, Met, Trp, Cys).

In some preferred embodiments, the peptide linker comprises one (or more) Gly-Ser elements. For example, in some of these embodiments, the peptide linker has the formula $(\text{Ser}_n\text{-Gly}_x)_y$, wherein n and $x \geq 1$, and $y \geq 1$. In some preferred embodiments, the peptide linker has the formula $(\text{Ser-Gly}_4)_y$, wherein $y = 1, 2, 3, 4, 5, 6, 7, 8$ or more. In some other preferred embodiments, the peptide linker includes a sequence having the formula $(\text{Ser-Gly}_4)_3$. In still other preferred embodiments, the peptide linker comprises a sequence of the formula $((\text{Ser-Gly}_4)_3\text{-Ser-Pro})$. Other peptide linker sequences are contemplated, including, but not limited to, $\text{Gly}_4\text{SerGly}_5\text{Ser}$, and $((\text{Ser}_4\text{-Gly})_3\text{-Ser-Pro})$.

In still further embodiments, the target molecule and the biocidal molecule comprising the fusion protein are fused directly without a linker sequence. In some embodiments, linker sequences are unnecessary where the fusion protein components have

non-essential N-or C-terminal amino acid regions that separate functional domains and prevent steric interference.

IV. Biocides

5 The present invention provides novel fusion proteins. In preferred embodiments, the recombinant fusion proteins comprise one or more biocide molecules (*e.g.*, a bactericidal enzyme) attached to the antibody portion of the construct via a linking group. The specificity of the monoclonal antibody portion of the construct targets the biocide molecule to a pathogen such as, for example, *E. coli* O157:H7, *Listeria monocytogenes*,
10 *Campylobacter jejuni*, or *Cryptosporidium parvum*.

 One benefit of the specific targeting ability of the fusion protein construct is that it allows for relative accumulation of biocide at locations where the targeted pathogens are challenging the animal. Increasing the local concentration of biocide relative to the targeted pathogens enhances the biocidal activity of the fusion protein construct. In particular, the
15 present invention contemplates that directing the biocide (*e.g.*, lysozyme, PLA2, and the like) to the immediate vicinity of the pathogen (*e.g.*, a bacterium) via the antibody portion of the construct effectively increasing the biocide's local concentration, thus providing a significantly greater biocidal (*e.g.*, bactericidal) effect than administering biocide alone (parasiticidal compounds). For example in the case of lysozyme, the affinity constant (K_m)
20 of lysozyme for its substrate is approximately 10^{-3} M, while that of phospholipase A2 is approximately 10^{-4} M. However, the K_d of a monoclonal antibody is usually in the range of 10^{-8} M to 10^{-11} M, thus antibodies have about 5 orders of magnitude higher affinity for their substrates than do biocidal molecules alone. Therefore, preferred embodiments of the present invention utilize monoclonal antibodies (or portions thereof) to specifically direct
25 biocide molecules to a target by taking advantage of the antibody's very high affinity for target pathogens. Additionally, directing the fusion protein constructs to target pathogens also reduces the possible deleterious effects to the animal caused by systemic administration of the biocidal molecules.

 In preferred embodiments, the directed biocidal approach described herein uses a
30 monoclonal antibody to direct a naturally occurring bactericidal enzyme to the target

pathogen. In some of these embodiments, the bactericidal enzyme(s) are components of the innate immune system. One such preferred bactericidal enzyme is lysozyme.

Lysozyme is naturally present in mammalian tissues and in secretions such as tears and mucus. Lysozyme is also found in many foods including, egg whites, cow milk, and human colostrum. The enzyme is widely reported to have antibacterial properties.

Lysozyme is a glycosidase that targets the polysaccharides of many bacterial cell walls rendering them more susceptible to osmotic lysis. Lysozyme is a 1,4- β -N-acetylmuramidase that cleaves the glycosidic bond between C-1 of N-acetylmuramic acid and C-4 of N-acetylglucosamine of the peptidoglycan layer present in many bacterial cell walls (*See e.g.*, M. Schindler *et al.*, *Biochemistry*, 16(3):423-431 [1977]). While it is not clear whether this cleavage contributes to the bactericidal action of lysozyme (K. During *et al.*, *FEBS Lett.*, 449(2-3):93-100 [1999]; and H.R. Ibrahim *et al.*, *FEBS Lett.*, 506(1):27-32 [2001]), it is widely accepted that lysozyme plays an important role in defense against bacterial infection. Lysozyme has also been shown to bind to the lipid A portion of bacterial endotoxin. This interaction prevents the endotoxin from inducing the release of inflammatory components by lymphocytes and macrophages (*See e.g.*, B. Reusens-Billen *et al.*, *Diabetes Res. Clin. Pract.*, 23(2):85-94 [1994]; K. Takada *et al.*, *Infect. Immun.*, 62(4):1171-1175 [1994]; and K. Takada *et al.*, *Circ. Shock*, 44(4):169-174 [1994]).

Other proteins that form part of the innate immune system, and especially those secreted by the intestinal Paneth cells, are contemplated for targeting the structural integrity of sporozoites. For example, phospholipase A2 (PLA2) is another naturally occurring bactericidal enzyme contemplated for use in certain embodiments of the present invention. Secretory type II phospholipase A2 (sPLA(2)-IIA) is a 14 kD enzyme synthesized in a number of gland cells, including Paneth cells of intestinal mucosa, prostate gland cells, and lacrimal glands. It is present in cellular secretions on mucosal surfaces including intestinal mucus, seminal plasma, and tears (X.D. Qu and R.I. Lehrer, *Infect. Immun.*, 66:2791-2797 [1998]; and X.D. Qu *et al.*, *Infect. Immun.*, 64:5161-5165 [1996]). Evidence suggests that phospholipase A2 has an important antibacterial role in addition to its inflammatory mediating role (*See e.g.*, A.G. Buckland and D.C. Wilton, *Biochim. Biophys. Acta*, 1488(1-2):71-82 [2000]). Elevated amounts of phospholipase A2 is found in patients with acute bacterial diseases (J.O. Gronoos *et al.*, *J. Infect. Dis.*, 185:1767-1772 [2002]). The enzyme

appears to effective in controlling *E coli*. infections when expressed in transgenic mice (*See e.g.*, V.J. Laine *et al.*, Infect. Immun., 68(1):87-92 [2000]). While the present invention is not limited to any mechanisms, PLA2 appears to hydrolyze membrane phospholipids, thus destroying the membranes of invading microbes. PLA2 serves as a critical component of the innate immune system, functioning in combination with lysozyme and the defensins to provide an effective barrier to invasion by a diverse range of organisms.

Mammalian cells are generally highly resistant to sPLA(2) IIA (R.S. Koduri *et al.*, J. Biol. Chem., 273:32142-32153 [1998]). The substrate specificity of the different members of the PLA2 family may be related to the differences in interfacial binding characteristics to charge-neutral phosphotidyl choline (PC) versus anionic phospholipids. Indeed, sPLA(2) family members sPLA2-V and -X bind efficiently and hydrolyze PC vesicles *in vitro* whereas the vesicles are a poor binding substrate for -IIA. Plasma membranes with a high PC content would therefore be stable in the presence of sPLA(2)-IIA. The composition of the phospholipids on the surface of the organism therefore contributes to the susceptibility of the organism to the action of sPLA2. Some parasitic eukaryotic organisms may evade the innate immune system by not stimulating the cells of the immune system to release biocidal enzymes and defensins (*e.g.*, *G. lamblia* and *C. albicans* appear not to stimulate Paneth cells). However, one recent report suggests that *Plasmodium* is susceptible to sPLA2 (Type III, from bee venom). Type III sPLA2 has an activity that is similar to the type IIA enzyme, but is a slightly larger molecule having N- and C-terminal extensions. Systemically, sPLA(2)-IIA has a role in generalized inflammatory responses. In acute inflammation, the levels of the enzyme are elevated many hundreds of fold, however, it appears to have no adverse effect at epithelial surfaces. *In vitro*, sPLA(2) apparently has no deleterious effect on various types of cultured mammalian cells. Healthy transgenic mice chronically over-expressing sPLA(2)-IIA have been produced and exhibit an elevated resistance to infection by gram positive organisms (V.J. Laine *et al.*, J. Immunol., 162:7402-7408 [1999]; and V.J. Laine *et al.*, Infect. Immun., 68:87-92 [2000]).

A number of inhibitors have been identified that have activity against *C. parvum* by targeting the parasite's metabolic pathways. These include, but are not limited to, metalloprotease inhibitors (P.C. Okhuysen *et al.*, Antimicrob. Agents Chemother., 40:2781-2784 [1996]) and serine protease antagonists (J.R. Forney *et al.*, J. Parasitol., 82:638-640

[1996]). Other enzymes essential to *C. parvum* infectivity provide useful inhibitor targets. These include, for example, phosphoinositide 3-kinase (J.R. Forney *et al.*, Infect. Immun., 67:844-852 [1999]) and cysteine proteinase (M.V. Nesterenko *et al.*, Microbios., 83:77-88 [1995]).

5 Other naturally occurring bactericidal molecules (*e.g.*, enzymes) contemplated for use in certain embodiments of the present invention, include, but are not limited to, lactoferrin, lactoperoxidase, bacterial permeability increasing protein (BPI), and Aprotinin. (*See e.g.*, B.A. Mannion *et al.*, J. Clin. Invest., 85(3):853-860 [1990]; A. Pellegrini *et al.*, Biochem. Biophys. Res. Commun., 222(2):559-565 [1996]; and P. Prohinar *et al.*, Mol.
10 Microbiol., 43(6):1493-1504 [2002]).

In some embodiments of the present invention, the biocide component of the fusion protein comprises an antimicrobial polypeptide (*See e.g.*, *Antimicrobial Peptide Protocols*, ed. W. M. Shafer, Humana Press, Totowa, NJ [1997]) or a pore forming agent. In some
15 embodiments, the antimicrobial peptide or pore forming agent is a compound or peptide selected from the following: magainin (*e.g.*, magainin I, magainin II, xenopsin, xenopsin precursor fragment, caerulein precursor fragment), magainin I and II analogs (PGLa, magainin A, magainin G, pexiganin, Z-12, pexiganin acetate, D35, MSI-78A, MG0 [K10E, K11E, F12W-magainin 2], MG2+ [K10E, F12W-magainin-2], MG4+ [F12W-magainin 2], MG6+ [f12W, E19Q-magainin 2 amide], MSI-238, reversed magainin II analogs [*e.g.*, 53D,
20 87-ISM, and A87-ISM], Ala-magainin II amide, magainin II amide), cecropin P1, cecropin A, cecropin B, indolicidin, nisin, ranalexin, lactoferricin B, poly-L-lysine, cecropin A (1-8)-magainin II (1-12), cecropin A (1-8)-melittin (1-12), CA(1-13)-MA(1-13), CA(1-13)-ME(1-13), gramicidin, gramicidin A, gramicidin D, gramicidin S, alamethicin, protegrin, histatin, dermaseptin, lentivirus amphipathic peptide or analog, parasin I, lycotoxin I or II,
25 globomycin, gramicidin S, surfactin, ralinomycin, valinomycin, polymyxin B, PM2 [(+/-) 1-(4-aminobutyl)-6-benzylindane], PM2c [(+/-) -6-benzyl-1-(3-carboxypropyl)indane], PM3 [(+/-)1-benzyl-6-(4-aminobutyl)indane], tachyplesin, buforin I or II, misgurin, melittin, PR-39, PR-26, 9-phenylnonylamine, (KLAKKLA)_n, (KLAKLAK)_n, where n = 1, 2, or 3, (KALKALK)₃, KLGKKLG)_n, and KAAKKAA)_n, wherein N = 1, 2, or 3, paradaxin, Bac 5,
30 Bac 7, ceratoxin, mdelin 1 and 5, bombin-like peptides, PGQ, cathelicidin, HD-5, Oabac5alpha, ChBac5, SMAP-29, Bac7.5, lactoferrin, granulysin, thionin, hevein and

knottin-like peptides, MPG1, 1bAMP, snakin, lipid transfer proteins, and plant defensins. Exemplary sequences for the above compounds are provided in Table 1. In some embodiments, the antimicrobial peptides are synthesized from L-amino acids, while in other embodiments, the peptides are synthesized from or comprise D-amino acids.

5

Table 1 Antimicrobial Peptides			
SEQ ID NO:	Name	Organism	Sequence
1	lingual antimicrobial peptide precursor (Magainin)	<i>Bos taurus</i>	MRLHHLLLALLFLVLSAGSGFTQGVR NSQSCRRNKGICVP IRCPGSMRQIGTCLGAQVKCCRRK
2	antimicrobial peptide PGQ	<i>Xenopus laevis</i>	GVLSNVIGYLKKLGTGALNAVLKQ
3	Xenopsin	<i>Xenopus laevis</i>	MYKGIFLCVLLAVICANSLATPSSDA DEDNDEVERYVRGW ASKIGQTLGKIAKVGLKELIQPKREA MLRSAEAQGKRPWIL

Table 1 Antimicrobial Peptides			
SEQ ID NO:	Name	Organism	Sequence
4	magainin precursor	<i>Xenopus laevis</i>	MFKGLFICSLIAVICANALPQPEASAD EDMDEREVRGIGKFLHSAGKFGKAF VGEIMKSKRDAEAVGPEAFADDEDLD EREVRGIGKFLHSAGKFGKAFVGEIM NSKRDAEAVGPEAFADDEDLDEREVR GIGKFLHSAGKFGKAFVGEIMNSKRD AEAVGPEAFADDEDLDEREVRGIGKFL HSAKKFGKAFVGEIMNSKRDAEAVG PEAFADEDLDEREVRGIGKFLHSAGK FGKAFVGEIMNSKRDAEAVGPEAFA DEDLDEREVRGIGKFLHSAGKFGK AFVGEIMNSKRDAEAVDDRRWVE
5	tachyplesin I	<i>Tachypleus gigas</i>	KWCFRVCYRGICYRRCR
6	tachyplesin II	<i>Tachypleus gigas</i>	RWCFRVCYRGICYRKCR
7	buforin I	<i>Bufo bufo gagarizans</i>	MSGRGKQGGKVRKAKTRSSRAGL QFPVGRVHRLLRKGNYAQRVGAGA PVYLAADVLEYLTAEILELAGNAARD NKKTRIIPRHLQLAVRNDEELNKLKG GVTIAQGGVLPNIQAVLLPKT ESSKPAKSK
8	buforin II	<i>Bufo bufo gagarizans</i>	TRSSRAGLQFPVGRVHRLLRK

Table 1 Antimicrobial Peptides			
SEQ ID NO:	Name	Organism	Sequence
9	cecropin A	<i>Bombyx mori</i>	MNFVRILSFVFALVLALGAVSAAPEP RWKLFKKIEKVGRNVRDGLIKAGPAI AVIGQAKSLGK
10	cecropin B	<i>Bombyx mori</i>	MNFAKILSFVFALVLALSMTSAAPEP RWKIFKKIEKMGRN IRDGIVKAGPAIEVLGSAKAIGK
11	cecropin C	<i>Drosophila melanogaster</i>	MNFYKIFVFVALILAISIGQSEAGWLK KLGKRIERIGQHT RDATIQGLGIAQQAANVAATARG
12	cecropin P1	<i>Sus scrofa</i>	SWLSKTAKKLENSAKKRISSEGIAIAIQ GGPR
13	indolicidin	<i>Bos taurus</i>	ILPWKWPWWPWRR
14	nisin	<i>Lactococcus lactis</i>	ITSISLCTPGCKTGALMGCNMKTATC HCSIHVSK
15	ranalexin	<i>Rana catesbeiana</i>	FLGGLIKIVPAMICAVTKKC
16	lactoferricin B	<i>Bos taurus</i>	FKCRRWQWRMKKLGAPSITCVRRAF
17	protegrin-1	<i>Sus scrofa</i>	RGGRLCYCRRRFCVCVGRX
18	protegrin-2	<i>Sus scrofa</i>	GGRLCYCRRRFCICVG
19	histatin precursor	<i>Homo sapiens</i>	MKFFVFALILALMLSMTGADSHAKR HHGYKRKFHEKHHSRGGYRSNYLY DN

Table 1 Antimicrobial Peptides			
SEQ ID NO:	Name	Organism	Sequence
20	histatin 1	<i>Macaca fascicularis</i>	DSHEERHHGRHGHKKYGRKFHEKH HSHRGYRSNYLYDN
21	dermaseptin	<i>Phyllomedusa sauvagei</i>	ALWKTMLKKLGTMALHAGKAALGA AADTISQTQ
22	dermaseptin 2	<i>Phyllomedusa sauvagei</i>	ALWFTMLKKLGTMALHAGKAALGA AANTISQGTQ
23	dermaseptin 3	<i>Phyllomedusa sauvagei</i>	ALWKNMLKGIGKLAGKAALGAVKK LVGAES
24	misgurin	<i>Misgurnus anguillicaudatus</i>	RQRVEELSKFSKKGAAARRRK
25	melittin	<i>Apis mellifera</i>	GIGAVLKVLTTGLPALISWISRKKRQ Q
26	pardaxin-1	<i>Pardachirus pavoninus</i>	GFFALIPKIISPLFKTLLSAVGSALSSS GEQE
27	pardaxin-2	<i>Pardachirus pavoninus</i>	GFFALIPKIISPIFKTLLSAVGSALSSS GGQE

Table 1 Antimicrobial Peptides			
SEQ ID NO:	Name	Organism	Sequence
28	bactenecin 5 precursor	<i>Bos taurus</i>	METQRASLSLGRCSLWLLLGLVLPS ASAQALSYREAVLR AVDQFNERSSSEANLYRLLELDPTPND DLDPGTRKPVSVFRV KETDCPRTSQQPLEQCDFKENGLVK QCVGTVTLDPSPNDQFDINCNELQSVR FRPPIRRPPIRPPFYPPFRPPIRPPIFPPIR PPFRPPLGPFPGRR
29	bactenecin precursor	<i>Bos taurus</i>	METPRASLSLGRWSLWLLLGLALPS ASAQALSYREAVLR AVDQLNEQSSEPNTYRLLELDQPPQD DEDPDSPKRVSFRVKETVCSRTTQQP PEQCDFKENGLLKRCEGTVTLQVR GNFDITCNNHQSIRITKQPWAPPQAA RLCRIVVIRVCR
30	ceratotoxin A	<i>Ceratitis capitata</i>	SIGSALKKALPVAKKIGKIALPIAKAA LP
31	ceratotoxin B	<i>Ceratitis capitata</i>	SIGSAFKKALPVAKKIGKAALPIAKA ALP

Table 1 Antimicrobial Peptides			
SEQ ID NO:	Name	Organism	Sequence
32	cathelicidin antimicrobial peptide	<i>Homo sapiens</i>	MKTQRNGHSLGRWSLVLLLLGLVMP LAIIAQVLSYKEAVL RAIDGINQRSSDANLYRLLDLDPRPT MDGDPDTPKPVSFT VKETVCPRTTQQSPEDCDFKKGDLV KRCMGTVTLNQARGSFDISCDKDNK RFALLGDFFRKSKEKIGKEFKRIVQRI KDFLRNLVPRTES
33	myeloid cathelicidin 3	<i>Equus caballus</i>	METQRNTRCLGRWSPLLLLLGLVIP ATTQALSYKEAVLRAVDGLNQRSSD ENLYRLLELDPLPKGDKDSDTPKPVS FMVKETVCPRIMKQTPEQCDFKENG LVKQCVGTVILDPVKDYFDASCDEP QRVKRFHSVGSLIQRHQQMIRDKSEA TRHGIRIITRPKLLLAS
34	myeloid antimicrobial peptide BMAP-28	<i>Bos taurus</i>	METQRASLSLGRWSLWLLLLGLALP SASAQALSYREAVLR AVDQLNEKSSEANLYRLLELDPPPKE DDENPNIPKPVSFVRVKETVCPRTSQQ SPEQCDFKENGLLKECVGTVTLDQV GSNFDITCAVPQSVGGLRSLGRKILR AWKKYGPIIVPIIRIG

Table 1 Antimicrobial Peptides			
SEQ ID NO:	Name	Organism	Sequence
35	myeloid cathelicidin 1	<i>Equus caballus</i>	METQRNTRCLGRWSPLLLLGLVIPP ATTQALSYKEAVLR AVDGLNQRSSDENLYRLLELDPLPKG DKDSDTPKPVSFMVKETVCPRIMKQ TPEQCDFKENGLVKQCVGTVILGPV KDHFDVSCGEPQQRVKRFGRLAKSFL RMRILLPRRKILLAS
36	SMAP 29	<i>Ovis aries</i>	METQRASLSLGRCSLWLLLLGLALPS ASAQVLSYREAVLRAADQLNEKSSE ANLYRLLELDPPPKQDDENSNIKPV SFRVKETVCPRTSQQPAEQCDFKENG LLKECVGTVTLDQVRNNFDITCAEPQ SVRGLRRLGRKIAHGVKKYGPTVLRI IRIAG
37	BNP-1	<i>Bos taurus</i>	RLCRIVVIRVCR
38	HNP-1	<i>Homo sapiens</i>	ACYCRIPACIAGERRYGTCTYQGRLW AFCC
39	HNP-2	<i>Homo sapiens</i>	CYCRIPACIAGERRYGTCTYQGRLWA FCC
40	HNP-3	<i>Homo sapiens</i>	DCYCRIPACIAGERRYGTCTYQGRLW AFCC
41	HNP-4	<i>Homo sapiens</i>	VCSCRLVFCRRTELRVGNCLIGGVSF TYCCTRV

Table 1 Antimicrobial Peptides			
SEQ ID NO:	Name	Organism	Sequence
42	NP-1	<i>Oryctolagus cuniculus</i>	VVCACRRALCLPRERRAGFCRIRGRI HPLCCRR
43	NP-2	<i>Oryctolagus cuniculus</i>	VVCACRRALCLPLERRAGFCRIRGRI HPLCCRR
44	NP-3A	<i>Oryctolagus cuniculus</i>	GICACRRRRCFPCNSERFSGYCRVNGAR YVRCCSRR
45	NP-3B	<i>Oryctolagus cuniculus</i>	GRCVCRKQLLCSYRERRIGDCKIRGV RFPFCCPR
46	NP-4	<i>Oryctolagus cuniculus</i>	VSCTCRRFSCGFGERASGSCTVNGG VRHTLCCRR
47	NP-5	<i>Oryctolagus cuniculus</i>	VFCTCRGFLCGSGERASGSCTINGVR HTLCCRR
48	RatNP-1	<i>Rattus norvegicus</i>	VTCYCRRTRCGFRERLSGACGYRGRI YRLCCR
49	Rat-NP-3	<i>Rattus norvegicus</i>	CSCRYSSCRFGERLLSGACRLNGRIY RLCC
50	Rat-NP-4	<i>Rattus norvegicus</i>	ACTCRIGACVSGERLTGACGLNGRIY RLCCR
51	GPNP	Guinea pig	RRCICTTRTCRFPYRRLGTCIFQNRVY TFCC

Table 1 Antimicrobial Peptides			
SEQ ID NO:	Name	Organism	Sequence
52	beta defensin-3	<i>Homo sapiens</i>	MRIHYLLFALLFLFLVPVPGHGGIINT LQKYYCRVRGGRC AVLSCLPKEEQIGKCSTRGRKCCRRK K
53	theta defensin-1	<i>Macaca mulatta</i>	RCICTRGFCRCLCRRGVC
54	defensin CUA1	<i>Helianthus annuus</i>	MKSSMKMFAALLVVMCLLANEMG GPLVVEARTCESQSHKFKGTCLSDTN CANVCHSERFSGGKCRGFRRRCFCT THC
55	defensin SD2	<i>Helianthus annuus</i>	MKSSMKMFAALLVVMCLLANEMG GPLVVEARTCESQSHKFKGTCLSDTN CANVCHSERFSGGKCRGFRRRCFCT THC
56	neutrophil defensin 2	<i>Macaca mulatta</i>	ACYCRIPACLAGERRYGTCTFYMGRV WAFCC
57	4 KDA defensin	<i>Androctonus australis hector</i>	GFGCPFNQGACHRHCRSIRRRGGYC AGLFKQTCTCYR
58	<i>defensin</i>	<i>Mytilus galloprovincialis</i>	GFGCPNNYQCHRHCKSIPGRCGGYC GGXHRLRCTCYRC

Table 1 Antimicrobial Peptides			
SEQ ID NO:	Name	Organism	Sequence
59	defensin AMP1	<i>Heuchera sanguinea</i>	DGVKLCDVPSGTWSGHCGSSSKCSQ QCKDREHFAYGGACH YQFPSVKCFCKRQC
60	defensin AMP1	<i>Clitoria ternatea</i>	NLCERASLTWTGNCGNTGHCDTQCR NWESAKHGACHKRGH WKCFCYFNC
61	cysteine-rich cryptdin-1 homolog	<i>Mus musculus</i>	MKKLVLLFALVLLAFQVQADSIQNT DEETKTEEQPGEKDQAVSVSFGDPQ GSALQDAALGWGRRCPQCPRCPSCP SCPRC PRCPRCKCNPK
62	beta-defensin-9	<i>Bos taurus</i>	QGVNRFVTCRINRGFCVPIRCPGHRR QIGTCLGPQIKCCR
63	beta-defensin-7	<i>Bos taurus</i>	QGVNRFVTCRINRGFCVPIRCPGHRR QIGTCLGPRIKCCR
64	beta-defensin-6	<i>Bos taurus</i>	QGVNHNVTCTRIYGGFCVPIRCPGRTR QIGTCFGRPVKCCRRW
65	beta-defensin-5	<i>Bos taurus</i>	QVVRNPQSCRWNMGVCIPISCPGNM RQIGTCFGPRVPCCR
66	beta-defensin-4	<i>Bos taurus</i>	QVVRNPQSCRWNMGVCIPFLCRVG MRQIGTCFGPRVPCCR
67	beta-defensin-3	<i>Bos taurus</i>	QGVNHNVTCTRINRGFCVPIRCPGRTR QIGTCFGPRIKCCRSW

Table 1 Antimicrobial Peptides			
SEQ ID NO:	Name	Organism	Sequence
68	beta-defensin-10	<i>Bos taurus</i>	QGVRSYLSWGNRGICLLNRCPPGRM RQIGTCLAPRVKCCR
69	beta-defensin-13	<i>Bos taurus</i>	SGISGPLSCGRNGGVCIPRCPVPMRQ IGTCFGRPVKCCRSW
70	beta-defensin-1	<i>Bos taurus</i>	DFASCHTNGGICLPNRCPPGHMIQIGIC FRPRVKCCRSW
71	coleopteracin	<i>Zophobas atratus</i>	SLQGGAPNFPQPSQQNGGWQVSPDL GRDDKGNTRGQIEIQNKGKDHDENA GWGKVIRGPNKAKPTWHVGGTYRR
72	beta defensin-3	<i>Homo sapiens</i>	MRIHYLLFALLFLFLVPVPGHGGIINT LQKYYCRVRGGRC AVL SCLPKEEQI GKCSTRGRKCCRRKK
73	defensin C	<i>Aedes aegypti</i>	ATCDLLSGFGVGDSACAAHCIARGN RGGYCNSKKVCVCRN
74	defensin B	<i>Mytilus edulis</i>	GFGCPNDYPCHRHCKSIPGRYGGYC GGXHRLRCTC
75	sapecin C	<i>Sarcophaga peregrina</i>	ATCDLLSGIGVQHSACALHCVFRGN RGGYCTGKGICVCRN
76	macrophage antibiotic peptide MCP-1	<i>Oryctolagus cuniculus</i>	MRTLALLAAILLVALQAQAEHVSVSI DEVVDQQPPQAEDQDVAIYVKEHES SALEALGVKAGVVCACRRALCLPRE RRAG FCRIRGRIHPLCCRR

Table 1 Antimicrobial Peptides			
SEQ ID NO:	Name	Organism	Sequence
77	cryptdin-2	<i>Mus musculus</i>	MKPLVLLSALVLLSFQVQADPIQNTD EETKTEEQSGEEDQAVSVSFGDREG ASLQEEsLRDLVCYCRTRGCKRRER MNGT CRKGHLMYTLCC
78	cryptdin-5	<i>Mus musculus</i>	MKTFVLLSALVLLAFQVQADPIHKTD EETNTEEQPG EEDQ AVSISFGGQEGSALHEELSKKLICYCR IRGCKRRERVFGT CRNLFLTFVFCCS
79	cryptdin 12	<i>Mus musculus</i>	LRDLVCYCRARGCKGRERMNGTCR KGHLLYMLCCR
80	defensin	<i>Pyrrhocoris apterus</i>	ATCDILSFQSQWVTPNHAGCALHCVI KGYKGGQCKITVCHCRR
81	defensin R-5	<i>Rattus norvegicus</i>	VT CYCRSTRCGFRERLSGACGYRGRI YRLCCR
82	defensin R-2	<i>Rattus norvegicus</i>	VTCS CRTSSCRFGERLSGACRLNGRI YRLCC
83	defensin NP-6	<i>Oryctolagus cuniculus</i>	GICACRRRFCLNFEQFSGYCRVNGAR YVRCCSRR
84	beta-defensin-2	<i>Pan troglodytes</i>	MRVLYLLFSFLFIFLMPLPGVFGGISD PVTCLKSGAICHP VFCPRRYKQIGTCGLPGTKCCKKP

Table 1 Antimicrobial Peptides			
SEQ ID NO:	Name	Organism	Sequence
85	beta-defensin-2	<i>Homo sapiens</i>	MRVLYLLFSFLFIFLMPLPGVFGGIGD PVTCLKSGAICHP VFCPRRYKQIGTCGLPGTKCCKKP
86	beta-defensin-1	<i>Homo sapiens</i>	MRTSYLLLFTLCLLSEMASGGNFLT GLGHRSDHYNCVSS GGQCLYSACPIFTKIQGTCYRGKAKC CK
87	beta-defensin-1	<i>Capra hircus</i>	MRLHHLLLVLFFLVLSAGSGFTQGIR SRRSCHRNGKVCAL TRCPRNMRQIGTCFGPPVKCCRKK
88	beta defensin-2	<i>Capra hircus</i>	MRLHHLLLALFFLVLSAGSGFTQGIIN HRSCYRNKGVCAP ARCPRNMRQIGTCHGPPVKCCRKK
89	defensin-3	<i>Macaca mulatta</i>	MRTLVLAAILLVALQAQAEPLQART DEATAAQEQIPTDNPEVVVSLAWDE SLAPKDSVPGLRKNMACYCRIPACL AGER RYGTCFYRRRVWAFCC
90	defensin-1	<i>Macaca mulatta</i>	MRTLVLAAILLVALQAQAEPLQART DEATAAQEQIPTDNPEVVVSLAWDE SLAPKDSVPGLRKNMACYCRIPACL AGER RYGTCFYLG RVWAFCC
91	neutrophil defensin 1	<i>Mesocricetus auratus</i>	VTFCFRRRGCASRERHIGYCRFGNTI YRLCCRR

Table 1 Antimicrobial Peptides			
SEQ ID NO:	Name	Organism	Sequence
92	neutrophil defensin 1	<i>Mesocricetus auratus</i>	CFCKRPVCDSGETQIGYCRLGNTFYR LCCRQ
93	Gallinacin 1-alpha	<i>Gallus gallus</i>	GRKSDCFRKNNGFCAFLKCPYLTLSG KCSRFHLCKRIW
94	defensin	<i>Allomyrina dichotoma</i>	VTCDLLSFEAKGFAANHSLCAAHCL AIGRRGGSCERGVICRR
95	neutrophil cationic peptide 1	<i>Cavia porcellus</i>	RRCICTTRTCRFPYRRLGTCIFQNRVY TFCC

In some embodiments of the present invention, the antimicrobial polypeptide is a defensin. In preferred embodiments, the compositions of the present invention comprise one or more defensins. In some of these embodiments, the antimicrobial polypeptide defensin is BNP1 (also known as bactanecin and bovine dodecapeptide). In certain

5 defensin is BNP1 (also known as bactanecin and bovine dodecapeptide). In certain embodiments, the defensin comprises the following consensus sequence: (SEQ ID NO:96 - $X_1CN_1CRN_2CN_3ERN_4CN_5GN_6CCX_2$, wherein N and X represent conservatively or nonconservatively substituted amino acids and $N_1 = 1$, $N_2 = 3$ or 4, $N_3 = 3$ or 4, $N_4 = 1, 2$, or 3, $N_6 = 5-9$, X_1 and X_2 may be present, absent, or equal from 1-2. The present invention is

10 not limited to any particular defensin. Representative defensins are provided in Tables 1 and 2.

Table 2 Defensins			
SEQ ID NO	Name	Organism	Sequence
38	HNP-1	Human	AC <u>Y</u> CRIPAC <u>I</u> AG <u>E</u> RRYGT <u>C</u> IYQGRLWA FCC
39	HNP-2	Human	<u>C</u> YCRIPAC <u>I</u> AG <u>E</u> RRYGT <u>C</u> IYQGRLWAF CC
40	HNP-3	Human	DC <u>Y</u> CRIPAC <u>I</u> AG <u>E</u> RRYGT <u>C</u> IYQGRLWA FCC
41	HNP-4	Human	VC <u>S</u> CRLVFCRRTEL <u>R</u> VGNCLIGGVSTY CCTRV
42	NP-1	Rabbit	VV <u>C</u> ACRRALCLPR <u>E</u> RRAG <u>F</u> CRIR <u>G</u> RIHP LCCRR
43	NP-2	Rabbit	VV <u>C</u> ACRRALCLPL <u>E</u> RRAG <u>F</u> CRIR <u>G</u> RIHP LCCRR
44	NP-3A	Rabbit	GI <u>C</u> ACRRRF <u>C</u> PNSE <u>R</u> FS <u>G</u> YCRVNG <u>A</u> RY VRCCSRR
45	NP-3B	Rabbit	GR <u>C</u> VCRKQLLC <u>S</u> YR <u>E</u> RRIG <u>D</u> CKIR <u>G</u> VR FPFCCPR
46	NP-4	Rabbit	VS <u>C</u> T <u>C</u> RRFSC <u>G</u> FG <u>E</u> RAS <u>G</u> SCTVNGVRH TLCCRR
47	NP-5	Rabbit	VF <u>C</u> T <u>C</u> RGFLC <u>G</u> SG <u>E</u> RAS <u>G</u> SCTINGVRHT LCCRR
48	RatNP-1	Rat	VT <u>C</u> Y <u>C</u> RRTRC <u>G</u> FR <u>E</u> RLSG <u>A</u> CGYR <u>G</u> RIY RLCCR

Table 2 Defensins			
SEQ ID NO	Name	Organism	Sequence
49	Rat-NP-3	Rat	<u>C</u> <u>S</u> <u>C</u> <u>R</u> <u>Y</u> <u>S</u> <u>S</u> <u>C</u> <u>R</u> <u>F</u> <u>G</u> <u>E</u> <u>R</u> <u>L</u> <u>L</u> <u>S</u> <u>G</u> <u>A</u> <u>C</u> <u>R</u> <u>L</u> <u>N</u> <u>G</u> <u>R</u> <u>I</u> <u>Y</u> <u>R</u> <u>L</u> <u>C</u> <u>C</u>
50	Rat-NP-4	Rat	<u>A</u> <u>C</u> <u>T</u> <u>C</u> <u>R</u> <u>I</u> <u>G</u> <u>A</u> <u>C</u> <u>V</u> <u>S</u> <u>G</u> <u>E</u> <u>R</u> <u>L</u> <u>T</u> <u>G</u> <u>A</u> <u>C</u> <u>G</u> <u>L</u> <u>N</u> <u>G</u> <u>R</u> <u>I</u> <u>Y</u> <u>R</u> <u>L</u> <u>C</u> <u>C</u> <u>R</u>
51	GPNP	Guinea pig	<u>R</u> <u>R</u> <u>C</u> <u>I</u> <u>C</u> <u>T</u> <u>T</u> <u>R</u> <u>T</u> <u>C</u> <u>R</u> <u>F</u> <u>P</u> <u>Y</u> <u>R</u> <u>R</u> <u>L</u> <u>G</u> <u>T</u> <u>C</u> <u>I</u> <u>F</u> <u>Q</u> <u>N</u> <u>R</u> <u>V</u> <u>Y</u> <u>T</u> <u>F</u> <u>C</u> <u>C</u>

In general, defensins are a family of highly cross-linked, structurally homologous antimicrobial peptides found in the azurophil granules of polymorphonuclear leukocytes (PMN's) with homologous peptides being present in macrophages. (*See e.g.*, Selsted *et al.*, Infect. Immun., 45:150-154 [1984]). Originally described as "Lysosomal Cationic Peptides" in rabbit and guinea pig PMN (Zeya *et al.*, Science, 154:1049-1051 [1966]; Zeya *et al.*, J. Exp. Med., 127:927-941 [1968]; Zeya *et al.*, Lab. Invest., 24:229-236 [1971]; Selsted *et al.*, [1984], *supra.*), this mixture was found to account for most of the microbicidal activity of the crude rabbit PMN extract against various microorganisms (Zeya *et al.*, [1966], *supra.*; Lehrer *et al.*, J. Infect. Dis., 136:96-99 [1977]; Lehrer *et al.*, Infect. Immun., 11:1226-1234 [1975]). Six rabbit neutrophil defensins have been individually purified and are designated NP-1, NP-2, NP-3A, NP-3B, NP-4, and NP-5. Their amino acid sequences were determined, and their broad spectra of activity were demonstrated against a number of bacteria (Selsted *et al.*, Infect. Immun., 45:150-154 [1984]), viruses (Lehrer *et al.*, J. Virol. 54:467 [1985]), and fungi (Selsted *et al.*, Infect. Immun., 49:202-206 [1985]; Segal *et al.*, 151:890-894 [1985]). Defensins have also been shown to possess mitogenic activity (*e.g.*, Murphy *et al.*, J. Cell. Physiol., 155:408-13 [1993]).

Four peptides of the defensin family have been isolated from human PMN's and are designated HNP-1, HNP-2, HNP-3, and HNP-4 (Ganz *et al.*, J. Clin. Invest., 76:1427-1435 [1985]; Wilde *et al.*, J. Biol. Chem., 264:11200-11203 [1989]). The amino acid sequences

of HNP-1, HNP-2, and HNP-3 differ from each other only in their amino terminal residues, while each of the human defensins are identical to the six rabbit peptides in 10 or 11 of their 29 to 30 residues. These are the same 10 or 11 residues that are shared by all six rabbit peptides. Human defensin peptides have been shown to share with the rabbit defensins a
 5 broad spectrum of antimicrobial activity against bacteria, fungi, and enveloped viruses (Ganz *et al.*, [1985], *supra*).

Three defensins designated RatNP-1, RatNP-2, and RatNP-4, have been isolated from rat. (Eisenhauer *et al.*, Infection and Immunity, 57:2021-2027 [1989]). A guinea pig defensin (GPNP) has also been isolated, purified, sequenced and its broad spectrum
 10 antimicrobial properties verified (Selsted *et al.*, Infect. Immun., 55:2281-2286 [1987]). Eight of its 31 residues were among those invariant in six rabbit and three human defensin peptides. The sequence of GPNP also included three nonconservative substitutions in positions otherwise invariant in the human and rabbit peptides. Of the defensins tested in a quantitative assay HNP-1, RatNP-1, and rabbit NP-1 possess the most potent antimicrobial
 15 properties, while NP-5 possesses the least amount of antimicrobial activity when tested against a panel of organisms in stationary growth phase. (Selsted *et al.*, Infect. Immun., 45:150-154 [1984]; Ganz *et al.*, J. Clin. Invest. 76:1427-1435 [1985]). Defensin peptides are further described in US 4,543,252; 4,659,692; and 4,705,777.

20 Accordingly, in some embodiments, the compositions of the present invention comprise one or more defensins selected from the group consisting of SEQ ID NOs: 37-95.

In preferred embodiments, suitable antimicrobial peptides comprise all or part of the amino acid sequence of a known peptide, more preferably incorporating at least some of the conserved regions identified in Table 2. In particularly preferred embodiments, the
 25 antimicrobial peptides incorporate at least one of the conserved regions, more usually incorporating two of the conserved regions, preferably conserving at least three of the conserved regions, and more preferably conserving four or more of the conserved regions. In preferred embodiments, the antimicrobial peptides comprise fifty amino acids or fewer, although there may be advantages in increasing the size of the peptide above that of the
 30 natural peptides in certain instances. In certain embodiments, the peptides have a length in the range from about 10 to 50 amino acids, preferably being in the range from about 10 to

40 amino acids, and most preferably being in the range from about 30 to 35 amino acids which corresponds generally to the length of the natural defensin peptides.

In some embodiments, the present invention provides antibodies (or portions thereof) fused to biocidal molecules (*e.g.*, lysozyme) (or portions thereof) suitable for use with processed food products as a whey based coating applied to food packaging and/or as a food additive. In still other embodiments, the compositions of the present invention are formulated for use as disinfectants for use in food processing facilities. Additional embodiments of the present invention provide human and animal therapeutics.

10 V. Applications

The methods and compositions of the present invention find use in a variety of applications, including, but not limited to, those described below.

A. Exemplary Target Pathogens

15 i) *Escherichia coli* O157:H7

Preferred embodiments of the present invention provide effective therapeutic treatments and prophylactic methods for combating the foodborne pathogen *E. coli* O157:H7. *E. coli* O157:H7 is a common component of the normal flora of the bovine gastrointestinal tract. Surveys of clinically normal cattle detect *E. coli* O157:H7 shedding in the feces of about 1-25% of animals. (D.D. Hancock *et al.*, *Epidemiol. Infect.*, 113(2):199-207 [1994]; MMWR Morb. Mortal. Wkly. Rep., 48(36):803-805 [1999]; and National Dairy Heifer Evaluation Project, USDA APHIS NAHMS [1994]). Human infection by *E. coli* O157:H7 most often occurs through the fecal-oral route, either directly through handling of infected cattle, or more commonly as a result consuming contaminated meat products. Small-scale outbreaks of *E. coli* O157:H7 disease have been associated with petting zoos and agricultural fairs. (See *e.g.*, G.C. Pritchard *et al.*, *Vet. Rec.*, 147:259-264 [2000]). Larger outbreaks of *E. coli* O157:H7 disease have been traced to the widespread dissemination and consumption of contaminated meat products such as ground beef. (J. Tuttle *et al.*, *Epidemiol. Infect.*, 122:185-192 [1999]).

Contamination of meat products with fecal matter harboring *E. coli* O157:H7 appears to occur primarily during slaughterhouse dehiding, evisceration, splitting, chilling,

and fabrication operations. Further dissemination of *E. coli* O157:H7 to otherwise uncontaminated meat products also occurs during grinding, processing, and transportation of meat products. Because of the severity of *E. coli* O157:H7 disease and the potential for the contamination of large quantities of otherwise wholesome meat by a relatively small amount
5 of contaminated meat, the recalls issued for potentially contaminated meat products are often very large.

Recent *E. coli* O157:H7 contaminated meat recalls, include the recall of 24 million pounds of ground beef by the Hudson Beef in 1997, and most recently the recall of 19 million pounds of ground beef by the ConAgra Beef Company in July 2002. Recalls of this
10 magnitude are obviously very costly; not only for actual value of the beef being destroyed, but also the logistical effort required to collect and dispose of the contaminated beef. More importantly, immeasurable costs arise from decreased consumer confidence in the meat packing industry and in the wholesomeness of food supply generally.

E. coli O157:H7 is particularly pathogenic because it produces a multi-unit
15 verotoxin (or a shiga-like toxin) protein that binds receptors in the kidney and gastrointestinal tract of man. This toxin produces a hemolytic uremic syndrome in children and the elderly, and a hemorrhagic colitis in adults. The Center for Disease Control reported over 70,000 cases of *E. coli* O157:H7 disease each year and 60 deaths annually. The symptoms of hemorrhagic colitis last an average of 8 days. This implies that over half a
20 million work days are lost per year due to *E. coli* O157:H7 infection.

E. coli O157:H7 disease is a costly and frustrating zoonosis, in part because its epidemiology is well understood yet very difficult to prevent. The recent massive dissemination of the organism in contaminated meat products is a function of the industrialization processes that are essential to providing affordable food.

25 Preferred embodiments of the present invention combat *E. coli* O157:H7, by providing chimeric murine-bovine monoclonal antibodies to provide passive immunity in host animals (*e.g.*, bovines), to induce specific immunity in host animals due to the bovine portion of the antibody, to topically control *E. coli* O157:H7 post harvest. Additional preferred embodiments provide chimeric murine-bovine monoclonal antibody fusion
30 proteins that directly reduce *E. coli* O157:H7 in host animals by providing highly controlled

and targeted bactericidal microenvironments that destroy the pathogens without affecting normal microbiological flora.

ii) *Listeria monocytogenes*

5 Ingestion of the bacterium *Listeria monocytogenes* probably occurs quite often, as the bacteria has been isolated in food products worldwide. (B. Lorber, Clin. Infect. Dis., 24:1-11 [1997]; and J.M. Farber and P.I. Peterkin, Microbiol. Rev., 55:476-511 [1991]). Development of Listeriosis, the invasive disease caused by *L. monocytogenes* ingestion, is determined primarily by the integrity of the host's immune system (predominantly cell-
10 mediated immune defects) and possibly also by inoculum size. (See e.g., P. Aureli *et al.*, New Engl. J. Med., 342:1236-1241 [2000]). *L. monocytogenes* crosses the mucosal barrier of the intestines and invades the bloodstream. The bacterium may disseminate to any organ, but has a clear predilection for the placenta and central nervous system (CNS), thus determining the main clinical syndromes caused by infection. Listeriosis is life-threatening
15 zoonosis, especially in human fetuses and neonates, the elderly, and patients with certain predisposing conditions. Many cases of Listeriosis probably go unreported especially in perinates and newborns.

L. monocytogenes is a difficult bacterium to control because it thrives in vacuum packed food products and at temperatures typically used in food refrigeration. (S. Liu *et al.*,
20 Appl. Environ. Microbiol., 68(4):1697-1705 [2002]). Thus, *L. monocytogenes* is a particular problem in ready to eat foods that consumers expect are safe to eat with no further cooking. Typical food products contaminated with *L. monocytogenes* include unpasteurized or low acid dairy products and ready-to-eat (RTE) meat products such as luncheon meat and pates.

25 Many of the larger listeriosis outbreaks have been associated with fresh dairy products, especially Mexican soft cheeses and other non-aged or fermented cheese products. (M.J. Linnan *et al.*, New Eng. J. Med., 319:823-828 [1988]). In specialty cheese production, *L. monocytogenes* has been found to accumulate in ripening rooms. (S.I. Pak *et al.*, *supra*). Outbreaks have also been linked to processed and deli meat products, including
30 turkey hot dogs, pate, and jellied tongue. (See e.g., C. Jacquet *et al.*, Appl. Env. Microbiol., 61:2242-2246 [1995]; J. McLaughlin *et al.*, Brit Med. J., 303:773-775 [1991]; and

Morbidity and Mortality Weekly Reports, 47:1085-1086 [1998]). *L. monocytogenes* is however, easily destroyed by cooking. As *Listeria* is heat labile, food products that cooked prior to eating and served hot have a lower risk profile.

The bacterium often contaminates food processing equipment, where it is typically found as a biofilm on steel and glass parts. *L. monocytogenes* tends to form biofilms on containers used to store food products. (A.C. Wong, J. Dairy Sci., 81(10):2765-2770 [1998]). It is also a common environmental contaminant of food storage facilities. (See e.g., M.S. Chae and H. Schraft, Int. J. Food. Microbiol., 62:103-111 [2000]). Thus, a pathogen originally considered an "environmental" organism on farms has invaded industrial food preparation facilities. Strains of *L. monocytogenes* have emerged with progressive resistance to antimicrobial agents. (C. Arizcun *et al.*, J. Food Prot., 61:731-734 [1988]). Contamination of food processing equipment and storage often seed small amounts of bacteria onto food products which multiply during refrigeration. The length of time food is kept refrigerated, both at the retail outlet and in home, and the actual storage temperatures influence the risk that even low levels of initial *L. monocytogenes* contamination will grow to become problematic. Outbreaks of listeriosis have been associated with breakdowns in the environmental controls at food processing facilities (e.g., during plant renovation). Given the capacity of *L. monocytogenes* to grow under refrigeration, even very low levels of *L. monocytogenes* contamination in food products as they leave the processor can ultimately result in inoculums large enough to cause lethal infections in the consumer. Consequently, the present invention contemplates controlling foodborne listeriosis requires focusing on post-processing food handling and storage.

L. monocytogenes is recognized as an apparent and inapparent infection of livestock and is widespread in agricultural environments. (See e.g., L. Hassan *et al.*, J. Dairy Sci., 83(11):2441-2447 [2000]). *L. monocytogenes* is widespread in the environment and shed in the feces and milk of inapparently infected cattle. (L. Hassan *et al.*, *supra*). Even low levels of *L. monocytogenes* contamination are enough to support the continued growth of the organism and are the primary source of human listeriosis.

Listeriosis is a serious disease. In milder its forms, listeriosis is a febrile illness with gastrointestinal signs but often progresses to bacteremia and meningitis with nervous system clinical manifestations including headache, loss of balance, and convulsions. Incubation

periods can be several weeks making epidemiologic investigation more difficult. Listeriosis is particularly serious for pregnant women, infants, and individuals with compromised immune systems. (See e.g., A. Schuchat *et al.*, Clinical Microbiol. Rev., 4:169-183 [1991]). Individuals with Acquired Immune Deficiency Syndrome (AIDS) are almost 300 times more likely to contract listeriosis than people with normally functioning immune systems. (J.G. Morris and M. Potter, Emerging Infectious Diseases, 3:435-441 [1997]). Diabetics and those affected by cancer and kidney disease are also at greater risk of contracting Listeriosis and are more prone to serious nervous system manifestations of the disease. Listeriosis in pregnant women can result in premature birth, still births, or birth of a critically infected child. Perinates and newborns are particularly at risk as the relative immaturity of their immune systems has been shown to contribute to the severity of disease (See e.g., L. Slutsker and A. Schuchat, Listeriosis in humans. In: Listeria, Listeriosis and Food Safety, E.T. Ryser and E.M. Marth eds. Marcel Dekker Inc., New York, New York pp. 75-95 [1999]). Human foodborne listeriosis tends to result in either sporadic cases or epidemics depending on whether a common food source infects a group of people (H.R. Ibrahim *et al.*, FEBS Lett., 506(1):27-32 [2001]). Contaminated meats, seafood, vegetables, fruits, and dairy products have all been the source of sporadic cases of listeriosis (S.I. Pak *et al.*, Prev. Vet. Med., 53(1-2):55-65 [2002]).

iii) *Cryptosporidium parvum*

Cryptosporidium parvum is a zoonotic apicomplexan parasite recognized as the cause of large outbreaks of acute diarrheal disease. The disease caused by *Cryptosporidium* infection is called cryptosporidiosis. Cryptosporidiosis has emerged as an important opportunistic infection in patients infected with HIV. With the advent of more effective HIV therapies, the association between *Cryptosporidium* infection and HIV has lessened in the US, however opportunistic *Cryptosporidiosis* following infection by HIV continues to be a major problem in developing countries.

Cryptosporidium is also recognized as a leading cause of traveler's diarrhea. An acutely debilitating diarrheal disease, accompanied by stomach cramps, cryptosporidiosis typically lasts 2-10 days. In the otherwise healthy host, cryptosporidiosis is rarely fatal, but deaths occur among the immunocompromised including AIDS patients, chemotherapy

patients, malnourished individuals, and the elderly, who may become chronically diarrheic and in whom the parasite may establish hard-to-eliminate hepatobiliary and pancreatic infections.

5 *C. parvum* infects cattle and other livestock usually within the first few hours or days of life. Infected animals can become long-term shedders of *C. parvum* oocysts. *C. parvum* is an economically important cause of diarrheal disease and mortality among calves which provide a significant reservoir for human infection. In swine, clinical disease cryptosporidiosis is less common, but *C. parvum* has been recognized as a highly prevalent contaminant of swine manure holding facilities.

10 *C. parvum* oocysts can survive for extended periods of time in water and soil contaminated from human or animal fecal shedding. The oocysts are not inactivated by chlorination, nor removed by many water filtration systems. Drinking water, recreational water contact, and fecally contaminated foodstuffs are the principal sources of infection for humans. Type 1 and 2 *C. parvum* genotypes are epidemiologically and genetically distinct,
15 although overlap occurs and heterogeneous infections can occur. Type 1 is transmitted from human to human, while type 2 is zoonotic and transmitted between cattle and other livestock, and humans. While genetic polymorphisms occur in both type 1 and 2 isolates, the extent of epitope homology between the genotypes and cross-protection is not fully understood. Dual infections may occur, but with no reproductive mixing of the genotypes.
20 Nevertheless, the present invention shows that three functionally defined antigens are conserved at the protein level between several type 1 and type 2 isolates. These antigens, CSL, P23 and GP25-200, were originally defined on type 2 isolates and previously shown to be the targets of neutralizing antibodies. The apparent conservation of antigens indicates that the compositions of the present invention using monoclonal antibodies to these
25 antigens as neutralizing agents, either alone or to target a biocide to the parasite surface, will have application to both Type 1 and 2 infections. The present invention further contemplates compositions comprising polyvalent antibody passive immunotherapies to treat epidemics of unknown origin.

Large outbreaks of human cryptosporidiosis demonstrate the potential for
30 *Cryptosporidium* to be used as a bioterrorism agent. A 1993 outbreak of cryptosporidiosis in Milwaukee resulted in over 400,000 cases of clinical disease and several dozen deaths,

following dissemination of *C. parvum* through the public water supply. The Milwaukee experience suggests that large waterborne or food borne outbreaks of cryptosporidiosis could be brought about by deliberate contamination. With the ratio of infective oocysts per gram of feces shed by an individual to the infective dose approximating one million to one (shedding 10^6 or 10^7 oocysts per gram, compared to an infective dose 10-100), the potential for producing major urban outbreaks is real.

C. parvum has several attributes that lend it for use as a potential bioterrorism agent: infectious oocysts are very hardy and easily transported; infective oocysts are shed in very large numbers but have a low infective dose; cryptosporidiosis is unlikely to be fatal to the terrorist handler; oocysts are readily available without access to reference collections or high security laboratories and can be easily propagated in neonatal ruminants (up to $\sim 10^{10}$ oocysts from a single calf); and widespread dissemination can be achieved in food or water. Given a high background incidence of *C. parvum* infections, an acute epidemic would be harder to trace back to a point source. Nevertheless, clinical signs are dramatic enough to cause panic, and to allow terrorist claims of responsibility to ring true. *C. parvum* thus fits the profile of an organism which might be deployed by a "low tech" terrorist group without access to a well-developed laboratory infrastructure.

The recent British detection of "ricin laboratories" indicate that low tech bioterrorism is today's reality. If the intent of bioterrorism is to produce mass hysteria rather than mass disease and fatalities, a few cases of cryptosporidiosis implicating contamination of a major food or water supplies could have a dramatic psychological effect.

Water distribution systems across the country are relatively difficult to secure. Security of water supply is also of concern in assuring a safe food supply. In an urban society dependent on complex food distribution chains, a point source contamination could affect people across a wide geographic area. For example, the recent nationwide recall of 28 million pounds of processed turkey due to *Listeria* contamination in a single processing plant illustrates that point source food contamination can have a very wide ripple effect. With only a moderate increment in microbiologic expertise, the effects of food bioterrorism could be devastating.

Dairy effluent is considered an important source of natural *C. parvum* infection, likewise, swine effluent is suspected as being reservoir for infection. Controlling infection

in animal populations would help reduce the environmental risk of natural infections. Accordingly, certain embodiments of the present invention provide compositions to control zoonotic pathogens in animal reservoirs and agricultural environments.

Prior to the present, there were no effective parasite-specific drug therapies to
5 control or curtail cryptosporidiosis in man or animals. Existing treatments are palliative and directed avoiding onset of dehydration. (S. Tzipori and H. Ward, *Microbes Infect.*, 4:1047 [2002]). Naturally occurring cases of cryptosporidiosis in human and animal hosts with normal immunological systems can be severe, but are typically self limiting. (C.L. Chappell
10 *et al.*, *Amer. J. Trop. Med. Hyg.*, 60:157-164 [1999]). In certain embodiments, colostral antibodies fed to calves limit infection and prevent clinical disease. In some other embodiments, polyclonal hyperimmune antibodies raised against *C. parvum* effectively limit clinical cases of the disease while allowing some active immunity to develop.

The present invention provides antibody-based immunoprophylaxis and immunotherapy that effectively control acute *C. parvum* infections. The present invention
15 contemplate that the efficacy of compositions and methods of passive immunotherapy comprising administering antibodies specifically developed against neutralization-sensitive epitopes is distinguishable from the host-produced antibodies in protection against natural infection, which depends on competent cell mediated immune responses (M. Riggs, *Microbes Infect.*, 4:1067 [2002]).

20 Preferred embodiments provide compositions and methods for administering passive immunotherapies against pathogens (*e.g.*, *C. parvum* infections). Faced with a population exposed to deliberately contaminated food or water, or in a battle theater setting, a rapidly deployable, rapidly effective, passive immunotherapies are strategically and clinically very valuable. In some of these embodiments, the present invention provides orally administered
25 monoclonal antibody compositions that specifically target pathogens (*e.g.*, parasites) and either prevent infection, or reduce an existing infection to subclinical levels and abbreviate existing clinical effects.

In some embodiments, the present invention provides monoclonal antibodies against defined apical complex and surface-exposed antigens to specifically neutralize infective
30 stages of *C. parvum in vitro* and *in vivo*. The present invention also provides

Previously unavailable recombinant antibodies to *C. parvum*. Prior to the present invention, high cost and inefficient production systems for recombinant and hybridoma monoclonals alike have generally removed widespread immunoprophylaxis and/or immunotherapies for cryptosporidiosis from serious clinical consideration.

5 Some preferred embodiments of the present invention make use of an extensive bank of hybridoma lines directed to *cryptosporidial* antigens. A large number of *C. parvum* antigens of distinct function have been identified and characterized. (M.W. Riggs, Microbes. Infect., 4:1067 [2002]). Several antigens in particular have shown potential for independent targeting to neutralize sporozoite and merozoite infectivity, including, but not
10 limited to, CSL, P23, and GP25-200. Briefly, CSL (~1300 kDa) is an apical complex-derived glycoprotein expressed on the surface of sporozoite and merozoite infective stages. After antibody binding to CSL, sporozoites release the antigen in membranous antibody-CSL complexes and are rendered non-infective. (M.W. Riggs *et al.*, J. Immunol., 158:1787-1795 [1997]). Since CSL has been shown to contain a ligand for a surface receptor on
15 human intestinal epithelial cells (See, R.C. Langer and M.W. Riggs, Infect. Immun., 67:5282-5291 [1999]; and R.C. Langer *et al.*, Infect. Immun., 69:1661-1670 [2001]), blocking of CSL is contemplated to account for the efficacy of anti-CSL antibodies in inhibiting sporozoite attachment. P23 (~23 kDa) is a surface protein of sporozoites and merozoites believed to be involved in motility and invasion processes (See, L.E. Perryman
20 *et al.*, Vaccine, 17:2142-2149 [1999]). Monospecific antibodies to P23 have been shown to curtail disease in neonatal calves. (L.E. Perryman *et al.*, *supra*). GP25-200 is a glycoprotein complex of variable size, found in the apical complex and on the surface of sporozoites and merozoites. (M.W. Riggs *et al.*, *supra*). Schaefer *et al.* demonstrated that when hybridoma derived monoclonal antibodies to CSL, P23, and GP25-200 were applied
25 singly, or in combination, significant sporozoite neutralization could be obtained. (D.A. Schaefer *et al.*, Infect. Immun., 68:2608-2616 [2000]).

 In some embodiments, optimal protection in neonatal mice is achieved by combining three antibodies: 3E2 (IgM) to target CSL; 3H2 (IgM) to target GP25-200; and IE10 (IgG1) to target P23. When these three antibodies are orally administered, individually, in the
30 neonatal mouse infection model, they are able to reduce intestinal infection by 50-60%. When these antibodies are administered as a polyvalent "cocktail" the three monoclonal

antibodies reduced intestinal infection by 86-93%. Preferred embodiments of the present invention provide recombinant analogues of 3E2, 3H2, and 1E10 antibodies. Additional preferred embodiments provide fusion proteins comprising cryptosporocidal enzymes and antibodies (e.g., IgG), or portions thereof, including, but not limited to, 3E2, 3H2, 1E10, and 4H9. 4H9 is a second antibody directed to GP25-200. It is an IgG1 antibody that is thought to recognize a different epitope on GP25-200 than does 3H2. In some embodiments, 4H9 is able to reduce infection in neonatal mice by ~50% when administered orally. Thus, in still other embodiments, compositions comprising 1E10 and 4H9 provide vehicles for delivering biocides to two different neutralization-sensitive molecules on the surface of sporozoites and merozoites.

The present invention contemplates that using a monoclonal antibody fusion protein to direct otherwise naturally occurring biocides to specific pathogenic organisms (e.g., *E. coli* O157:H7, *L. monocytogenes*, *Cryptosporidium parvum* and the like) has wide applicability in human and animal health. The present invention further contemplates compositions to control other pathogenic feedlot organisms including, but not limited to, *E. coli* K99 in calves and *E. coli* K88 in piglets.

In still further embodiments, the present invention provides embodiments to control other pathogens responsible for foodborne illnesses and other emerging infectious diseases as a component of the Nation's food security and bioterrorism response. For example, some embodiments of the present invention are focused on controlling potential foodborne bioterrorism agents such as, *Clostridium botulinum*, *Clostridium perfringens*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus pneumoniae*, *Staphylococcus saprophyticus*, *Shigella dysenteriae*, *Salmonella typhi*, *Salmonella paratyphi*, *Salmonella enteritidis*, fungal agents and the like.

iii). Other Exemplary Target Pathogens

In some other embodiments, the present methods and compositions are directed to specifically controlling (e.g., therapeutic treatments or prophylactic measures) diseases caused by the following pathogens: *Bartonella henselae*, *Borrelia burgdorferi*, *Campylobacter jejuni*, *Campylobacter fetus*, *Chlamydia trachomatis*, *Chlamydia pneumoniae*, *Chlamydia psittaci*, *Simkania negevensis*, *Escherichia coli* (e.g., O157:H7

and K88), *Ehrlichia chafeensis*, *Clostridium botulinum*, *Clostridium perfringens*,
Clostridium tetani, *Enterococcus faecalis*, *Haemophilus influenzae*, *Haemophilus ducreyi*,
Coccidioides immitis, *Bordetella pertussis*, *Coxiella burnetii*, *Ureaplasma urealyticum*,
Mycoplasma genitalium, *Trichomatis vaginalis*, *Helicobacter pylori*, *Helicobacter*
5 *hepaticus*, *Legionella pneumophila*, *Mycobacterium tuberculosis*, *Mycobacterium bovis*,
Mycobacterium africanum, *Mycobacterium leprae*, *Mycobacterium asiaticum*,
Mycobacterium avium, *Mycobacterium celatum*, *Mycobacterium celonae*, *Mycobacterium*
fortuitum, *Mycobacterium genavense*, *Mycobacterium haemophilum*, *Mycobacterium*
intracellulare, *Mycobacterium kansasii*, *Mycobacterium malmoeense*, *Mycobacterium*
10 *marinum*, *Mycobacterium scrofulaceum*, *Mycobacterium simiae*, *Mycobacterium szulgai*,
Mycobacterium ulcerans, *Mycobacterium xenopi*, *Corynebacterium diphtheriae*,
Rhodococcus equi, *Rickettsia aeschlimannii*, *Rickettsia africae*, *Rickettsia conorii*,
Arcanobacterium haemolyticum, *Bacillus anthracis*, *Bacillus cereus*, *Lysteria*
monocytogenes, *Yersinia pestis*, *Yersinia enterocolitica*, *Shigella dysenteriae*, *Neisseria*
15 *meningitidis*, *Neisseria gonorrhoeae*, *Streptococcus bovis*, *Streptococcus hemolyticus*,
Streptococcus mutans, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Staphylococcus*
aureus, *Staphylococcus epidermidis*, *Staphylococcus pneumoniae*, *Staphylococcus*
saprophyticus, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Salmonella typhi*, *Salmonella*
paratyphi, *Salmonella enteritidis*, *Treponema pallidum*, Human rhinovirus, Human
20 coronavirus, Dengue virus, Filoviruses (e.g., Marburg and Ebola viruses), Hantavirus, Rift
Valley virus, Hepatitis B, C, and E, Human Immunodeficiency Virus (e.g., HIV-1, HIV-2),
HHV-8, Human papillomavirus, Herpes virus (e.g., HV-I and HV-II), Human T-cell
lymphotrophic viruses (e.g., HTLV-I and HTLV-II), Bovine leukemia virus, Influenza virus,
Guanarito virus, Lassa virus, Measles virus, Rubella virus, Mumps virus, Chickenpox
25 (Varicella virus), Monkey pox, Epstein Bahr virus, Norwalk (and Norwalk-like) viruses,
Rotavirus, Parvovirus B19, Hantaan virus, Sin Nombre virus, Venezuelan equine
encephalitis, Sabia virus, West Nile virus, Yellow Fever virus, causative agents of
transmissible spongiform encephalopathies, Creutzfeldt-Jakob disease agent, variant
Creutzfeldt-Jakob disease agent, *Candida*, *Cryptococcus*, *Cryptosporidium*, *Giardia*
30 *lamblia*, *Microsporidia*, *Plasmodium vivax*, *Pneumocystis carinii*, *Toxoplasma gondii*,
Trichophyton mentagrophytes, *Enterocytozoon bieneusi*, *Cyclospora cayetanensis*,

Encephalitozoon hellem, *Encephalitozoon cuniculi*, among other viruses, bacteria, archaea, protozoa, fungi, and the like).

The present invention is not limited to the exemplary microorganisms described herein. One skilled in the art understands that the methods and compositions of the present invention are suitable for the targeting of any microorganism or group or class of microorganism.

B. Biofilms

In some embodiments, the methods and compositions of the present invention target bacteria present as a biofilm. *Listeria monocytogenes* can form biofilms on a variety of materials used in food processing equipment and other food and non-food contact surfaces (Blackman, J Food Prot 1996; 59:827-31; Frank, J Food Prot 1990; 53:550-4; Kryszinski, J Food Prot 1992; 55:246-51; Ronner, J Food Prot 1993; 56:750-8). Biofilms can be broadly defined as microbial cells attached to a surface, and which are embedded in a matrix of extracellular polymeric substances produced by the microorganisms. Biofilms are known to occur in many environments and frequently lead to a wide diversity of undesirable effects. For example, biofilms cause fouling of industrial equipment such as heat exchangers, pipelines, and ship hulls, resulting in reduced heat transfer, energy loss, increased fluid frictional resistance, and accelerated corrosion. Biofilm accumulation on teeth and gums, urinary and intestinal tracts, and implanted medical devices such as catheters and prostheses frequently lead to infections (Characklis WG. Biofilm processes. In: Characklis WG and Marshall KC eds. New York: John Wiley & Sons, 1990:195-231; Costerton et al., Annu Rev Microbiol 1995; 49:711-45).

Biofilm formation is a serious concern in the food processing industry because of the potential for contamination of food products, leading to decreased food product quality and safety (Kumar CG and Anand SK, Int J Food Microbiol 1998; 42:9-27; Wong, J Dairy Sci 1998; 81:2765-70; Zottola and Sasahara, Int J Food Microbiol 1994; 23:125-48). The surfaces of equipment used for food handling or processing are recognized as major sources of microbial contamination. (Dunsmore et al., J Food Prot 1981; 44:220-40; Flint et al., Biofouling 1997; 11:81-97; Grau, In: Smulders FJM ed. Amsterdam: Elsevier, 1987:221-234; Thomas et al., In: Smulders FJM ed. Amsterdam: Elsevier, 1987:163-180). Biofilm

bacteria are generally hardier than their planktonic (free-living) counterparts, and exhibit increased resistance to antimicrobial agents such as antibiotics and disinfectants. It has been shown that even with routine cleaning and sanitizing procedures consistent with good manufacturing practices, bacteria can remain on equipment, food and non-food contact surfaces and can develop into biofilms. In addition, *L. monocytogenes* attached to surfaces such as stainless steel and rubber, materials commonly used in food processing environments, can survive for prolonged periods (Helke and Wong, J Food Prot 1994; 57:963-8). This would partially explain their ability to persist in the processing plant. Common sources of *L. monocytogenes* in processing facilities include equipment, conveyors, product contact surfaces, hand tools, cleaning utensils, floors, drains, walls, and condensate (Tomkin et al., Dairy, Food Environ Sanit 1999; 19:551-62; Welbourn and Williams, Dairy, Food Environ Sanit 1999; 19:399-401).

C. Plant Pathogens

In still further embodiments, the present invention provides methods and compositions targeted towards plant pathogens. Plant fungi have caused major epidemics with huge societal impacts. The Irish potato famine, with its consequent economic disaster and human population displacement, was the result of the sudden introduction of the fungus *Phytophthora infestans*.

South American cocoa crops are under threat of two major fungal diseases: witches broom caused by *Crinipellis pernicioso* and frosty pod (*Moniliophthora roreri*), which together threaten the viability of the chocolate production industry in the western hemisphere.

Phytophthora blight, caused by the oomycete *Phytophthora capsici*, has become one of the most serious threats to production of cucurbits (cucumbers, squash, pumpkins) and peppers, both in the United States and worldwide (Erwin, D. C., and Ribeiro, O. K. 1996. *Phytophthora Diseases Worldwide*. American Phytopathological Society, St. Paul, MN).

Banana crops worldwide are affected by Black Sigatoka is caused by the ascomycete, *Mycosphaerella fijiensis*. *Fusarium* scab affects small grain crops (wheat and barley). *Ganoderma* spp fungi have produced deaths of ornamental palms, as do several

species of *Phytophthora* (Elliott and Broschat, T. K. 2001. *Palms* 45:62-72; Nagata and Aragaki, M. 1989. *Plant Dis.* 73:661-663).

Plants are also affected by bacteria and viruses. *Burkholderia cepacia* is a bacterium which produces economic losses to onion crops (Burkholder 1950. *Phytopathology* 40:115-118). Numerous plant viruses cause significant crop losses worldwide. Exemplary of such plant viruses are soybean mosaic virus, bean pod mottle virus, tobacco ring spot virus, barley yellow dwarf virus, wheat spindle streak virus, soil born mosaic virus, wheat streak virus in maize, maize dwarf mosaic virus, maize chlorotic dwarf virus, cucumber mosaic virus, tobacco mosaic virus, alfalfa mosaic virus, potato virus X, potato virus Y, potato leaf roll virus and tomato golden mosaic virus.

D. Sanitation

In yet other embodiments, the methods and compositions of the present invention find use in the sanitation of household and other areas. *Listeria* spp are common contaminants of the domestic environment. As many as 47% of households sampled were contaminated, with dishcloths, and drain areas being common sites of contamination. (Beumer et al., *Epidemiol Infect.* 1996 Dec;117(3):437-42).

Pseudomonas aeruginosa is frequently isolated from showers and baths and hot tubs (Zichichi et al., *Int J Dermatol.* 2000 Apr;39(4):270-3; Silverman and Nieland, *J Am Acad Dermatol.* 1983 Feb;8(2):153-6).

Where a family member has an infectious disease, transmission may occur through contamination of domestic objects (Barker et al., *J Appl Microbiol.* 2000 Jul;89(1):137-44).

Domestic food preparation and storage areas are also a source of bacterial food contaminants. The public is showing increasing awareness of the need to control domestic microbial contamination (Mattick et al., *Int J Food Microbiol.* 2003 Aug 25;85(3):213-26; Kusumaningrum et al., *Int J Food Microbiol.* 2003 Aug 25;85(3):227-36).

E. Building structures

In other embodiments, the present invention provides methods of targeting building structures. There has been increasing public attention to the potential health risks of mold exposure, particularly in wet buildings. A variety of molds have been isolated from both

damaged homes and businesses, including agents that secrete toxigenic materials.

Stachybotrys chartarum is a fungus that has become notorious as a mycotoxin producer that can cause animal and human mycotoxicosis. Indeed, over the past 15 years in North America, evidence has accumulated implicating this fungus as a serious problem in homes and buildings and one of the causes of the "sick building syndrome." (Mahmoudi et al., J Asthma. 2000 Apr;37(2):191-8).

Legionella spp. bacteria replicate in manmade water containing structures, especially when these are heated, such as industrial cooling towers, heating and air conditioning systems. Legionnaires disease pneumonia is contracted by susceptible individuals that breathe water droplets from such sources. Preventive and remedial treatment of water containing structures is needed to eliminate the source of infection to building inhabitants (Shelton et al., AIHAJ. 2000 Sep-Oct;61(5):738-42).

F. Military and Bioterrorism Applications

The methods and compositions of the present invention further find use in military and bioterrorism applications. For example, in some embodiments, the methods and compositions of the present invention are used in the decontamination of surfaces exposed to unknown bacteria and potentially other microorganisms (e.g., military equipment and personal protective gear).

In yet other embodiments, microorganisms engineered for use in combatting bioterror agents (e.g., *B. Anthracis*, smallpox, etc.) are targeted by the methods and compositions of the present invention.

The methods and compositions of the present invention further find use in combating unknown and drug resistant organisms. The prevalence of bacteria that resist standard antibiotic therapy is increasing rapidly. Furthermore, the ability to engineer organisms with multiple drug resistance to standard antibiotics creates a significant threat in bioweapons development. Because the broad spectrum antimicrobials of the present invention are suitable for use against broad classes of pathogens, they can respond to unknown bacteria and their bactericidal effect is independent of antibiotic resistance mechanisms.

G. Medical Applications

The methods and compositions of the present invention additionally find use in the treatment of subjects (e.g., humans) infected with a microorganism (e.g., food borne pathogens). The methods and compositions of the present invention are particularly well suited for use against antibiotic resistant organisms are targeted by the methods and compositions of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to constructs that encode novel microorganism targeting molecules (e.g., innate immune receptors ligands or monoclonal antibodies), novel fusion proteins, and chimeric monoclonal antibodies and to methods of using and producing the same. In particular, the present invention relates to methods of producing novel monoclonal antibody biocide (e.g., bactericidal enzymes) fusion proteins in transgenic animals (e.g., bovines) and in cell cultures. The present invention also relates to therapeutic and prophylactic methods of using monoclonal antibody biocide fusion proteins in health care (e.g., human and veterinary), agriculture (e.g., animal and plant production), and food processing (e.g., beef carcass processing). The present invention also relates to methods of using monoclonal antibody biocide fusion proteins in various diagnostic applications in number of diverse fields such as agriculture, medicine, and national defense.

Certain embodiments of the present invention relate to the production of novel monoclonal antibodies and chimeric monoclonal antibody fusion proteins in host cells containing multiple integrated copies of an integrating vector. Preferred embodiments of the present invention utilize integrating vectors (*i.e.*, vectors that integrate via an integrase or transposase or have the capability to code for these enzymes) to create cell lines containing a high copy number of a nucleic acid encoding a gene of interest. The transfected genomes of the high copy number cells are stable through repeated passages (e.g., at least 10 passages, preferably at least 50 passages, and most preferably at least 100 passages). Furthermore, in preferred embodiments, the host cells of the present invention are capable of producing high levels of protein (e.g., more than 1 pg/cell/day, preferably

more than 10 pg/cell/day, more preferably more than 50 pg/cell/day, and most preferably more than 100 pg/cell/day).

Additional embodiments provide methods for the production of transgenic non-human animals that express novel proteins in their tissues (*e.g.*, mammary glands). In preferred embodiments, the transgenic animals are non-human ruminant (*Ruminantia*) mammals. In other preferred embodiments, the transgenic animals are ungulates. In particularly preferred embodiments, the mammals are female ruminants (*e.g.*, bovines) that preferentially express the novel proteins in their mammary glands. In some additional embodiments, the novel protein compositions produced in the transgenic animals are collected, purified, and subsequently incorporated into a variety of additional compositions (*e.g.*, food additives, pharmaceuticals, disinfecting agents, *etc.*) and/or used in a variety of therapeutic or prophylactic methods. In some embodiments, the proteins of interest (the novel fusion proteins disclosed herein) are mixed with colostrum (or colostrum substitute(s)) and subsequently feed to nursing feedlot animals (*e.g.*, beef calves, piglets, lambs, kids, and the like). In other embodiments, the proteins of interest are formulated with one or more carriers (*e.g.*, whey) and used in the meat processing industry either a topical disinfecting agent applied to animal carcasses or as an edible supplement mixed into finished meat products. In still further embodiments, the proteins of interest (*e.g.*, novel monoclonal antibodies and chimeric monoclonal antibody fusion proteins disclosed herein) are purified from the lactation of transgenic non-human animals and subsequently processed and formulated for administration to subjects (*e.g.*, humans and non-human animals) as therapeutic or prophylactic medicaments.

Further embodiments of the present invention provide methods for producing transgenic non-human animals by the introduction of exogenous DNA into pre-maturation oocytes and mature, unfertilized oocytes (*i.e.*, pre-fertilization oocytes) using retroviral vectors that transduce dividing cells (*e.g.*, vectors derived from murine leukemia virus [MLV], Moloney murine leukemia virus [MMLV], and the like). In addition, the present invention provides methods and compositions for cytomegalovirus promoter-driven, as well as, mouse mammary tumor LTR expression of various recombinant proteins. The present invention is not limited however to the aforementioned constructs, promoters, and other genetic elements. Indeed, the present invention provides numerous examples of

contemplated genetic constructs (*e.g.*, retroviral vectors) and methods of producing stably transfected cell lines (*e.g.*, mammalian, amphibian, insect, and plant) and transgenic non-human animals (*e.g.*, bovines).

In some preferred embodiments, retrovector transgenic technologies (described in greater detail herein) are used to overcome problems inherent in earlier methods for creating transgenic mammals. In preferred embodiments, unlike earlier transgenic methods, genes of interest (*e.g.*, genes encoding at least a portion of a recombinant antibody) are introduced into unfertilized oocytes (*e.g.*, bovine oocytes). After entry of the retroviral RNA into the cell, and reverse transcription into DNA, the integration of the DNA provirus into the host cell genome is mediated by the retroviral integrase and specific nucleotide sequences at the ends of the retroviral genome. By introducing genes to the oocyte (*e.g.*, a bovine oocyte) at metaphase II arrest, the vector has access to the oocyte DNA when there is no nuclear membrane in place. The present invention contemplates this technology negates the need for dividing cells for retroviral integration to occur. Depending on the conditions, such integrations can occur at one or several independent sites in the genome and are transmitted in standard Mendelian patterns upon subsequent animal (*e.g.*, bovine) breeding. The integrated gene is transcribed like other indigenous cell genes, and the proteins it encodes are expressed at high levels. In still other preferred embodiments, the retrovector backbone used lacks the genes essential for viral structure and enzyme functions, therefore the retroviral constructs are replication defective. In yet other embodiments, the present invention uses constructs that preclude the need for a selectable marker. Importantly, preferred embodiments contemplate that the removal of selectable markers (*e.g.*, antibiotic-dependent selection markers) provides a significant advantage, especially upon consideration of regulatory requirements for transgenic livestock.

In some of preferred embodiments directed to producing transgenic animals, the contemplated approach has major advantages. For example, the efficiency of transgenic live births using the contemplated transgenic methods is high *e.g.*, from about 25% -75% of animals (cattle) born when genes without a selection marker are used. Additionally, in preferred embodiments, retroviral genes insert as single copies, thus decreasing the risk of genetic instability upon subsequent cell replication, which tends to splice out tandem repeats of genes typical in pronuclear injection and nuclear transfer technologies. The present

invention further contemplates in some preferred embodiments, where transgenes are inserted prior to fertilization the risk of producing mosaic animals, which are only transgenic in some tissues but not all, is greatly reduced.

Exemplary compositions and methods of the present invention are described in more detail in the following sections: I. Production of recombinant antibodies; II. Production of recombinant chimeric antibodies; III. Production of pathogen specific monoclonal antibodies in a multigenic expression system; IV. Comparison of murine and chimeric murine-bovine antibodies; and V. Transgenic animal technologies; VI. Considerations for combating *Cryptosporidium* and other parasites; VII. Transgenic plant technologies; and VIII. Pharmaceutical compositions.

I. Production of Recombinant Antibodies

The present invention contemplates obtaining hybridoma cell lines that produce monoclonal antibodies against particular pathogens of interest (*e.g.*, *E. coli* strain O157:H7) from one or more sources (*e.g.*, ATCC). The cell lines are subsequently used to isolate the heavy and light chain genes that encode for pathogen (*e.g.*, *E. coli* O157:H7 and *Listeria monocytogenes*) specific monoclonal antibodies according to standard molecular biology methods.

For example, in one embodiment, hybridoma cell line ATCC HB 10452, which makes monoclonal antibody 4E8C12 specific for *E. coli* O157:H7 and O26:H11, are grown according to the depositors instructions. The cells are maintained in cRPMI at 37 °C and 5% CO₂ atmosphere and split biweekly at a 1:10 ratio. In another example, monoclonal antibody hybridomas to the pathogen *L. monocytogenes* from the cell line ATCC 4689-4708 are likewise grown according to the depositors instructions. In some embodiments, candidate monoclonal antibodies are chosen based upon their binding affinity to the pathogen of interest (*e.g.*, *L. monocytogenes*) as well as their binding specificity that in certain instances includes as many different pathogen serotypes as possible. In some other embodiments, candidate monoclonals preferably show no or only weak cross-reaction with other species of bacteria and mammalian cells.

These cultures are expanded and grown in roller bottles under the described conditions to allow production of approximately milligram amounts of purified monoclonal

antibodies. In preferred embodiments, the antibodies are purified using any suitable protocol such as ammonium sulfate precipitation. In some embodiments, the purified monoclonal antibodies are used to perform various *in vitro* functionality tests. For example, the present invention contemplates using purified monoclonal antibodies to perform affinity and specificity tests in order to select for the antibodies that have the best binding properties to the surface of the pathogen of interest (*e.g.*, *L. monocytogenes*) and/or that include binding to a broad range of serotypes. Contemplated functionality tests include, but are not limited to, enzyme-linked immunosorbent assays (ELISA) and competitive ELISA assays. In one embodiment, varying concentrations of different monoclonal antibodies are allowed to bind to immobilized heat killed pathogens (*e.g.*, *L. monocytogenes*). In another embodiment using a competitive assay, various concentrations of competing antigen are added to the wells of test plate and the binding of the monoclonal antibodies is measured. In yet another embodiment, quantitative immunofluorescence assays are used to allow the determination of binding affinity based on fluorescence intensity per cell. The present invention contemplates that by determining the affinity of the monoclonal antibodies based on their binding capacity to the pathogen of interest (*e.g.*, *L. monocytogenes*), the present invention allows the selection of the one monoclonal antibody that is best for topical applications against viable pathogens.

Cells from the highest affinity hybridoma clone will be used to extract total RNA with the purpose of isolating the monoclonal antibody-specific heavy and light chain gene transcripts. Upon total RNA extraction, the RNA is reverse transcribed using standard molecular biology kits and protocols, such as the RIBOCLONE cDNA synthesis system from Promega (Promega Corp., Madison, WI). Preferably, the procedures used create double stranded cDNA of all RNA transcripts in a cell, including the transcripts from the murine heavy and light chain genes. The total cDNA is used as a template to specifically amplify the mouse IgG2a heavy chain and the Igk light chain. Site-directed mutagenesis primers are used to amplify these sequences. The present invention contemplates that the use of these primers adds short sequences of DNA, and introduces suitable restriction sites thus allowing direct cloning of the product into the retrovector backbone.

In preferred embodiments, once the genes for the murine heavy and light chain have been isolated, they are separated by an IRES element and inserted into the retrovector

expression system under the control of the simian cytomegalovirus and the bovine alpha-lactalbumin promoter. In particularly preferred embodiments, the genes for the murine heavy and light antibody chains are cloned into the GPEX gene product expression system under the control of the simian cytomegalovirus (sCMV) promoter (Gala Design, Inc.,
5 Middleton, WI) or other suitable multigenic gene expression systems. This process allows for the production of cell lines that secrete high levels of the monoclonal antibodies.

In particular, the heavy chain followed by an internal ribosome entry site (IRES) element are cloned into the retrovector backbone at the same site. Similarly, the light chain is then cloned into the retrovector backbone. Once the retroviral construct is complete,
10 quality control sequencing will confirm that all the elements are present. The present invention contemplates that the use of the IRES element in between heavy and light chain genes yields fully functional antibodies expressed and secreted into the medium at exceptionally high levels (*e.g.*, >100pg/cell/day in CHO cells). In some preferred embodiments, after the retroviral constructs are complete, quality control sequencing is used
15 to confirm that all the elements are present. The retrovector construct are then used to transform host cells along with the plasmid that encodes the vesicular stomatitis virus glycoprotein (VSV-G) used for pseudotyping the retrovirus. This procedure creates intermediate level viral titer that is used to infect production cell lines (*e.g.*, 293H or CHO cells). The population of transduced cells are subjected to clonal selection based on the
20 antibody levels present in the medium supernatant. The clone with the highest level of antibodies secreted into the supernatant is selected to produce milligram amounts of murine monoclonal antibody 4E8C12. In preferred embodiments, the recombinant antibodies are purified from cell supernatants using standard techniques well known to those in the art.

Figure 1 shows one contemplated retroviral construct for expression of murine and
25 chimeric bovine murine antibodies with lysozyme. In some cell culture expression embodiments, the alpha-lactalbumin promoter is replaced with simian cytomegalovirus promoter.

II. Production of Recombinant Chimeric Antibodies

30 In some embodiments, the bovine IgG1 and IgG2 heavy chain genes are used to modify the constructs made above to produce constructs encoding chimeric bovine-murine

antibodies. For example, in one contemplated embodiment, the constant portion of the murine heavy chain gene is replaced with the constant portion of the bovine heavy chain gene to create a chimeric bovine-murine monoclonal antibodies. A suitable bovine heavy chain IgG1 sequence may be selected from, but is not limited to, the following GenBank
5 Accession Numbers: BD105809; S82409; U32264; U32263; U32262; U32261; U32260; U32259; U32258; U32257; U32256; U32255; U32254; U32253; U32252; U32251; U32250; U32249; U34749; U34748; U32852; U32851; U32850; U36824; U36823; S82407; X62917; X62916; and X16701. Likewise, a suitable bovine heavy chain IgG2 sequence may be selected from, but is not limited to, the following GenBank
10 Accession Numbers: S82409; S82407; Z37506; and X16702. In preferred embodiments, GenBank Accession No. S282409 (SEQ ID NO:1) provides bovine IgG1/IgG2 sequences. (*See*, I. Kacskovics and J.E. Butler, *Mol. Immunol.*, 33(2):189-195 [1996]). Preferably, the murine IgG2a heavy chain gene will be replaced by the bovine sequence for IgG1 or IgG2a. Thus, modified with bovine IgG1/IgG2 sequences, the vectors described above are used in
15 subsequent cloning steps.

In preferred embodiments, following sequence analysis of the construct, the constructs are used to create vectors for the transduction of production cell lines (*e.g.*, 293H) and packaging cell lines (*e.g.*, 293gp). Standard clonal analysis techniques are used to select for clones that produce high levels of the bovine-murine chimeric antibody. Once a
20 top clone has been selected, enough chimeric antibody will be produced from this clone to conduct functionality tests with the derived chimeric monoclonal antibody.

In preferred embodiments, production cell lines that secrete high levels of the monoclonal antibodies are made from the above-mentioned constructs. The retroviral construct containing the chimeric murine-bovine monoclonal antibody genes are used to
25 transduce at least one production cell line (*e.g.*, the 293H production cell line). Upon transduction and expansion, the cell pool is subjected to limited dilution cloning to select for clones that produce high levels of the chimeric monoclonal antibody as determined by standard assay techniques (*e.g.*, ELISA assays). One of the top clones is used to produce chimeric murine-bovine monoclonal antibodies in milligram amounts that are subsequently
30 used in the functionality tests described below.

The present invention further contemplates the production of retrovector packaging cell lines that produce high titers of retrovector containing the gene for the monoclonal antibodies in preparation for making transgenic animals, such as bovines. For example, the retrovector construct containing the chimeric murine-bovine monoclonal antibody genes are used to transduce a packaging cell line (*e.g.*, 293gp packaging cell line). The transduced packaging cell pool is then subjected to limiting dilution cloning and clones that produce the highest infectious viral titers are used for virus production. After a thorough quality control of the top virus titer producing clone, which ensures that the construct is complete, an appropriate amount of pseudotyped virus are purified and cryopreserved for use in oocyte injections.

III. Production of Pathogen Specific Monoclonal Antibodies in a Multigenic Expression System

In certain preferred embodiments, the production of *L. monocytogenes*-specific monoclonal antibody is conducted in the GPEX gene product expression system (Gala Design, Inc., Middleton, WI). In an initial step, the transduced production cell pool is subjected to clonal analysis to select the top antibody producing clones. Preferably, the retrovector construct will be used to transform host cells along with the plasmid that encodes the vesicular stomatitis virus glycoprotein (VSV-G) used for pseudotyping the retrovirus. This procedure creates intermediate level viral titer used to infect production cell lines (*e.g.*, 293H and CHO cells among others). The population of transduced cells is then subjected to a clonal selection, based on antibody levels present in the medium supernatant.

In additional embodiments, the selected clones are then expanded and used to produce sufficient quantities of monoclonal *L. monocytogenes*-specific antibodies to perform one or more functionality studies similar to those mentioned above.

The clone with the highest level of antibody secreted into the supernatant is then chosen to produce milligram amounts of recombinant murine monoclonal antibody against *L. monocytogenes*. Additional experiments with the purified monoclonal antibodies, similar to those mentioned above are contemplated. The objective of these experiments is to determine whether the production of the selected high-affinity monoclonal antibody affects the binding capacity when compared to the original hybridoma-derived antibody. Since the

present invention contemplates using a mammalian expression system, no changes in affinity of the GPEX produced monoclonal antibody are expected.

IV. Comparison of Murine and Chimeric Murine-bovine Antibodies

5 In preferred embodiments, the present invention contemplates additional functionality testing of the purified murine monoclonal antibody as compared to the hybridoma-derived product. For example, in one embodiment, a number of tests are conducted to demonstrate that the 4E8C12 monoclonal is highly specific for *E. coli* strain O157:H7 and strain O26:H11 and no other related strains or species. In some of these
10 embodiments, the assays contemplated for determining the specific bactericidal activity are divided into two phases. First, the bactericidal activity of the monoclonal antibody and fusion proteins are tested *in vitro* for inactivation of the pathogenic strain (*e.g.*, *E. coli* O157:H7). Second, the monoclonal antibody and fusion proteins are evaluated by adding to formulations in turkey slurries.

15 In particular, to assess *in vitro* inactivation, *E. coli* O157:H7 (five food and outbreak isolates) are grown in trypticase soy broth (TSB) until late log phase (~24 h). The cells are harvested by centrifugation, washed in 67 mM sodium phosphate buffer, pH 6.6 (PB), and strains mixed in approximately equal concentrations. The *E. coli* mixture is then added to a level of 10⁵ per ml to PB. The monoclonal conjugates are added starting at concentrations
20 that correspond to the bactericidal concentration of lysozyme and phospholipase A2 alone and down at least 3 logs. The suspensions are incubated at 4° and 10° C and cell viability determined at 0, 1, 4, 8 and 24 h by direct plating on TSB and MacConkey sorbitol agars. The cell suspensions are examined microscopically for clumping. If clumping is observed, further experimental techniques are used to separate the cells (*e.g.*, addition of surfactants,
25 such as Tween 80, changing pH, and mild sonication). Controls without added monoclonal conjugates are also contemplated for testing. All conditions are tested in triplicate and standard deviations of viability are determined.

In some embodiments, cooked, uncured, and unsmoked turkey breast is obtained from a manufacturer. Slurries of this meat product are prepared as described by Schlyter *et al.*, Int. J. Food Microbiol., 19(4):271-281 (1993) adjusted to the appropriate brine content,
30 and pasteurized to 68°C. Two levels of filter-sterilized monoclonal conjugates, depending

on *in vitro* results, are added to the slurries after pasteurization. Flasks are cooled to 4°C and subsequently inoculated with *E. coli* O157:H7 (five strain mixture of food and outbreak isolates) to yield about a 10⁵ cfu/ml slurry, and dispensed 3 ml per sterile polystyrene tube for incubation at 4 and 10°C for up to 4 weeks. In preferred embodiments, triplicate samples per variable are assayed weekly for changes in *E. coli* O157:H7 populations using direct plating on MacConkey sorbitol agar techniques.

In addition to the bactericidal tests, the present invention further contemplates additional experiments to determine whether the chimeric bovine-murine antibodies contemplated are more effective than their murine counterparts in mediating pathogen ingestion by phagocytes. While there is a substantial amount of data available on the efficacy of humanizing therapeutic murine antibodies in order to improve beneficial reactions between immune cells and target cells (for example ADCC, phagocytosis, antigen presentation) in humans, however, the efficacy of a chimeric bovine-murine antibodies in mediating ingestion and killing of a pathogen in cattle has yet to be determined.

Accordingly, the present invention provides functional assays of bovine monocyte/macrophage to measure killing/ingestion of *E. coli* O157:H7 in the presence of the murine monoclonal antibody, or the chimeric antibody, or no antibody. It is expected that the chimeric bovine-murine antibody of the present invention are superior in mediating phagocytosis compared to murine only versions.

In yet other embodiments, the present invention contemplates the purification of sufficient quantities of retrovectors containing genes for the chimeric monoclonal antibodies to conduct further functional assays and additional tests.

In still other embodiments, based upon the results obtained in the above-mentioned assays and tests, further clonal analysis of packaging cell lines that express the chimeric antibody are contemplated. Briefly, a high viral titer producing clone is chosen and expanded. The expanded culture are subsequently induced to produce infective viral particles and viral preparations to enrich viral particles to a titer of approximately 1-5x10⁸ cfu/ml. Such titers have proven effective in producing transgenic animals when used for oocyte injection in transgametic systems.

V. Transgenic Animal Technologies

The methods and compositions used in certain embodiments of the present invention for creating transgenic animals (*e.g.*, bovines and other ungulates) for expression of the biocidal fusion proteins are described in greater detail below.

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A. Retroviruses and Retroviral Vectors

Retroviruses (family Retroviridae) are divided into three groups: the spumaviruses (*e.g.*, human foamy virus); the lentiviruses (*e.g.*, human immunodeficiency virus and sheep visna virus) and the oncoviruses (*e.g.*, MLV, Rous sarcoma virus).

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Retroviruses are enveloped (*i.e.*, surrounded by a host cell-derived lipid bilayer membrane) single-stranded RNA viruses that infect animal cells. When a retrovirus infects a cell, its RNA genome is converted into a double-stranded linear DNA form (*i.e.*, it is reverse transcribed). The DNA form of the virus is then integrated into the host cell genome as a provirus. The provirus serves as a template for the production of additional viral genomes and viral mRNAs. Mature viral particles containing two copies of genomic RNA bud from the surface of the infected cell. The viral particle comprises the genomic RNA, reverse transcriptase and other *pol* gene products inside the viral capsid (which contains the viral *gag* gene products), which is surrounded by a lipid bilayer membrane derived from the host cell containing the viral envelope glycoproteins (also referred to as membrane-associated proteins).

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The organization of the genomes of numerous retroviruses is well known in the art and this has allowed the adaptation of the retroviral genome to produce retroviral vectors. The production of a recombinant retroviral vector carrying a gene of interest is typically achieved in two stages. First, the gene of interest is inserted into a retroviral vector which contains the sequences necessary for the efficient expression of the gene of interest (including promoter and/or enhancer elements which may be provided by the viral long terminal repeats [LTRs] or by an internal promoter/enhancer and relevant splicing signals), sequences required for the efficient packaging of the viral RNA into infectious virions (*e.g.*, the packaging signal [Psi], the tRNA primer binding site [-PBS], the 3' regulatory sequences required for reverse transcription [+PBS] and the viral LTRs). The LTRs contain sequences required for the association of viral genomic RNA, reverse transcriptase and integrase

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functions, and sequences involved in directing the expression of the genomic RNA to be packaged in viral particles. For safety reasons, many recombinant retroviral vectors lack functional copies of the genes that are essential for viral replication (these essential genes are either deleted or disabled); the resulting virus is said to be replication defective.

5 Second, following the construction of the recombinant vector, the vector DNA is introduced into a packaging cell line. Packaging cell lines provide viral proteins required in *trans* for the packaging of the viral genomic RNA into viral particles having the desired host range (*i.e.*, the viral-encoded gag, pol and env proteins). The host range is controlled, in part, by the type of envelope gene product expressed on the surface of the viral particle.

10 Packaging cell lines may express ecotropic, amphotropic or xenotropic envelope gene products. Alternatively, the packaging cell line may lack sequences encoding a viral envelope (env) protein. In this case the packaging cell line will package the viral genome into particles that lack a membrane-associated protein (*e.g.*, an env protein). In order to produce viral particles containing a membrane associated protein that will permit entry of

15 the virus into a cell, the packaging cell line containing the retroviral sequences is transfected with sequences encoding a membrane-associated protein (*e.g.*, the G protein of vesicular stomatitis virus [VSV]). The transfected packaging cell will then produce viral particles that contain the membrane-associated protein expressed by the transfected packaging cell line; these viral particles, which contain viral genomic RNA derived from one virus

20 encapsidated by the envelope proteins of another virus are said to be pseudotyped virus particles.

 Viral vectors, including recombinant retroviral vectors, provide a more efficient means of transferring genes into cells as compared to other techniques such as calcium phosphate-DNA co-precipitation or DEAE-dextran-mediated transfection, electroporation or

25 microinjection of nucleic acids. It is believed that the efficiency of viral transfer is due in part to the fact that the transfer of nucleic acid is a receptor-mediated process (*i.e.*, the virus binds to a specific receptor protein on the surface of the cell to be infected). In addition, the virally transferred nucleic acid once inside a cell integrates in controlled manner in contrast to the integration of nucleic acids which are not virally transferred; nucleic acids transferred

30 by other means such as calcium phosphate-DNA co-precipitation are subject to rearrangement and degradation.

The most commonly used recombinant retroviral vectors are derived from the amphotropic Moloney murine leukemia virus (MoMLV) (Miller and Baltimore, Mol. Cell. Biol., 6:2895 [1986]). The MoMLV system has several advantages: 1) this specific retrovirus can infect many different cell types, 2) established packaging cell lines are available for the production of recombinant MoMLV viral particles and 3) the transferred genes are permanently integrated into the target cell chromosome. The established MoMLV vector systems comprise a DNA vector containing a small portion of the retroviral sequence (the viral long terminal repeat or "LTR" and the packaging or "psi" signal) and a packaging cell line. The gene to be transferred is inserted into the DNA vector. The viral sequences present on the DNA vector provide the signals necessary for the insertion or packaging of the vector RNA into the viral particle and for the expression of the inserted gene. The packaging cell line provides the viral proteins required for particle assembly (Markowitz *et al.*, J. Virol., 62:1120 [1988]).

Despite these advantages, existing retroviral vectors based upon MoMLV are limited by several intrinsic problems: 1) they do not infect non-dividing cells (Miller *et al.*, Mol. Cell. Biol., 10:4239 [1992]), 2) they produce low titers of the recombinant virus (Miller and Rosman, BioTechn., 7: 980 [1989]; and Miller, Nature 357: 455 [1992]) and 3) they infect certain cell types (*e.g.*, human lymphocytes) with low efficiency (Adams *et al.*, Proc. Natl. Acad. Sci. USA 89:8981 [1992]). The low titers associated with MoMLV-based vectors has been attributed, at least in part, to the instability of the virus-encoded envelope protein. Concentration of retrovirus stocks by physical means (*e.g.*, ultracentrifugation and ultrafiltration) leads to a severe loss of infectious virus.

The low titer and inefficient infection of certain cell types by MoMLV-based vectors has been overcome by the use of pseudotyped retroviral vectors which contain the G protein of VSV as the membrane associated protein. Unlike retroviral envelope proteins which bind to a specific cell surface protein receptor to gain entry into a cell, the VSV G protein interacts with a phospholipid component of the plasma membrane (Mastromarino *et al.*, J. Gen. Virol., 68:2359 [1977]). Because entry of VSV into a cell is not dependent upon the presence of specific protein receptors, VSV has an extremely broad host range.

Pseudotyped retroviral vectors bearing the VSV G protein have an altered host range characteristic of VSV (*i.e.*, they can infect almost all species of vertebrate, invertebrate and

insect cells). Importantly, VSV G-pseudotyped retroviral vectors can be concentrated 2000-fold or more by ultracentrifugation without significant loss of infectivity (Burns *et al.*, Proc. Natl. Acad. Sci. USA, 90:8033 [1993]).

The VSV G protein has also been used to pseudotype retroviral vectors based upon the human immunodeficiency virus (HIV) (Naldini *et al.*, Science 272:263 [1996]). Thus, the VSV G protein may be used to generate a variety of pseudotyped retroviral vectors and is not limited to vectors based on MoMLV.

The present invention is not limited to the use of the VSV G protein when a viral G protein is employed as the heterologous membrane-associated protein within a viral particle.

The G proteins of viruses in the Vesiculovirus genera other than VSV, such as the Piry and Chandipura viruses, that are highly homologous to the VSV G protein and, like the VSV G protein, contain covalently linked palmitic acid (Brun *et al.*, Interviol., 38:274 [1995]; and Masters *et al.*, Virol., 171:285 [1990]). Thus, the G protein of the Piry and Chandipura viruses can be used in place of the VSV G protein for the pseudotyping of viral particles. In addition, the VSV G proteins of viruses within the Lyssa virus genera such as Rabies and Mokola viruses show a high degree of conservation (amino acid sequence as well as functional conservation) with the VSV G proteins. For example, the Mokola virus G protein has been shown to function in a manner similar to the VSV G protein (*i.e.*, to mediate membrane fusion) and therefore may be used in place of the VSV G protein for the pseudotyping of viral particles (Mebatsion *et al.*, J. Virol., 69:1444 [1995]). Viral particles may be pseudotyped using either the Piry, Chandipura or Mokola G protein as described in the art with the exception that a plasmid containing sequences encoding either the Piry, Chandipura or Mokola G protein under the transcriptional control of a suitable promoter element (*e.g.*, the CMV intermediate-early promoter; numerous expression vectors containing the CMV IE promoter are available, such as the pcDNA3.1 vectors [Invitrogen]) is used in place of pHCMV-G. Sequences encoding other G proteins derived from other members of the Rhabdoviridae family may be used; sequences encoding numerous rhabdoviral G proteins are available from the GenBank database.

B. Integration of Retroviral DNA

The majority of retroviruses can transfer or integrate a double-stranded linear form of the virus (the provirus) into the genome of the recipient cell only if the recipient cell is cycling (*i.e.*, dividing) at the time of infection. Retroviruses that have been shown to infect
5 dividing cells exclusively, or more efficiently, include MLV, spleen necrosis virus, Rous sarcoma virus and human immunodeficiency virus (HIV; while HIV infects dividing cells more efficiently, HIV can infect non-dividing cells).

It has been shown that the integration of MLV virus DNA depends upon the host cell's progression through mitosis and it has been postulated that the dependence upon
10 mitosis reflects a requirement for the breakdown of the nuclear envelope in order for the viral integration complex to gain entry into the nucleus (Roe *et al.*, EMBO J., 12:2099 [1993]). However, as integration does not occur in cells arrested in metaphase, the breakdown of the nuclear envelope alone may not be sufficient to permit viral integration; there may be additional requirements such as the state of condensation of the genomic DNA
15 (Roe *et al.*, *supra*).

C. Introduction of Retroviral Vectors into Gametes Before the Last Meiotic Division

The nuclear envelope of a cell breaks down during meiosis as well as during mitosis.
20 Meiosis occurs only during the final stages of gametogenesis. The methods of the present invention exploit the breakdown of the nuclear envelope during meiosis to permit the integration of recombinant retroviral DNA and permit for the first time the use of unfertilized oocytes (*i.e.*, pre-fertilization and pre-maturation oocytes) as the recipient cell for retroviral gene transfer for the production of transgenic animals. Because infection of
25 unfertilized oocytes permits the integration of the recombinant provirus prior to the division of the one cell embryo, all cells in the embryo will contain the proviral sequences.

Oocytes which have not undergone the final stages of gametogenesis are infected with the retroviral vector. The injected oocytes are then permitted to complete maturation with the accompanying meiotic divisions. The breakdown of the nuclear envelope during
30 meiosis permits the integration of the proviral form of the retrovirus vector into the genome of the oocyte. When pre-maturation oocytes are used, the injected oocytes are then cultured

in vitro under conditions that permit maturation of the oocyte prior to fertilization *in vitro*. Conditions for the maturation of oocytes from a number of mammalian species (*e.g.*, bovine, ovine, porcine, murine, caprine) are well known to the art. In general, the base medium used herein for the *in vitro* maturation of bovine oocytes, TC-M199 medium, may be used for the *in vitro* maturation of other mammalian oocytes. TC-M199 medium is supplemented with hormones (*e.g.*, luteinizing hormone and estradiol) from the appropriate mammalian species. The amount of time a pre-maturation oocyte must be exposed to maturation medium to permit maturation varies between mammalian species as is known to the art. For example, an exposure of about 24 hours is sufficient to permit maturation of bovine oocytes while porcine oocytes require about 44-48 hours.

Oocytes may be matured *in vivo* and employed in place of oocytes matured *in vitro* in the practice of the present invention. For example, when porcine oocytes are to be employed in the methods of the present invention, matured pre-fertilization oocytes may be harvested directly from pigs that are induced to superovulate as is known to the art. Briefly, on day 15 or 16 of estrus the female pig(s) is injected with about 1000 units of pregnant mare's serum (PMS; available from Sigma and Calbiochem). Approximately 48 hours later, the pig(s) is injected with about 1000 units of human chorionic gonadotropin (hCG; Sigma) and 24-48 hours later matured oocytes are collected from oviduct. These *in vivo* matured pre-fertilization oocytes are then injected with the desired retroviral preparation as described herein. Methods for the superovulation and collection of *in vivo* matured (*i.e.*, oocytes at the metaphase 2 stage) oocytes are known for a variety of mammals (*e.g.*, for superovulation of mice, see Hogan *et al.*, *supra* at pp. 130-133 [1994]; for superovulation of pigs and *in vitro* fertilization of pig oocytes see Cheng, Doctoral Dissertation, Cambridge University, Cambridge, United Kingdom [1995]).

Retroviral vectors capable of infecting the desired species of non-human animal, which can be grown and concentrated to very high titers (*e.g.*, 1×10^8 cfu/ml) are preferentially employed. The use of high titer virus stocks allows the introduction of a defined number of viral particles into the perivitelline space of each injected oocyte. The perivitelline space of most mammalian oocytes can accommodate about 10 picoliters of injected fluid (those in the art know that the volume that can be injected into the perivitelline space of a mammalian oocyte or zygote varies somewhat between species as

the volume of an oocyte is smaller than that of a zygote and thus, oocytes can accommodate somewhat less than can zygotes).

The vector used may contain one or more genes encoding a protein of interest; alternatively, the vector may contain sequences that produce anti-sense RNA sequences or ribozymes. The infectious virus is microinjected into the perivitelline space of oocytes (including pre-maturation oocytes) or one cell stage zygotes. Microinjection into the perivitelline space is much less invasive than the microinjection of nucleic acid into the pronucleus of an embryo. Pronuclear injection requires the mechanical puncture of the plasma membrane of the embryo and results in lower embryo viability. In addition, a higher level of operator skill is required to perform pronuclear injection as compared to perivitelline injection. Visualization of the pronucleus is not required when the virus is injected into the perivitelline space (in contrast to injection into the pronucleus); therefore injection into the perivitelline space obviates the difficulties associated with visualization of pronuclei in species such as cattle, sheep and pigs.

The virus stock may be titered and diluted prior to microinjection into the perivitelline space so that the number of proviruses integrated in the resulting transgenic animal is controlled. The use of a viral stock (or dilution thereof) having a titer of 1×10^8 cfu/ml allows the delivery of a single viral particle per oocyte. The use of pre-maturation oocytes or mature fertilized oocytes as the recipient of the virus minimizes the production of animals which are mosaic for the provirus as the virus integrates into the genome of the oocyte prior to the occurrence of cell cleavage.

In order to deliver, on average, a single infectious particle per oocyte, the micropipets used for the injection are calibrated as follows. Small volumes (*e.g.*, about 5-10 pl) of the undiluted high titer viral stock (*e.g.*, a titer of about 1×10^8 cfu/ml) are delivered to the wells of a microtiter plate by pulsing the micromanipulator. The titer of virus delivered per a given number of pulses is determined by diluting the viral stock in each well and determining the titer using a suitable cell line (*e.g.*, the 208F cell line) as described in the art. The number of pulses which deliver, on average, a volume of virus stock containing one infectious viral particle (*i.e.*, gives a MOI of 1 when titered on 208F cells) are used for injection of the viral stock into the oocytes.

Prior to microinjection of the titered and diluted (if required) virus stock, the cumulus cell layer is opened to provide access to the perivitelline space. The cumulus cell layer need not be completely removed from the oocyte and indeed for certain species of animals (*e.g.*, cows, sheep, pigs, mice) a portion of the cumulus cell layer must remain in contact with the oocyte to permit proper development and fertilization post-injection. Injection of viral particles into the perivitelline space allows the vector RNA (*i.e.*, the viral genome) to enter the cell through the plasma membrane thereby allowing proper reverse transcription of the viral RNA.

10 **D. Detection of the Retrovirus Following Injection into Oocytes or Embryos**

The presence of the retroviral genome in cells (*e.g.*, oocytes or embryos) infected with pseudotyped retrovirus may be detected using a variety of means. The expression of the gene product(s) encoded by the retrovirus may be detected by detection of mRNA corresponding to the vector-encoded gene products using techniques well known to the art (*e.g.*, Northern blot, dot blot, *in situ* hybridization and RT-PCR analysis). Direct detection of the vector-encoded gene product(s) is employed when the gene product is a protein which either has an enzymatic activity (*e.g.*, α -galactosidase) or when an antibody capable of reacting with the vector-encoded protein is available.

Alternatively, the presence of the integrated viral genome may be detected using Southern blot or PCR analysis. For example, the presence of the LZRNL or LSRNL genomes may be detected following infection of oocytes or embryos using PCR as follows. Genomic DNA is extracted from the infected oocytes or embryos (the DNA may be extracted from the whole embryo or alternatively various tissues of the embryo may be examined) using techniques well known to the art. The LZRNL and LSRNL viruses contain the *neo* gene and the following primer pair can be used to amplify a 349-bp segment of the *neo* gene: upstream primer: 5'-GCATTGCATCAGCCATGATG-3' (SEQ ID NO:103) and downstream primer: 5'-GATGGATTGCACGCAGGTTC-3' (SEQ ID NO:104). The PCR is carried out using well known techniques (*e.g.*, using a GeneAmp™ kit according to the manufacturer's instructions [Perkin-Elmer]). The DNA present in the reaction is denatured by incubation at 94EC for 3 min followed by 40 cycles of 94EC for 1 min, 60EC for 40 sec and 72EC for 40 sec followed by a final extension at 72EC for 5 min. The PCR products

may be analyzed by electrophoresis of 10 to 20% of the total reaction on a 2% agarose gel; the 349-bp product may be visualized by staining of the gel with ethidium bromide and exposure of the stained gel to UV light. If the expected PCR product cannot be detected visually, the DNA can be transferred to a solid support (*e.g.*, a nylon membrane) and
5 hybridized with a ³²P-labeled *neo* probe.

Southern blot analysis of genomic DNA extracted from infected oocytes and/or the resulting embryos, offspring and tissues derived therefrom is employed when information concerning the integration of the viral DNA into the host genome is desired. To examine the number of integration sites present in the host genome, the extracted genomic DNA is
10 typically digested with a restriction enzyme, which cuts at least once within the vector sequences. If the enzyme chosen cuts twice within the vector sequences, a band of known (*i.e.*, predictable) size is generated in addition to two fragments of novel length which can be detected using appropriate probes.

15 E. Detection of Foreign Protein Expression in Transgenic Animals

The present invention also provides transgenic animals that are capable of expressing foreign proteins in their milk, urine and blood. The transgene is stable, as and shown to be passed from a transgenic bull to his offspring. In addition, the transgenic animals produced according to the present invention express foreign proteins in their body
20 fluids (*e.g.*, milk, blood, and urine). Thus, the present invention further demonstrates the utility of using the MoMLV LTR as a promoter for driving the constitutive production of foreign proteins in transgenic cattle. It is also contemplated that such a promoter could be used to control expression of proteins that would prevent disease and/or infection in the transgenic animals and their offspring, or be of use in the production of a consistent level of
25 protein expression in a number of different tissues and body fluids.

For example, it is contemplated that the MoMLV LTR of the present invention will find use in driving expression of antibody to pathogenic organisms, thereby preventing infection and/or disease in transgenic animals created using the methods of the present invention. For example, it is contemplated that antibodies directed against organisms such
30 as *E. coli*, *Salmonella ssp.*, *Streptococcus ssp.*, *Staphylococcus spp.*, *Mycobacterium spp.*, produced by transgenic animals will find use preventing mastitis, scours, and other diseases

that are common problems in young animals. It is also contemplated that proteins expressed by transgenic animals produced according to the present invention will find use as bacteriostatic, bactericidal, fungistatic, fungicidal, viricidal, and/or anti-parasitic compositions. Thus, it is contemplated that transgenic animals produced according to the present invention will be resistant to various pathogenic organisms. Furthermore, the milk produced by female transgenic animals would contain substantial antibody levels. The present invention contemplates that these antibodies are useful in the protection of other animals (e.g., through passive immunization methods).

VI. Considerations for Combating *Cryptosporidium* and Other Parasites

A. Production of Transgenic Expression System for Monoclonal 3E2 Antibodies Against *C. parvum*

In certain embodiments, the present invention uses an established hybridoma line (as described herein) as a source for the 3E2 genes for insertion into a replication defective retrovector. While the present invention is not limited to any mechanism, it is contemplated that 3E2 has especially potent neutralizing capabilities against sporozoites because it is of the IgM isotype. It is thought that through binding to repetitive epitopes of the CSL antigen the circumsporozoite precipitate (CSP)-like reaction is induced (M.W. Riggs *et al.*, J. Immunol., 143:1340-1345 [1989]) that renders the sporozoite non-infective. IgM antibodies exist in several forms, one, in unstimulated B-lymphocytes they are membrane-bound and, two, upon stimulation of the B-lymphocyte, IgM is secreted as a pentamer joined by the J-chain. J-chain expression plays an important role in inducing the pentamerization process of IgM. In studies done by Niles *et al.*, high expression of the J-chain resulted in a high percentage of pentameric IgM. (M.J. Niles *et al.*, Proc. Natl. Acad. Sci. USA, 92:2884-2888 [1995]). A third possible configuration for IgM was shown to be a hexamer. (A. Cattaneo and M.S. Neuberger, EMBO J., 6:2753-2758; and T.D. Randall *et al.*, Eur. J. Immunol., 20:1971-1979 [1990]). In one embodiment, the present invention specifically provides a cloning strategy that addresses the pentamer and hexamer configurations. In some embodiments, the hexamer configuration of IgM is contemplated to provide better efficacy against *Cryptosporidium* sporozoites than IgG.

In some embodiments, an IgM isotype control (of irrelevant specificity) is constructed in parallel following the cloning strategy described herein. Briefly, the retrovectors are pseudotyped with VSVg to give pantropic infectivity and used to achieve gene transfer to bovine oocytes and to CHO cells (component C). For transgenic expression
5 in mammals (e.g., bovines), as opposed to expression in cell culture, the construct is designed to remove antibiotic-based selection markers (i.e., undesirable in an animal population), and to insert a promoter that links expression closely to lactation thus restricting expression to the mammary cells. In some embodiments, an alphalactalbumin promoter is used for this purpose. To assure high probability of infection and transgene
10 integration into the oocyte genome, very high retrovector titer is needed for injection into the very small perivitelline space. It is contemplated that using pseudotyped VSVG vector envelope stabilizes the vector and increases the ability to concentrate vector sufficiently for injection in picoliter amounts. Preferably, transgenic embryos are produced by injection of unfertilized oocytes, *in vitro* fertilization, and transfer to recipient animals (e.g., surrogate
15 bovine mothers). After transgenic offspring have been verified as transgenic and grown to 6-8 months, a hormone regimen is used to initiate lactation.

A consideration in using retrovectors is the need to provide assurances that no reversion, recombination, or mutation of replication defective retrovectors to viral competence has occurred. Thus, in preferred embodiments a testing protocol is followed for
20 testing packaging cell lines and transgenic offspring.

In some embodiments, two different IRES elements are used to reduce the likelihood of recombination events that can be triggered by different identical sequences in a vector. The use of the IRES element in between heavy and light chain genes has been tested extensively and proven to yield fully functional antibodies, expressed and secreted into the
25 medium at high levels (up to 100 pg/cell/day in CHO cells in serum free medium).

B. Selection and Testing of Biocides, and Preparation of Vector for *Cryptosporidium* Neutralizing Monoclonal Antibodies and Fusion Proteins

30 In some additional embodiments, additional antibodies are selected from a large previously reported test panel. (See, X.D. *et al.*, Infect. Immun., 64:5161-5165 [1996]). For

example, 1E10 is an IgG1 isotype, that targets the P23 antigen; 3H2 is an IgM, that targets the GP25-200 antigen. Because, in some embodiments, IgG may be preferred for biocide fusion proteins, The present invention also expresses the 4H9 antibody. 4H9 is an IgG that targets GP25-200, but a different epitope than 3H2. (D.A. Schaefer *et al.*, Infect. Immun., 5 68:2608-2616 [2000]). In one embodiment, a 4 different antibody-biocide fusion types (Figure 2) from each IgG antibody are constructed. These molecules are expressed in the GPEX cell culture system (Gala Design, Middleton, WI) and tested for their efficacy against sporozoites *in vitro* and *in vivo*. The considerations pertaining to production of tricistronic constructs for IgM, discussed above with respect to 3E2, are also relevant to 3H2.

10 To select appropriate biocides, the present invention contemplates expanding the preliminary testing of sporozoite neutralization by potential biocides to include additional candidates, and comparison of human PLA2 to bee venom PLA2.

In some preferred embodiments, molecular modeling is used to guide the structural assembly of the fusion molecules. The relative geometry of a monoclonal antibody 15 molecule with a molecule of biocidal activity attached to the C-terminus is similar to that of complement binding to the Fc region of the MAb HC when bound to a pathogen, which results in destruction of the membrane. Thus, the present invention contemplates using the *C. parvum* binding site affinity of the MAb molecule to bring the biocidal activity into close apposition to its substrate by attachment of the biocide to the C-terminus of the monoclonal 20 heavy chain.

Secretory PLA2 is a relatively small molecule (~14 kDa) and is comparable in size to one of the CH1 or CH2 domains of an antibody molecule. As an alternative, an N-terminal extension linker on the PLA2 portion of the molecule is created to move the phospholipase domains a short distance from the MAb molecule. One linker contemplated 25 for use for constructing single chain monoclonal-cytokine fusion proteins is a -(Gly4-Ser)3-extension (~16-20 angstrom extension). (See *e.g.*, C.R. Robinson and R.T. Sauer, Proc. Natl. Acad. Sci. USA, 95:5929-5934 [1998]). This is a relatively neutral sequence that is flexible and does not have a strong structure-forming propensity. In another exemplary embodiment, the present invention inserts a proline into the middle of the extension arm to 30 provide a "kink", with freedom to rotate in the extension chain and thus allow different geometrical relationships between the biocide and the antibody molecule.

C. Expression of Monoclonal Antibodies and Monoclonal Antibody-Biocide Fusions in Cell Culture and Animal Models

In some preferred embodiments, the present invention provides animal based expression systems for producing large quantities of present compositions, while, in other preferred embodiments, the present invention provides high yielding cell lines, prepared. In some embodiments, cell based production is more expensive and on a smaller scale than production in transgenic animals (e.g., bovines), significant quantities of antibodies and fusion products are rapidly obtainable as compared to the proposed transgenic-derived products.

After expression and testing *in vitro* the present invention, contemplates scale up production using roller bottles to make sufficient recombinant product to test in mice. Then the most promising compounds, based on their efficacy in mice, are tested in an animal model where clinical disease is observed. The neonatal mouse model provides an essential, cost-effective means for the initial *in vivo* evaluation of product efficacy in reducing intestinal infection levels and is widely accepted for this purpose. However, *C. parvum* infection in neonatal mice does not cause diarrhea or other signs of disease, hence the need for subsequent evaluation in a clinical model for compositions having demonstrated anti-cryptosporidial activity in mice.

In some embodiments, piglets are selected as the clinical model of choice because of their small size, availability in adequate numbers to permit comparative studies and statistical analysis, and development of intestinal lesions resulting in acute watery diarrhea, dehydration, malabsorption, and weight loss when infected with *C. parvum* (C.W. Kim, Cryptosporidiosis in Pigs and Horses. In: J.P. Dubey, C.A. Speer, and R. Fayer eds. Boca Raton, FL: CRC Press, pp. 105-111 [1990]). Importantly, as monogastrics, the pathogenesis and control of cryptosporidiosis in piglets is thought to closely model that of human infections and response to treatment. (S. Tzipori, Adv. Parasitol., 40:187-221 [1998]).

Criteria for determining efficacy in piglets include, but are not limited to, clinical signs, weight loss, fecal volume and dry matter, and fecal oocyst quantitation and duration of shedding. Following euthanasia at 10 days post infection, extensive histopathological examination complete the data set.

VII. Transgenic Plant Technologies

In some embodiments, the fusion proteins of the present invention are expressed in transgenic organisms such as transgenic plants having a transgene inserted into its nuclear or plastidic genome. Techniques of plant transformation are known as the art. (See e.g., Wu
 5 and Grossman, *Methods in Enzymology*, Vol. 153, Recombinant DNA Part D, Academic Press [1987], and EP 693554). Foreign

nucleic acids can be introduced into plant cells or protoplasts by several methods. For example, nucleic acid can be mechanically transferred by microinjection directly into plant cells by use of micropipettes. In some embodiments, foreign nucleic acid can also be
 10 transferred into plant cells by using polyethylene glycol to form a precipitation complex with the genetic material that is taken up by the cell. (See e.g., Paszkowski *et al.*, *J. EMBO*, 3:2712-2722 [1984]). In other embodiments, foreign nucleic acid are introduced into plant cells by electroporation. (See e.g., Fromm *et al.*, *Proc. Nat. Acad. Sci. USA*, 82:5824 [1985]). Briefly, plant protoplasts are electroporated in the presence of plasmids or nucleic
 15 acids containing the relevant genetic construct. Electrical impulses of high field strength reversibly permeabilize the plant cell's biomembranes thus allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and form a plant callus. Preferably, selection of the transformed plant cells with the transformed gene is accomplished using phenotypic markers.

20 In certain other embodiments, the cauliflower mosaic virus (CaMV) is used as a vector to introduce foreign nucleic acids into plant cells. (See e.g., Hohn *et al.*, "Molecular Biology of Plant Tumors," Academic Press, New York, pp. 549-560 [1982]; and US 4,407,956). CaMV viral DNA genome is inserted into a parent bacterial plasmid creating a recombinant DNA molecule which can be
 25 propagated in bacteria. The recombinant plasmid can be further modified by introduction of the desired DNA sequence. The modified viral portion of the recombinant plasmid is then excised from the parent bacterial plasmid, and used to inoculate the plant cells or plants.

High velocity ballistic penetration by small particles can be used to introduce foreign nucleic acid into plant cells. Nucleic acid is disposed within the matrix of small beads or
 30 particles, or on the surface. (See e.g., Klein *et al.*, *Nature*, 327:70-73 [1987]). Although

typically only a single introduction of a new nucleic acid segment is required, this method also provides for multiple introductions.

A nucleic acid can be introduced into a plant cell by infection of a plant cell, an explant, an inieristem or a seed with *Agrobacterium tumefaciens* transformed with the nucleic acid. Under appropriate conditions, the transformed plant cells are grown to form shoots, roots, and develop further into plants. The nucleic acids can be introduced into plant cells, for example, by means of the Ti plasmid of *Agrobacterium tumefaciens*. The Ti plasmid is transmitted to plant cells upon infection by *Agrobacterium tumefaciens*, and is stably integrated into the plant genome. (See *e.g.*, Horsch *et al.*, Science, 233:496-498 [1984]; and Fraley *et al.*, Proc. Nat. Acad. Sci. USA, 80:4803 [1983]).

Plants from which protoplasts can be isolated and cultured to give whole regenerated plants can be transformed so that whole plants are recovered which contain the transferred foreign gene. All plants that can be produced by regeneration from protoplasts can also be transfected using the process according to the invention (*e.g.*, cultivated plants of the genera *Fragaria*, *Lotus*, *Medicago*, *Onobrychis*, *Trifolium*, *Trigonella*, *Vigna*, *Citrus*, *Geranium*, *Manihot*, *Daucus*, *Arabidopsis*, *Brassica*, *Raphanus*, *Sinapis*, *Atropa*, *Capsicum*, *Hyoscyarnus*, *Lycopersicon*, *Nicotiana*, *Solarium*, *Petunia*, *Digitalis*, *Majorana*, *Ciohorium*, *Helianthus*, *Lactuca*, *Bromus*, *Asparagus*, *Antirrhinum*, *Hererocallis*, *Nemesia*, *Pelargoniwn*, *Panicum*, *Pennisetum*, *Ranunculus*, *Senecio*, *Salpiglossis*, *Cucumis*, *Browaalia*, *Glycine*, *Lolium*, *Zea*, *Triticum*, *Sorghum*, *Datura*, *Solanum*, *Beta*, *Pisum*, *Phaseolus*, *Allium*, *Avena*, *Hordeum*, *Oryzae*, *Setaria*, *Secale*, *Sorghum*, *Triticum*, *Musa*, *Cocos*, *Cydonia*, *Pyrus*, *Malus*, *Phoenix*, *Elaeis*, *Rubus*, *Fragaria*, *Prunus*, *Arachis*, *Saccharum*, *Coffea*, *Camellia*, *Ananas*, or *Vitis*). In general, protoplasts are produced in accordance with conventional methods. (See *e.g.*, US 4,743,548; 4,677,066, 5,149,645; and 5,508,184).

Plant tissue may be dispersed in an appropriate medium having an appropriate osmotic potential (*e.g.*, 3 to 8 wt. % of a sugar polyol) and one or more polysaccharide hydrolases (*e.g.*, pectinase, cellulase, *etc.*), and the cell wall degradation allowed to proceed for a sufficient time to provide protoplasts. After filtration the protoplasts may be isolated by centrifugation and may then be resuspended for subsequent treatment or use.

Plant regeneration from cultured protoplasts is described in Evans *et al.*, "Protoplasts Isolation and Culture," Handbook of Plant Cell Cultures 1:124-176 (MacMillan Publishing Co. New York 1983); M.R. Davey, "Recent Developments in the Culture and Regeneration of Plant Protoplasts," Protoplasts (1983)-Lecture Proceedings, *pp.* 12-29, (Birkhauser, Basel 1983); P.J. Dale, "Protoplast Culture and Plant Regeneration of Cereals and Other Recalcitrant Crops," Protoplasts (1983)-Lecture Proceedings, *pp.* 31-41, (Birkhauser, Basel 1983); and H. Binding, "Regeneration of Plants," Plant Protoplasts, *pp.* 21-73, (CRC Press, Boca Raton 1985).

Regeneration from protoplasts varies from species to species of plants, but generally a suspension of transformed protoplasts containing copies of the exogenous sequence is first generated. In certain species, embryo formation can then be induced from the protoplast suspension, to the stage of ripening and germination as natural embryos. The culture media can contain various amino acids and hormones, such as auxins and cytokinins. It can also be advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Shoots and roots normally develop simultaneously. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is fully reproducible and repeatable.

In vegetatively propagated crops, the mature transgenic plants can be propagated by the taking of cuttings or by tissue culture techniques to produce multiple identical plants for trailing, such as testing for production characteristics. Selection of a desirable transgenic plant is made and new varieties are obtained thereby, and propagated vegetatively for commercial sale. In seed propagated crops, the mature transgenic plants can be self crossed to produce a homozygous inbred plant. The inbred plant produces seed containing the gene for the newly introduced foreign gene activity level. These seeds can be grown to produce plants that have the selected phenotype. The inbreds according to this invention can be used to develop new hybrids. In this method, a selected inbred line is crossed with another inbred line to produce the hybrid.

Parts obtained from a transgenic plant, such as flowers, seeds, leaves, branches, fruit, and the like are covered by the invention, provided that these parts include cells which have been so transformed. Progeny and variants, and mutants of the regenerated plants are also included within the scope of this invention, provided that these parts comprise the

introduced DNA sequences. Progeny and variants, and mutants of the regenerated plants are also included within the scope of this invention.

Selection of transgenic plants or plant cells can be based upon a visual assay, such as observing color changes (*e.g.*, a white flower, variable pigment production, and uniform
 5 color pattern on flowers or irregular patterns), but can also involve biochemical assays of either enzyme activity or product quantitation. Transgenic plants or plant cells are grown into plants bearing the plant part of interest and the gene activities are monitored, such as by visual appearance (for flavonoid genes) or biochemical assays (Northern blots); Western
 10 blots; enzyme assays and flavonoid compound assays, including spectroscopy. (*See e.g.*, Harborne *et al.*, (Eds.) "The Flavonoids, Vols: 1 and 2, Acad. Press 1975). Appropriate plants are selected and further evaluated. Methods for generation of genetically engineered plants are further described in US 5,283,184; 5,482,852, and EPO Application EP 693,554.

15 **VIII. Pharmaceutical Compositions**

The present invention provides novel methods and compositions for treating diseases characterized by pathogenic infection comprising administering subjects (*e.g.*, bovines, humans, and other mammals) a pharmaceutical and/or nutraceutical composition comprising
 20 chimeric recombinant antibodies either in food based (*e.g.*, whey protein) carriers, or common pharmaceutical carriers, including any sterile, biocompatible pharmaceutical carrier (*e.g.*, saline, buffered saline, dextrose, water, and the like) to subjects.

In some embodiments, the methods of the present invention comprise administering the compositions of the present invention in suitable pharmaceutical carriers. In some
 25 embodiments, these pharmaceutical compositions contain a mixture of at least two types of antibody-biocide compositions co-administered to a subject. In still further embodiments, the pharmaceutical compositions comprise a plurality of antibody-biocide compositions administered to a subject under one or more of the following conditions: at different periodicities, different durations, different concentrations, different administration routes,
etc.

30 In some preferred embodiments, the compositions and methods of the present invention find use in treating diseases or altered physiological states characterized by

pathogenic infection. However, the present invention is not limited to ameliorating (*e.g.*, treating) only these types of conditions in a subject. Indeed, various embodiments of the present invention are directed to treating a range of physiological symptoms and disease etiologies in subjects generally characterized by infection with a pathogen (*e.g.*, bacteria, 5 archaeae, viruses, mycoplasma, fungi, *etc.*).

Depending on the condition being treated, these pharmaceutical compositions are formulated and administered systemically or locally. Techniques for formulation and administration are found in the latest edition of "Remington's Pharmaceutical Sciences" (Mack Publishing Co, Easton Pa.). Accordingly, the present invention contemplates 10 administering pharmaceutical compositions in accordance with acceptable pharmaceutical delivery methods and preparation techniques. For example, some compounds of the present invention are administered to a subject intravenously in a pharmaceutically acceptable carrier such as physiological saline. For injection, the pharmaceutical compositions of the invention are formulated in aqueous solutions, preferably in physiologically compatible 15 buffers (*e.g.*, Hanks' solution, Ringer's solution, or physiologically buffered saline). For tissue or cellular administration, penetrants appropriate to the particular barrier to be permeated are preferably used in the formulations. Such penetrants are generally known in the art. Standard methods for intracellular delivery of pharmaceutical agents are used in other embodiments (*e.g.*, delivery via liposomes). Such methods are well known to those 20 skilled in the art.

In some embodiments, present compositions are formulated for parenteral administration, including intravenous, subcutaneous, intramuscular, and intraperitoneal. In some embodiments, these compositions optionally include aqueous solutions (*i.e.*, water-soluble forms). Additionally, suspensions of the active compounds may also be 25 prepared as oily injection suspensions as appropriate. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable 30 stabilizers or agents that increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Therapeutic co-administration of some contemplated compositions is also be accomplished using gene therapy techniques described herein and commonly known in the art.

In other embodiments, the present compositions are formulated using
5 pharmaceutically acceptable carriers and in suitable dosages for oral administration. Such carriers enable the compositions to be formulated as tablets, pills, capsules, dragees, liquids, gels, syrups, slurries, suspensions and the like, for oral or nasal ingestion by a patient to be treated.

Pharmaceutical preparations for oral use can be obtained by combining the active
10 compounds (*e.g.*, chimeric antibody biocide fusion proteins) with a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, *etc.*; cellulose such as methyl cellulose,
15 hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; and gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid or a salt thereof such as sodium alginate.

Ingestible formulations of the present compositions may further include any material
20 approved by the United States Department of Agriculture for inclusion in foodstuffs and substances that are generally recognized as safe (GRAS), such as, food additives, flavorings, colorings, vitamins, minerals, and phytonutrients. The term "phytonutrients" as used herein, refers to organic compounds isolated from plants that have a biological effect, and includes, but is not limited to, compounds of the following classes: isoflavonoids, oligomeric
25 proanthcyanidins, indol-3-carbinol, sulforaphane, fibrous ligands, plant phytosterols, ferulic acid, anthocyanocides, triterpenes, omega 3/6 fatty acids, polyacetylene, quinones, terpenes, catechins, gallates, and quercitin.

Dragee cores are provided with suitable coatings such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel,
30 polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee

coatings for product identification or to characterize the quantity of active compound, (*i.e.*, dosage).

Compositions of the present invention that can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients mixed with fillers or binders such as lactose or starches, lubricants such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycol with or without stabilizers.

In some embodiments of the present invention, therapeutic agents are administered to a patient alone, or in combination with one or more other drugs or therapies (*e.g.*, antibiotics and antiviral agents *etc.*) or in pharmaceutical compositions where it is mixed with excipient(s) or other pharmaceutically acceptable carriers. In one embodiment of the present invention, the pharmaceutically acceptable carrier is pharmaceutically inert.

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. For example, an effective amount of therapeutic compound(s) may be that amount that destroys or disables pathogens as compared to control pathogens.

In addition to the active ingredients, preferred pharmaceutical compositions optionally comprise pharmaceutically acceptable carriers, such as, excipients and auxiliaries that facilitate processing of the active compounds into preparations that can be used pharmaceutically.

In some embodiments, the pharmaceutical compositions used in the methods of the present invention are manufactured according to well-known and standard pharmaceutical manufacturing techniques (*e.g.*, by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes).

Dosing is dependent on severity and responsiveness of the disease state to be treated, with the course of treatment lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved. Optimal dosing schedules are calculated from measurements of composition accumulation in the subject's body. The

administering physician can easily determine optimum dosages, dosing methodologies and repetition rates. Optimum dosages may vary depending on the relative potency of compositions agents, and can generally be estimated based on the EC_{50} s found to be effective in *in vitro* and *in vivo* animal models. Additional factors that may be taken into account, include the severity of the disease state; the age, weight, and gender of the subject; the subject's diet; the time and frequency of administration; composition combination(s); possible subject reaction sensitivities; and the subject's tolerance/response to treatments. In general, dosage is from 0.001 μ g to 100 g per kg of body weight, and may be given once or more daily, weekly, monthly or yearly. The treating physician can estimate repetition rates for dosing based on measured residence times and concentrations of the drug in bodily fluids or tissues. Following successful treatment, it may be desirable to have the subject undergo maintenance therapy to prevent the recurrence of the disease state, wherein the therapeutic agent is administered in maintenance doses, ranging from 0.001 μ g to 100 g per kg of body weight, once or more daily, weekly, or other period.

For any compound used in the methods of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. Then, preferably, dosage can be formulated in animal models (particularly murine or rat models) to achieve a desirable circulating concentration range that results in increased PKA activity in cells/tissues characterized by undesirable cell migration, angiogenesis, cell migration, cell adhesion, and/or cell survival. A therapeutically effective dose refers to that amount of compound(s) that ameliorate symptoms of the disease state (*e.g.*, pathogenic infection). Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index, and it can be expressed as the ratio LD_{50}/ED_{50} . Compounds that exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and additional animal studies can be used in formulating a range of dosage, for example, mammalian use (*e.g.*, humans). The dosage of such compounds lies preferably, however the present invention is not limited to this range, within a range of circulating concentrations that include the ED_{50} with little or no toxicity.

Guidance as to particular dosages and methods of delivery is provided in the literature (*See*, US 4,657,760; 5,206,344; or 5,225,212).

Administration of some agents to a patient's bone marrow may necessitate delivery in a manner different from intravenous injections.

5

EXAMPLES

The present invention provides the following non-limiting examples to further describe certain contemplated embodiments of the present invention.

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EXAMPLE 1

Effects of PLA2 on Sporozoite Infectivity

This experiment describes the effects of PLA2 on sporozoite infectivity. Briefly, sporozoites were incubated in an isotonic saline solution (37 °C, 30 min) with a range of concentrations of PLA2 isolated from honey bee venom (Sigma-Aldrich Corp., St. Louis, MO, 1.5 U/μg protein). Control sporozoites were identically incubated in buffer containing concentration-matched BSA but no PLA2. Sporozoites were then washed in medium and inoculated onto replicate Caco-2 human intestinal epithelial cell monolayers. 24 h later, infection was quantified in test and control monolayers by immunofluorescence assay as described herein and the mean percent reduction of infection calculated. The results indicate that PLA2 achieved a highly significant reduction of sporozoite infectivity at a concentration as low as 0.014 U/ml (Figure 3, $P < 0.0005$). Percent viability of Caco-2 cells following exposure to PLA2-treated sporozoites (86%) was similar to that of uninoculated control cells (91%) at 24 hrs, as determined by trypan blue dye exclusion. This finding suggests little if any toxic effect of residual PLA2 on host cells. The data suggests that PLA2 is a viable candidate for antibody fusion. Through fusion of PLA2 to a monoclonal antibody with a high affinity for sporozoites, such as 1E10 or 4H9, lethal concentrations of biocide are deliverable to the sporozoite surface with relatively low amounts of biocide being used.

EXAMPLE 2

Target Antigens CSL, P23, and GP25-200 are Conserved in both Type 1 and Type 2 *C. parvum* Isolates

Western blotting of type 1 and type 2 *C. parvum* was performed to evaluate
5 expression of the antigens and epitopes defined by the monoclonal antibodies proposed
herein. For these studies, human *C. parvum* isolates were obtained from Peruvian patients
and genotyped by nested PCR primers designed to amplify a region within the 18S rRNA
gene, followed by RFLP analysis of the amplicons to differentiate Type 1 from Type 2
according to G.D. Sturbaum *et al.*, Appl. Environ. Microbiol., 67:2665-2668 [2001]). Two
10 human isolates determined to be of the Type 1 genotype were evaluated by Western blot for
recognition by monoclonal antibodies 3E2 (anti-CSL), 1E10 (anti-P23), and 3H2 (anti-
GP25-200) using previously described methods. (M.W. Riggs *et al.*, Infect. Immun.,
62:1927-1939 [1994]). The Iowa Type 2 isolate (J. Heine *et al.*, J. Infect. Dis., 150:768-775
[1984]) was examined in parallel. Each monoclonal bound to both Type 1 isolates. In
15 addition, the molecular weights and immunoreactivities of the Type 1 antigens recognized
by each monoclonal were indistinguishable from those recognized in the Type 2 isolate in
blots of antigen resolved by either 2-12% or 4-20% reducing SDS-PAGE. Importantly,
these findings suggest conservation of the antigens and epitopes defined by 3E2, 1E10, and
3H2 between Type 1 and Type 2 *C. parvum*, and are consistent with the functional role
20 ascribed to each antigen.

EXAMPLE 3

Various Genetic Engineering Techniques

The present example describes the isolation of the genes for the heavy, light, and J-
25 chains from the 3E2 murine hybridoma cell line, cloning into the retrovector backbone in
two configurations (for cell culture expression and for transgenic production), and clonal
analysis of the vector producing packaging lines to identify high titer lines maintaining the
fidelity of the protein.

In one embodiment, cells from the 3E2 hybridoma are used to extract total RNA
30 with the purpose of isolating the monoclonal antibody-specific heavy, light and J-chain
transcripts. Upon total RNA extraction, the RNA is reverse transcribed to create cDNA

using standard molecular biology protocols. The total cDNA is then used as a template to specifically amplify the mouse IgM-heavy and light chains as well as the J chain. Site-directed mutagenesis primers are used to amplify the sequences. The use of these primers adds short sequences of DNA that introduce suitable restriction sites that allow direct
5 cloning of the product into the retrovector backbone.

As mentioned herein, in some embodiments, two retroviral constructs are made containing the hybridoma-derived antibody genes. Preferably, one is a bicistronic construct, aimed at producing hexameric IgM. This construct bears the genes for IgM heavy and light chain and upon expression in a cell, spontaneous hexamer formation of IgM takes place.
10 The elements of this and other contemplated constructs are shown in Figures 4A-4D. The construct in Figure 4A provides a bicistronic antibody. The construct provided in Figure 4B is tricistronic and contains, in addition to the IgM heavy and light chain, the J-chain that for pentamer formation of IgM. (Figure 4B).

To create the tricistronic vector the following cloning steps are performed. First, the
15 heavy chain (HC) is cloned into the multiple cloning site (MCS) of the disclosed retrovector backbone. Second, the genes for the encephalomyocarditis virus (EMCV) IRES (internal ribosome entry site) element, the signal peptide (SP) and the IgM light chain (LC) are combined. Preferably, the IRES is engineered to optimize the secondary initiation of protein synthesis, thus allowing consistent performance in obtaining equimolar expression
20 of heavy and light chains. The genes for the foot-and-mouth disease virus (FMDV) IRES element, the SP and the J-chain gene are combined in parallel. (*See e.g.*, M. Harries *et al.*, J. Gene Med., 2:243-249 [2000]; and X.Y. Wen *et al.*, Cancer Gene Ther., 8:361-370 [2001]). Third, the IRES-SP-LC element is cloned into the backbone after the HC. Fourth, the third element of the construct, the J-chain, preceded by the second IRES is cloned into the
25 backbone. The present invention contemplates that using two different IRES elements reduces the likelihood of recombination events that are usually triggered by different identical sequences in a vector. The use of the IRES element in between heavy and light chain genes has been extensively tested and has proven to yield fully functional antibodies, expressed and secreted into the medium at exceptionally high levels (up to 100 pg/cell/day
30 in CHO cells in serum free medium).

Once the retroviral constructs are complete, quality control sequencing is used to confirm that all the elements are present. The retrovector construct are then used to transfect production cell lines using the GPEX system (Gala Design, Inc., Middleton, WI). Following production in the GPEX system, purified product (either pentameric or hexameric IgM 3E2) is tested using standard *in vitro* inhibition tests described herein and/or of known technologies.

In some embodiments, for the r3E2 monoclonal antibody to be expressed in the milk of cows, lactation specific promoter based on the bovine alpha-lactalbumin promoter is used (G.T. Bleck and R.D. Bremel, Gene, 126:213-218 [1993]), and the neo-selectable marker is removed from the construct. (See, Figures 4C and 4D). In a standard cloning step, the sCMV promoter used in the GPEX system is replaced with the alpha-lactalbumin promoter. Clonal analysis performed on the packaging cell lines to identify the antibody-producing clones that give the highest expression. In additional embodiments, an IgM isotype control (of irrelevant specificity) is constructed in parallel following the same cloning strategy.

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EXAMPLE 4

Production of Vector and Injection into Bovine Oocytes to Make Transgenic Embryos for Transfer to Recipient Animals (Cattle)

After quality assurance, the alphalactalbumin- bearing construct is used to transfect the 293 gp packaging cell line along with the plasmid encoding for the VSVg surface glycoprotein for pseudotyping the viral particles. (See, A.W. Chan *et al.*, Proc. Natl. Acad. Sci. USA, 95:14028-14033 [1998]). 293gp packaging cells used in this process are derived from working stocks supplied by Gala Designs, Inc. (Middleton, WI) for cGMP production. The resulting viral supernatant is used to infect packaging cells at a low virus to cell ratio so as to achieve single insertions of the virus. The packaging cell pool are then subjected to a clonal analysis and supernatants of single clones are monitored for high titer viral particle production. One clone is chosen based on viral titer and quality assurance for use in the TRANSGAMETIC system (Gala Design, Inc., Middleton, WI). Bovine oocytes are harvested and grown in culture to metaphase 2 arrest (16 hrs). At this stage, which is prolonged in the bovine oocyte, the nuclear membrane has dispersed, allowing vector to gain access to the nucleus. Pseudotyped vector injected into the perivitelline space infects

30

the oocyte and provirus is integrated into the oocyte's haploid DNA. Upon viral particle injection, the oocytes are fertilized using sexed semen, which allows for almost 100 % female calves to be born. The transgenic frequency is expected to be between 25-75%. Preferably, embryos are biopsied and screened by PCR for presence of the transgene prior to
5 transfer into recipients, to optimize the transfer of transgene positive embryos.

In vitro tests are done to confirm that the tricistronic expression of the 3E2 IgM antibody has no influence on specificity and affinity of this antibody. Following positive *in vitro* tests of the recombinant 3E2 monoclonal antibody and achievement of high numbers of transgenic embryos, the embryos are transferred into surrogate mothers. Accordingly,
10 groups of young mature female cattle (heifers) are hormonally synchronized to receive embryos at seven days post fertilization. Cattle are observed throughout pregnancy and ultrasounds are conducted to confirm pregnancy and sex of the embryo at 70 days. After the 280 day gestation period, calves are delivered by cesarian section (a routine surgery performed under epidural anesthesia in a standing surviving cow) and tested for the
15 transgene.

EXAMPLE 5

Confirmation of Transgene Presence

Following birth of the offspring they are tested for presence of the transgene and
20 raised to near puberty. Lactation is then hormonally induced to identify the best protein expression and to provide product for evaluation.

At the age of approximately 8 months, lactation is induced in transgenic heifers, using a hormonal regimen, and milk analyzed for expression of the r3E2 product. Product is collected, purified from whey, and quantified for efficacy studies in mice and piglets.

25 A progestin implant is used to simulate a short pseudopregnancy and then initiate milking in peripubertal (6-8 months old) heifers. Heifers should yield up to 250-1000 ml per day of milk, increasing rapidly to approximate a first lactation heifer yield of 15-20 liters a day. Subsequent fertility is not impaired. Milk product is tested for the presence of murine antibody using established Western blot and ELISA procedures. The animals are
30 milked until enough product is obtained to conduct efficacy testing in mice and the neonatal pig model using assays described herein.

For quantification to carry out efficacy testing in pigs, monoclonal antibody is purified after fat removal from milk by continuous flow centrifuge while the milk is at animal body temperature. A skim milk product is used for further processing. In some embodiments, size exclusion chromatography and tangential-flow ultrafiltration allow purification of sufficient amounts. MAbs are recovered from the milk serum with affinity chromatography or size exclusion chromatography with a similar efficiency as from cell culture fluids.

EXAMPLE 6

Evaluation of Efficacy of Milk Production

In some embodiments, efficacy studies of milk production are preformed in neonatal mouse and piglet models respectively. *In vivo* efficacy assays for *C. parvum* neutralizing r3E2 are preformed in mice. Studies of the effect of milk expressed 3E2 on the infectivity of *C. parvum* sporozoites in mice are performed as described herein.

In vivo efficacy assays for *C. parvum* neutralizing r3E2 are conducted in piglets. These studies are performed following the same protocol as described herein. Three groups of 8 piglets are assigned to treatment (milk derived r3E2), isotype rIgM control, and placebo control groups. Dosages, experimental regimens, and blinded evaluations are conducted as described herein.

EXAMPLE 7

Founder Animals

Lines of founder animals are identified for propagation to develop production herds. Suitable high expressing transgenic founder animals (*e.g.*, cattle) are identified and superovulated for propagation of a herd of production animals for large scale production of r3E2. Yields of r3E2 in milk are compared between founder animals and the best animal(s) selected for super ovulation and insemination. Embryos are harvested and stored in liquid nitrogen for future herd expansion.

EXAMPLE 8

Identification of Candidate for Expression as Recombinant Antibody Biocide Fusion Proteins

In some embodiments, various biocides are evaluated for potential neutralizing
5 activity against *C. parvum* sporozoites using the *in vitro* assay described in herein.
Candidate biocides include, but are not limited to: PLA2, both from human and bee venom;
protease inhibitors such as leupeptin, aprotinin, antipain, amastatin, and soybean trypsin
inhibitor; lysozyme; and phosphatidylinositol-specific phospholipase C. The preceding
protease inhibitor candidates were selected based on their reported activity against *C.*
10 *parvum*. (See e.g., J.R. Forney *et al.*, J. Parasitol., 82:638-640 [1996]; J.R. Forney *et al.*, J.
Parasitol., 83:771-774 [1997]; and P.C. Okhuysen *et al.*, Antimicrob. Agents Chemother.,
40:2781-2784 [1996]).

For this assay, isolated sporozoites are incubated (15 min, 37°C) with an individual
biocide in isotonic buffer over a range of concentrations that would theoretically be
15 achievable at the sporozoite surface by targeted delivery as a MAb-biocide fusion protein.
PLA2 concentrations are based in part on preliminary data which showed that < 0.02
units/ml was effective in neutralization. In parallel, viability of control sporozoites after
incubation with the selected biocide concentrations is determined by fluorescein diacetate
assay. (See, M.W. Riggs *et al.*, Infect. Immun., 62:1927-1939 [1994]). Following
20 incubation with biocide, sporozoites are washed, and then inoculated onto individual Caco-2
human intestinal epithelial cell monolayers grown in microscopy grade 96-well plates (10
replicates per treatment). For comparison, control monolayers are inoculated with
sporozoites identically incubated with: 1) MEM; 2) murine hybridoma-derived neutralizing
MAb 3E2 as a positive control; or 3) non-toxic control proteins such as BSA, each
25 concentration-matched to the biocide being tested. Samples are then processed and
evaluated as described herein. The mean numbers of intracellular parasite stages per host
cell in test and control cultures is examined for significant differences using ANOVA. Each
experiment is performed three times. In parallel experiments, to monitor potential host cell
toxicity of residual biocide, control monolayers are inoculated with the final wash medium
30 from biocide incubation tubes to which no sporozoites were added, but which otherwise
have been processed identically to test samples. Cell viability in control and sporozoite

inoculated monolayers is determined 24 hrs post-inoculation using an acridine orange-ethidium bromide viability assay and epifluorescence microscopy. (See, R.C. Duke and J.J. Cohen, Morphological and biochemical assays of apoptosis. John Wiley & Sons, New York, NY, [2002]).

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EXAMPLE 9

Isolation of Genes for Antibody Heavy, Light chains, and J chains

In some embodiments, the genes for antibody heavy, light chains, and J chains where applicable, are isolated from the 1E10, 3H2, and 4H9 hybridoma cell lines. The genes are
10 cloned into the GPEX retrovector (Gala Design, Inc., Middleton, WI) as standalone antibody constructs for each antibody, and for the IgG1s 1E10 and 4H9, as fusions to a biocide gene. In some embodiments, 4 structurally different antibody-biocide fusion variants are considered for 1E10 and 4H9. At the same time, vectors with a promoter suitable for transgenic expression are prepared.

15 The following hybridoma cell lines are used for antibody gene extraction: 3H2, which expresses an IgM against GP25-200; 4H9, which expresses an IgG1 against GP25-200; and 1E10, which expresses an IgG1 against P23. An isotype control for IgG is constructed and prepared in parallel using a hybridoma of irrelevant specificity. Total RNA is extracted from cells with the purpose of isolating the monoclonal antibody-specific heavy
20 and light chain genes as described herein. The immunoglobulin genes are be cloned into the GPEX retrovector backbone as bicistronic constructs; in the case of 3H2, the present invention contemplates apply a cloning strategy identical to the one applied for the 3E2 constructs described herein. (See, Figures 5A-5D). Standalone recombinant constructs of each antibody are produced. In addition, two IgG isotypes are engineered to contain a
25 biocide attached to either the N-terminus or the C-terminus of the antibody. The cDNA for the biocide found to be most effective in neutralizing *C. parvum* sporozoites *in vitro*, and least toxic to host cells is acquired through either the NIH Mammalian Gene Collection (human PLA2) or synthesized (Blue Heron Biotechnology, Seattle). The PLA2, or other biocide, cDNA is expanded through standard amplification in *E. coli* laboratory strains.
30 Plasmid are extracted and sequenced for quality control purposes. The biocide genes are then cloned into 4 different antibody fusion constructs using glycine-serine (G4S)3-4

linkers. When expressed in the GPEX system, these constructs produce: a full size antibody with a biocide fusion to either the N-terminus (Figure 5A) or to the C-terminus (Figure 5B) of the heavy chain; or a single chain antibody with a biocide fusion to the N-terminus of the light chain (Figure 5C) or to the C-terminus of the heavy chain (Figure 5D). The antibody-biocide fusions are tested for their efficacy in mediating neutralization and killing of sporozoites *in vitro* and reducing infection *in vitro* and *in vivo*.

Constructs are also prepared for the production of transgenic embryo monoclonal antibody to be expressed in the milk of cows, using a lactation specific promoter based on the bovine alpha-lactalbumin promoter (G.T. Bleck and R.D. Bremel, Gene, 126:213-218 [1993]), and the neo-selectable marker is removed from the construct (Figure 4). In a standard cloning step, the sCMV promoter used in the GPEX system (Gala design, Inc., Middleton, WI) will be replaced with the alpha-lactalbumin promoter.

Retrovector constructs are used to transduce host cells and produce pseudotyped replication deficient retrovector. Pool populations of transduced cells are subjected to a clonal selection, based on antibody levels present in the medium supernatant determined by *C. parvum* ELISA. Clones with the highest level of antibody secreted into the supernatant are chosen to produce milligram amounts of recombinant murine monoclonal antibody and monoclonal antibody-biocide fusions against *C. parvum*.

Constructs needed for transgenic cattle production are also prepared. Constructs contain lactation specific promoter based on the bovine alpha-lactalbumin promoter (G.T. Bleck and R.D. Bremel, *infra*), and no neo-selectable marker (Figure 4). In a standard cloning step, the sCMV promoter used in the GPEX system is replaced with the alpha-lactalbumin promoter.

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EXAMPLE 10

Cloning of Vector Constructs

In some embodiments, the above vector construct, and those for antibody 3E2 are clonally selected and expressed in the GPEX cell culture system (Gala Design, Inc., Middleton, WI) to obtain adequate quantities of assembled antibody or antibody-biocide fusion protein for testing *in vitro* and *in vivo*.

30

Briefly, the retrovector constructs prepared above are used to transform host cells along with the plasmid that encodes the vesicular stomatitis virus glycoprotein (VSV-G) used for pseudotyping the retrovirus. This procedure creates intermediate level viral titer that is used to infect production cell lines (CHO cells). CHO cells used in this process are derived from a working stock used to established a cGMP production. The population of transduced cells is subjected to a clonal selection, based on antibody levels present in the medium supernatant. Antibody levels are determined by standard ELISA methods using sporozoite lysate antigen prepared as described in Schaefer *et al.* (D.A. Schaefer *et al.*, Infect. Immun., 68:2608-2616 [2000]). The clones with the highest level of antibody secreted into the supernatant are chosen to produce milligram amounts of recombinant monoclonal antibody.

Using the GPEX cell culture in a roller bottle system, gram scale quantities of rMAbs 3E2, 3H2, 1E10, 4H9, and the rMAb-fusion parasitocides are expressed. Based on a 30 pg/cell/day average, one roller bottle produces approximately 20 mg product per week.

In some embodiments, complete product purification is unnecessary to formulate oral immunotherapies, especially when milk derived. However, in some embodiments, for the purposes of standardization of tests, purification of the monoclonals from tissue culture medium follows protocols established for other monoclonals. Briefly, harvested media is filtered through a 0.45 micron sterile filter to remove cells and the immunoglobulins (IgG1, IgG2, and IgG4) and are captured using a protein A affinity column, or in case of IgM, using HiTrap™ IgM Purification columns (Amersham Biosciences, Piscataway, NJ) or for the purification of single chain antibodies Thiophilic Resin columns (BD Biosciences Clontech, Palo Alto, CA). After washing, the immunoglobulins are eluted by low pH and the pooled eluate fractions are neutralized to pH 7.5. In some embodiments, a second chromatography step is employed to remove contaminants, host cell DNA and to act as a viral clearance step. This typically utilizes anion exchange chromatography (*e.g.*, Q-Sepharose™). The final polishing step utilizes size exclusion chromatography (*e.g.*, Sephadex™200), to separate aggregates from monomers. Antibody are further concentrated or formulated as required.

EXAMPLE 11

**Recombinant Monoclonal Antibodies and Monoclonal Antibody Biocide Fusion
Products Efficacy in Neutralizing Sporozoites *In vitro***

In some embodiments, recombinant monoclonal antibodies and monoclonal antibody
5 biocide fusion products expressed herein are tested for their efficacy in neutralizing
sporozoites *in vitro*.

Prior to testing in neutralization assays, the monoclonals are evaluated for retention
of sporozoite and merozoite reactivity by IFA, and for antigen specificity by Western
immunoblot. (See e.g., M.W. Riggs *et al.*, Infect. Immun., 62:1927-1939 [1994]; M.W.
10 Riggs *et al.*, J. Immunol., 158:1787-1795 [1997]).

In vitro* neutralization assay for *C. parvum

To quantify specific neutralizing activity of each of the four MABs and the fusion
biocides against the infective sporozoite stage, an *in vitro* neutralization assay is used. (See,
15 R.C. Langer *et al.*, Infect. Immun., 67:5282-5291 [1999]). The antibody-biocide fusions
based on the r1E10 and r4H9 antibodies in the four configurations depicted in Figure 5, and
full size versions of r1E10, r4H9, and r3H2 are each tested individually. For this assay,
isolated sporozoites are incubated with the selected MAB (10 µg/ml final concentration),
then inoculated onto individual Caco-2 human intestinal epithelial cell monolayers (ATCC
20 HTB37) (M. Pinto *et al.*, Biol. Cell, 47:323-330 [2002]). Prior to inoculation, monolayers
of Caco-2 cells are grown to ~90% confluency in microscopy grade 96-well tissue culture
plates. For comparison, control monolayers are inoculated with sporozoites which have
been identically incubated with: 1) tissue culture medium (MEM); 2) murine hybridoma-
derived neutralizing monoclonal; or 3) isotype- and concentration- matched recombinant
25 control MAb of irrelevant specificity. Ten replicates are performed for each treatment.
After incubation, inoculation medium is aspirated from monolayers and replaced with
MEM. At 24 hrs post-inoculation, monolayers are washed, fixed, blocked, and processed
for automated immunofluorescence assay (IFA) using MAb 4B10 and AlexaFluor™488
affinity-purified goat anti-mouse IgM to detect intracellular stages. MAb 4B10, prepared
30 against *C. parvum* as previously described (M.W. Riggs *et al.*, J. Immunol. 158:1787-1795
[1997]), recognizes all parasite stages in Caco-2 cells through 72 hrs post-inoculation.

(R.C. Langer and M.W. Riggs, Infect. Immun., 67:5282-5291 [1999]). Intestinal epithelial cell nuclei are counterstained with 300 nM 4,6-diamidino-2-phenylindole. Using an Olympus-IMT2 inverted microscope equipped for automated digital image capture, 50 standardized visual fields per well are read and stored on the program computer.

- 5 Intracellular parasite stages and epithelial cell nuclei are then quantified using Compix SimplePCI software (Compix, Inc., Cranberry Township, PA). Mean numbers of intracellular parasite stages per host cell in test and control cultures are examined for significant differences using ANOVA. Each experiment is performed three times. BSL-2 precautions are observed to prevent accidental infection of project personnel with *C.*
- 10 *parvum*.

***Cryptosporidium parvum* propagation for use in the proposed studies**

- The Iowa *C. parvum* isolate (J. Heine *et al.*, J. Infect. Dis., 150:768-775 [1984]) (genotype 2, bovine origin) has been maintained since 1988 by propagation in newborn
- 15 *Cryptosporidium*-free calves (M.W. Riggs *et al.*, J. Immunol., 143:1340-1345 [1989]; and M.W. Riggs and L.E. Perryman, Infect. Immun., 55:2081-2087 [1987]). This well-characterized isolate is infectious for humans and animal models, including neonatal mice and pigs. (See *e.g.*, J. Heine *et al.*, J. Infect. Dis., 150:768-775 [1984]; R.C. Langer and M.W. Riggs, Infect. Immun., 67:5282-5291 [1999]; H.W. Moon and W.J. Bemrick, Vet.
- 20 Pathol., 18:248-255 [1981]; and S. Tzipori H. and Ward, Microbes. Infect., 4:1047 [2002]). Parasites are obtained by propagation in newborn calves as previously described. (M.W. Riggs and L.E. Perryman, *supra*). Oocysts are isolated from the feces of experimentally infected calves as previously described, and stored in 2.5% KCr₂O₇ (4 °C) (M.J. Arrowood K. and Donaldson *et al.*, J. Eukaryot. Microbiol., 43:895 [1996]; and M.W. Riggs and L.E.
- 25 Perryman, *supra*). To obtain isolated sporozoites, oocysts are hypochlorite-treated prior to excystation, then passed through a sterile polycarbonate filter. For mouse and piglet experiments, oocysts are used within 30 days of isolation and disinfected with 1% peracetic acid prior to administration.

EXAMPLE 12

In vivo Neutralizing Activity Assays

Each of the monoclonal antibody-biocide fusions based on the r1E10 and r4H9 antibodies and monoclonal antibodies r3E2, r1E10, r4H9, and r3H2 determined to have significant *in vitro* sporozoite neutralizing activity, is individually tested to quantify *in vivo* efficacy against infection. The neonatal mouse model is used. (See, M.W. Riggs MW and L.E. Perryman, Infect. Immun., 55:2081-2087 [1987]; and D.A. Schaefer *et al.*, Infect. Immun., 68:2608-2616 [2000]). Groups of 15 six-day-old specific pathogen free ICR mice (Harlan Sprague Dawley) are administered 5×10^4 oocysts (50 X mouse ID₅₀) by gastric intubation. After 48 hrs, culture-derived r3E2 (4 mg MAb/ml, 75 μ l) are given by intubation. Every 12 hrs thereafter, mice are administered additional r3E2 (4 mg MAb/ml, 100 μ l), for a total of eight treatments. Cimetidine (10 mg/kg) are included with all treatments. For comparison, groups of 15 six-day-old control mice are infected and treated identically with: 1) murine hybridoma-derived neutralizing 3E2, or 2) isotype- and concentration- matched recombinant control MAb of irrelevant specificity. After euthanasia at 140-142 hrs post-inoculation, the jejunum, ileum, cecum, and colon are collected from each mouse and processed for histopathology. Sections are coded and examined by the same investigator, without knowledge of treatment group, for *C. parvum* stages in mucosal epithelium. Scores are assigned to longitudinal sections representing the entire length of: i) terminal jejunum; ii) ileum; iii) cecum; and (iv) colon, then summed to an infection score for each mouse. (See, M.W. Riggs MW and L.E. Perryman, Infect. Immun., 55:2081-2087 [1987]; and D.A. Schaefer *et al.*, Infect. Immun., 68:2608-2616 [2000]). Each experiment is performed twice. Mean infection scores within each experiment are analyzed by Student's one-tailed t test. Mean infection scores between experiments are analyzed by ANOVA. Additionally, all intestinal sections and sections of stomach, liver, and kidney from mice treated with antibody-biocide fusions are examined by an ACVP Board-Certified Veterinary Pathologist to determine if any lesions suggestive of biocide-host toxicity are present.

EXAMPLE 13***In vivo* Efficacy Assays of rMAbs and rMAb-Fusion****Parasiticides in a Neonatal Piglet Model**

This example provides *in vivo* efficacy assays for *C. parvum* neutralizing rMAb.

5 Newborn male piglets for the proposed studies are obtained by project personnel at the time of parturition from sows in which the perineum has been thoroughly cleaned using standard methods equivalent to pre-surgical preparation. Piglets, collected as born and colostrum-deprived, are immediately placed in disinfected isolation crates for transport to BSL-2 isolation facilities. Precautions are taken to prevent animal exposure to an exogenous
10 source of *C. parvum* and other potential diarrheal agents. (See e.g., L.E. Perryman *et al.*, Mol. Biochem. Parasitol., 80:137-147 [1996]; L.E. Perryman *et al.*, Vaccine, 17:2142-2149 [1999]; and M.W. Riggs and L.E. Perryman, Infect. Immun., 55:2081-2087 [1987]). Following arrival at BSL-2 isolation facilities, piglets are assigned to either treatment (8 piglets) or control groups (8 piglets) by blind code. Group assignments and coding are
15 made by an independent third party not be involved in conducting the experiments, data collection, or interpretation of results. All personnel involved with the experiments have no knowledge of piglet group assignments. Codes are revealed only at completion of the study. Testing of rMAbs and rMAb-biocide fusion proteins, individually and in combination to be selected, proceeds as follows.

20

Testing of individual rMAbs

To allow accurate comparisons between activities of the six rMAb constructs being evaluated, the concentration of each is standardized on an equimolar basis. Using the experimental design for rMAb 3E2 as an example, each construct is evaluated, individually,
25 as follows. One group of 8 piglets is administered 10⁷ oocysts by gastric intubation at 24 hrs of age. Forty-eight hours later, each piglet receives 250 mg culture-derived rMAb 3E2 by intubation. At 12 hrs and every 12 hrs thereafter, each piglet is administered 50 mg additional rMAb 3E2 for a total of 10 treatments (750 mg MAb r3E2 total/piglet). Omeprazole (PRILOSEC, Astra-Merck) [1mg/kg] is administered 6-8 hrs prior to each
30 rMAb treatment to block production of gastric acid according to a regimen previously shown to elevate gastric pH in pigs to ~7 (D.L. Foss and M.P. Murtaugh, Vaccine, 17:788-

801 [1999]). As an additional precaution against gastric degradation, rMAb is formulated in NaHCO₃ buffer prior to administration. For comparison, a group of 8 control piglets is identically infected with 10⁷ oocysts and administered recombinant isotype control MAb construct according to the same treatment regimen as the principals. Piglets are confined, individually, in elevated metabolic isolation cages equipped with fecal collection pans, and maintained on ESBILAC (PetAg, Inc., Hampshire, IL) for the duration of the experiment. To prevent urine from contaminating feces for subsequent analyses, a diversion device is attached and sealed around the prepuccial orifice of each piglet to divert urine into a drainage outlet. Piglets are examined twice daily by a veterinarian, without knowledge of treatment group, and assigned numerical scores based on clinical assessment for symptoms of depression, anorexia, and dehydration. Piglet weights at the time of infection and at the end of the experiment are also recorded. The total volume of feces excreted and percent dry matter for successive 24 hrs fecal collections is determined to provide an objective, quantitative index of diarrhea for each piglet. Fecal samples are examined for oocysts prior to challenge and daily thereafter by IFA using oocyst-specific MAb 4D3 to determine pre-patent and patent periods as previously described. (See, M.W. Riggs *et al.*, Antimicrob. Agents Chemother., 46:275-282 [2002]). Total oocyst counts (number oocysts per ml of feces X total ml feces) for each piglet is determined from samples of well-mixed feces collected over successive 12 hrs periods (M.W. Riggs *et al.*, *supra*). Feces from each piglet is examined for possible bacterial and viral enteropathogens by standard methods. Piglets are euthanized 10 days post-infection, or before if clinically indicated. Sections of duodenum, jejunum, ileum, cecum, and colon from identically sampled sites in each piglet are collected for histopathology. Sections are coded and examined histologically without knowledge of treatment group by an ACVP board-certified veterinary pathologist. Villus length to crypt depth ratios and the density of organisms per unit length of mucosa is determined as previously described (See, M.W. Riggs *et al.*, Infect. Immun., 62:1927-1939 [1994]; M.W. Riggs. and L.E. Perryman, Infect. Immun., 55:2081-2087 [1987]). Infection scores of 0, 1, 2 or 3 (0, no infection; 1, < 33% of mucosa infected; 2, 33 to 66% of mucosa infected; and 3, > 66% of mucosa infected) are assigned to longitudinal sections from the (i) terminal jejunum, (ii) ileum, (iii) cecum, and (iv) proximal colon, then summed to obtain an infection score (0 to 12) for each piglet. (M.W. Riggs. and L.E. Perryman, *supra*).

Additionally, all intestinal sections, and sections of stomach, liver, and kidney from piglets treated with rMAb-biocide constructs are examined by an ACVP Board-Certified Veterinary Pathologist to determine if any lesions suggestive of biocide-host toxicity are present.

Clinical, parasitologic, and histologic data is analyzed statistically by ANOVA using the

5 General Linear Models Program of SAS.

Testing of combined rMAbs

Following evaluation of the individual rMAbs above, the necessary data is available to decide which rMAbs are the best candidates for testing in combination for additive

10 efficacy. Based on previous findings in mice, an optimal combination comprises up to three MAbs, one against each of the three target antigens (CSL, P23, GP25-200) (L.E. Perryman *et al.*, Mol. Biochem. Parasitol., 80:137-147 [1996]). Because the neutralizing activity of anti-CSL MAb 3E2 is profoundly greater than that of all other Mabs against *C. parvum*, in some embodiments, this MAb is an important component in the selected combination.

15 Either rMAb 1E10 or rMAb 1E10-biocide fusion, whichever demonstrates greater efficacy in the above experiments, is included in the combination to target P23. In other embodiments, to target GP25-200, rMAb 3H2, rMAb 4H9, or rMAb 4H9-biocide fusion, whichever demonstrates the greatest efficacy in the above experiments, is included. Thus, in one embodiment, the combination to be evaluated contains rMAbs 3E2 + 1E10
20 (standalone or biocide fusion) + 3H2 or 4H9 (standalone or biocide fusion).

Previous studies on MAb combinations show that hybridoma-derived MAbs 3E2, 1E10, 3H2, and 4H9 recognize distinct epitopes and do not inhibit binding of each other to *C. parvum*. Nevertheless, it is useful to repeat binding inhibition experiments with the recombinant candidates selected for combination testing to confirm that they do not inhibit
25 binding of each other due to steric hindrance or other influences introduced by recombinant expression. In brief, this is evaluated by ELISA using biotin-labeled (Sulfo-NHS-biotin, Pierce) and unlabeled rMAb candidates as previously described. (See, L.E. Perryman *et al.*, *supra*). Immulon™-4 96-well ELISA plates are coated with solubilized sporozoite antigen, washed, and blocked. Plates are incubated with an individual unlabeled rMAb, then
30 biotinylated competitor rMAb, washed, and developed with peroxidase-labeled Streptavidin

and substrate. Mean ODs of replicate wells for each treatment and control group are analyzed for significant differences.

After determining that the three rMAbs selected for combination testing in piglets do not significantly inhibit binding of each other, they are combined and the concentration of each standardized on an equimolar basis to match that previously evaluated individually. Efficacy testing of the combined rMAbs in piglets then proceeds as described for individual rMAbs above. In brief, one group of 8 piglets are infected with 10^7 oocysts at 24 hrs of age and receive the combined rMAbs in NaHCO₃ buffer 48 hrs later by intubation. At 12 hrs and every 12 hrs thereafter, piglets receive additional combined rMAbs for a total of 10 treatments. For comparison, a group of 8 control piglets are identically infected and administered an appropriate isotype control rMAb combination according to the same treatment regimen. Clinical assessment scores, piglet weights, total volume of feces excreted and percent dry matter for successive 24 hr collections, pre-patent and patent periods, and total oocyst counts are determined as above. Ten days post-infection, sections of duodenum, jejunum, ileum, cecum, and colon from each piglet are collected, and examined histologically to assess lesions and assign infection scores. Tissues are also examined to determine if any lesions suggestive of biocide-host toxicity are present. Clinical, parasitologic, and histologic data is analyzed statistically as described above. Data from testing of the individual rMAbs is compared with data from testing of the rMAb combination by one-way ANOVA stratified by treatment group.

EXAMPLE 14

Targeted Biocides Using Innate Receptor Recognition

This Example describes the construction and analysis of fusion proteins of an innate receptor and a biocide.

A. Genetic engineering of sCD14, LBP, SP-D and MBL into retrovirus backbone for secretion

All the innate receptor molecules were previously cloned and produced in various cell lines as recombinant molecules. The gene for the human CD14 receptor is obtained

from total RNA extracted from human PBMCs. Following reverse transcription of the RNA into cDNA, the specific gene for CD14 is cloned by PCR. Primers are designed to amplify the sequence starting with the signal peptide sequence down to the first codon of the GPI anchor. The GPI anchor is excluded to facilitate secretion. The GPI anchor sequence is replaced by a portion of the human immunoglobulin Fc region. Adding Ig domains to proteins does not interfere with proper folding; an added Fc portion can contribute to the stability of a fusion protein and extend its half-life (*e.g.*, Chang *et al.*, Surgery 2002; 132:149-56). The hinge region, CH2 and CH3 domains of human immunoglobulin are already part of a construct library and are transferred to the CD14 receptor construct.

Constructs that contain the Fc portion with a biocide already connected are available from prior work.

LPS binding protein (LBP; accession number NM_004139) naturally occurs as a secreted protein. The amino acid sequence of the secreted protein is known (Schumann *et al.*, Science 1990; 249:1429-31) and the secreted form has been produced as a recombinant protein (Han *et al.*, J Biol Chem 1994; 269:8172-5; Theofan *et al.*, J Immunol 1994; 152:3623-9). The gene for LBP is cloned from the mammalian gene collection construct into the retroviral construct. As LBP is a secreted protein, it is not necessary to attach an immunoglobulin Fc portion to stabilize it. The linker-biocide portion is attached directly to the C-terminus of LBP.

SP-D and MBL are from the defense collectin family. These molecules form multimers, increasing their overall avidity to the pathogen surface. Surfactant protein D, SP-D has a glomerular structure at its c-terminal end. This structure is thought to interact with LPS either in solution or as part of a pathogen surface. If the linker-biocide portion is added to the c-terminal end it will most likely be very close to the binding site. An N-terminally attached biocide version of the fusion protein is also produced. SP-D can obtain a cross-like tetrameric conformation. Based on electron microscopy images (Holmskov *et al.*, Annu Rev Immunol 2003; 21:547-78) bound SP-D adopts a fairly two-dimensional structure when bound to its specific surface, bringing the N-termini close to the surface as well. An N-terminally attached biocide is thus accessible enough to destroy a bacterial membrane.

The mannan-binding lectin (MBL) is also a collectin, forming multimeric complexes to achieve a highly efficient binding to microorganism surfaces. The gene for MBL is obtained from the human gene collection, accession number 67483. Like the other collectins MBL is a soluble secreted protein therefore not requiring modifications to make it soluble. The gene is used in its native confirmation and the biocide is added via the linker. The same cloning strategy as that described above for SP-D is used.

B. Expression of riR in vitro – test binding to bacterial components

The retroviral constructs containing the genes for the innate receptor (or the complete construct containing the Fc-portion plus biocide) are co-transfected with the VSV-G envelope plasmid into the packaging cell line and infectious retroviral particles are produced by transient expression. Packaging cells are grown to exponential phase and then exposed to a calcium chloride solution containing a mixture of the VSV-G encoding plasmid and the retroviral construct containing the immunoglobulin genes. Cells are then grown for 16-24 hours until pseudotyped retrovector is harvested from the supernatant over a period of several days. The titers resulting are typically in the range of 10^5 - 10^6 infectious units per ml culture media. Media is concentrated to be used at high multiplicity of infection on the target production cell line (CHO or 293 cells). Once these production cells have been exposed to high titer retrovirus (transduction), they start to express product (monoclonal antibody or others) typically in the range of 10-25 $\mu\text{g/ml}$ for well-expressed monoclonal antibody molecules in standard plastic tissue culture vessels. The pool population of transduced production cells are subjected to clonal analysis to obtain high-level producing clones.

The recombinant innate receptor molecules (riR) are tested for their interaction with target cells (bacteria), first by enzyme linked immunosorbent assay (ELISA). For this assay, the cell product is first quantified. The natural antigen (*e.g.*, whole *E. coli* cells) is used as a capture agent to monitor specificity. A similar assay has been established to monitor *Listeria* surface antigens. Briefly, antigenic fractions or whole bacteria are used to coat 96-well plates. After washing, samples containing riR are incubated at serial dilutions with the antigen. Secondary conjugates are used to quantify binding. Flow cytometry analysis allows the measurement of the number of riR molecules interacting with whole

microorganism cells. Assays are designed to compare all the different recombinant innate receptor candidates simultaneously against one or multiple different targets (*e.g.* different *E. coli* isolates). These assays identify which candidates (clonal lines) are suitable for the cloning procedures described below.

5

C. Reengineer riR construct to contain biocide fusion portion

Upon determining which of the riR retain good binding capabilities when expressed in mammalian tissue culture, the corresponding genetic constructs are modified to contain the gene for one of the two biocides. Biocides are attached to the riRs at either the N-terminus or at the C-terminus of the riR. The CD14 riR is made with a C-terminal Fc-portion that helps stabilize it. Therefore CD14 is made as a C-terminal biocide only. The other candidates LBP, MBL and SP-D are made as both N-terminal and C-terminal fusions. Figure 6 shows the components of these constructs featuring a (Gly₄Ser)₃ linker which has been used widely. The design of the linker is optimized for functionality of the fusion protein. In some embodiments, the linker is modified as described (George and Heringa, Protein Eng 2002; 15:871-9). Alternatively, the linker is designed with a symmetric sequence of (Gly₄Ser)₂-P-P-(Gly₄Ser)₂ placing the most favored amino acid pair (a structure breaking Pro-Pro) in the middle of the non-helical linkers. Phospholipase and lysozyme constructs are expressed in cell culture to use as controls.

20

D. Expression of riR-biocide fusions and binding tests

The constructs for the riR-biocide fusions are introduced into a mammalian tissue culture system as described above. Upon production of milligram amounts of riR-biocide fusions, binding tests including ELISA and flow cytometry are done. In addition precise affinity measurements are undertaken using surface plasmon resonance (SPR) on a Biacore 2000 machine. This allows for the determination of which riR-biocide binds best to its target, and also if the linker and fusion elements change the original affinity. Multiple bacterial surface-binding innate immunity receptors are expressed in mammalian tissue culture. In addition, multiple riR-biocide fusion proteins are assembled.

30

EXAMPLE 15

Targeted Biocides using Antibody Targeting PAMPs

This Example describes the construction and analysis of constructs comprising monoclonal antibodies and biocides.

5

A. Antigens for immunization

Antigens that are not specific to one bacterial isolate but rather common to a broad group are identified (*e.g.*, all Gram negatives or all Gram positives, or all of a major family), in order to obtain a broadly reactive monoclonal antibody. Such targets include structural components such as lipopolysaccharides, peptidoglycans, porins that are common in structure and function among the classes of bacteria (Feng *et al.*, J Gen Microbiol 1990; 136 (Pt 2):337-42; Klebba *et al.*, J Biol Chem 1990; 265:6800-10; Peters *et al.*, Infect Immun 1985; 50:459-66). Preferred targets are genetically defined and can either be purified directly or over-expressed in an *E.coli* expression system. Both of these methods can be used to immunize mice (Feng *et al.*, J Gen Microbiol 1990; 136 (Pt 2):337-42; He *et al.*, Appl Environ Microbiol 1996; 62:3325-32). For dominant structural components minimal purification is needed to arrive at an antigen preparation that has a strong probability of providing cross reactive antibodies. In some embodiments, *E.coli* O157:H7 and *L. monocytogenes* are utilized as PAMP “donors”.

20

B. Immunize mice to produce monoclonal broad spectrum antibody

An array of monoclonal antibodies is prepared by Neoclone (Madison, WI). The monoclonal antibodies are used to screen for reactivity with an array of target organisms.

25

C. Antibody binding tests against wide variety of microorganisms, selection of best candidates

Monoclonal antibodies are obtained as ascitic fluid containing high levels of monoclonal antibody plus the corresponding immortalized B-cells. The antibody contained in the ascitic fluid is used to perform specificity and affinity tests on various targets.

Multiple strains of pathogenic *E. coli*, *Salmonella*, *Listeria*, and *Lactobacillus* are used for testing. Initial tests include ELISA assays to establish general binding patterns. The

antigenic structures that were used for the immunization of the mice leading to these monoclonals are well defined and the distribution patterns over the surface of a bacterial cell are known. Based on this and what is known about other monoclonals to similar or the same antigenic structures, the quantity of interaction expected can be estimated. In addition, commercially available monoclonal antibodies to these structures are used as controls (e.g., those available from Inotek Pharmaceuticals, Beverly, MA). It is contemplated that some of the monoclonals will have a broad range of cross-reactions with other organisms. This has been described on various occasions, especially between *E. coli* and other *Enterobacteriaceae* (Feng *et al.*, supra; Klebba *et al.*, J Biol Chem 1990; 265:6800-10), but also between *E. coli* and *Salmonella* (O-antigens) (Westerman *et al.*, J Clin Microbiol 1997; 35:679-84). The cross reactivity increases the number of potential target organisms that can be treated with one single broad-reacting monoclonal antibody.

D. Clone immunoglobulin genes from candidates into retroviral backbone – express in vitro – test binding

The heavy and light chain variable regions of the immunoglobulin genes are amplified out of the cDNA by PCR using a commercially available Ig-primer kit. These primers consist of degenerate upper primers specific to the signal-peptide sequence at the 5'-end of the immunoglobulin heavy- or light chain and lower primers to different sections in the constant region, depending on the section that is to be isolated. A lower primer to the joining region is chosen to amplify just the variable region. Alternatively, a lower primer to the 3' UTR of the heavy- or light chain gene is chosen to amplify the entire framework. Once the PCR products are obtained using high-fidelity polymerase that ensures that error-free amplicons are generated, they are cloned into blunt end PCR cloning kits that are commercially available. Upon cloning of these fragments, multiple clones are analyzed by sequencing and sequences aligned with each other for comparison. Once the complete constructs have been confirmed by sequencing they are used in the first round of transfections that result in high titer pseudotyped retrovector for the transduction of production cell lines.

E. Reengineer the antibody constructs to contain biocide fusions

The heavy chain gene is removed from the construct and replaced with a fusion that consists of the heavy chain gene, a C-terminal linker element and the gene of either human lysozyme or human phospholipase A (Figure 7). The heavy chain-linker-lysozyme element
5 is separated from the light chain gene via the IRES (internal ribosome binding site) element that enables individual translation of both products. The C terminus is used as it is least likely to give rise to steric hindrance at the antibody binding site. The (Gly₄Ser)₃ linker is used.

10 F. Expression of biocide fusions and binding tests

The retrovector is used to transduce production cell lines (CHO or 293) and obtain cells that have stably integrated copies of the construct. Upon clonal analysis to select clones with the highest production of fusion protein, the products are again tested for affinity and specificity as described in the above examples. Surface plasmon resonance analysis is used
15 to determine exactly what influence the linker and biocide fusion portion have on the binding affinity of the antibody portion.

EXAMPLE 16**Targeted biocides using secretory IgA and pentameric IgM as delivery molecules**

20 The example describes the construction and analysis of constructs comprising IgA and IgMs and biocides.

A. Obtain secretory component (SC) through engineering of pIgR gene and clone into retroviral backbone

25 The cloning strategy for the murine polymeric immunoglobulin receptor (pIgR) gene (accession U06431) is based on published information about the sequence, structure and interaction of murine pIgR with IgA (Piskurich *et al.*, J Immunol 1995; 154:1735-47). As a source for the pIgR gene total RNA is isolated from murine liver cells, which have been shown to produce high levels of pIgR (Piskurich *et al.*, supra). After reverse transcribing
30 the RNA, PCR is used to obtain the gene for the pIgR. Primers specific to the 3' UTR region and to the region upstream of position 2020 of the transmembrane region are

designed and used to amplify a truncated version of the pIgR. The downstream primer is designed to introduce a stop codon right after amino acid position 594. The sequence corresponds to the cleaved secretory component found in circulation. A similar procedure has been published to make human secretory IgA (Rindisbacher *et al.*, J Biol Chem 1995; 270:14220-8).

B. Create secretory component-producing cell line by using existing J-chain-biocide producing cell line

The truncated pIgR gene obtained as described above is cloned into the retroviral backbone behind the simian CMV promoter as described earlier. CHO cells that already produce J-chain linked to biocide are superinfected with the pIgR construct and ELISA and Western Blot-based clonal analysis are performed to find clones that produce similar amounts of J-chain and secretory component. The resulting SC and J-chain-biocide producing cell line is used as a recipient cell line for the IgA constructs.

C. Use this cell line as recipient for IgA constructs

The next step towards a secretory IgA-producing cell line is to transduce the SC+J-chain-biocide producing cell line with reengineered IgA that is made by genetic assembly as described below. This construct is the first “fully artificially” produced mouse secretory IgA that incorporates all three components, the secretory component, the J-chain and a rearranged IgA, in one single production cell line. In addition it is the first time that the J-chain is engineered into a fusion protein.

D. Express secretory IgA-biocide fusion in vitro and test binding

Production cell populations producing sIgA-biocide are subjected to clonal analysis in order to choose the highest producers. The selection of these clones focuses on dimeric secretory IgA-biocide detection. In order to make sure that the reengineered sIgA-biocide maintains its binding capabilities to bacterial surfaces, supernatants containing sIgA-biocide are tested by ELISA and flow cytometry as described above. Once satisfactory binding characteristics have been confirmed, candidates are chosen to go into the extensive testing series described below.

E. Engineer variable regions from PAMP-reactive immunoglobulins onto constant IgA or IgM region

The constant IgA regions are obtained through extraction from IgA producing hybridomas. The immortalized monoclonal antibody-producing cell lines generated as described above are the source for the variable region genes. To obtain the variable regions from these cells the same procedures as described above with the exception that lower primers that anneal to the hinge region so that only the variable portion of the gene is amplified are used. The products are then cloned in frame into the existing IgA and IgM constant region constructs. These constructs representing anti-PAMP IgA are used to make sIgA-biocide fusion in cells that make SC and J-chain-biocide. Since the IgM does not incorporate the secretory component, the constructs representing anti-PAMP on an IgM framework are used to transduce production cells that make only the J-chain-biocide.

F. Express pentameric IgM-biocide fusion in vitro and test binding

The anti-PAMP IgM constructed above is used to transduce JC-biocide producing production cells in analogy to the procedures described in Example 14. Product is tested for the pentameric structure and binding capabilities to PAMPs as well as whole bacterial cells. Candidates that are chosen based on their good binding and structure are taken into the extensive testing series described below.

EXAMPLE 17

Bactericidal Testing System

This example describes testing methods for the analysis of candidate fusion proteins identified as described above. Constructs developed as described above are evaluated under the same conditions. Three test systems are used, as described below:

A. Testing in Bacterial Cell Culture

The lowest stringency test evaluates the bactericidal effect on test organisms in culture. Cultures of the test organism are exposed to a range of concentrations of the test product and control media for times ranging from 1-24 hours at 4°C and 10°C. Aliquots in triplicate are re-plated on a growth medium to quantify the residual bacteria. The cell suspensions are also examined microscopically for clumping; various techniques are used to separate cells for quantification.

B. Testing in Biofilms and Ground Meat

For *Listeria* and *Lactobacillus*, the efficacy of constructs is tested in a biofilm model system; for *Lactobacillus*, *E.coli* and *Salmonella* the efficacy of bacterial killing is tested in a ground meat suspension.

Protocols for biofilm development and antimicrobial testing are known (Somers *et al.*, 88th Ann. Meet. International Association for Food Protection, Minneapolis, MN. Abstract P055. 2001). Stainless steel is used as a prototype surface for initial evaluation of the anti-listerial activity. Biofilms comprising a cocktail of *L. monocytogenes* or *Lactobacillus* isolates are established on 1cm² pre-sterilized steel chips. Biofilm chips are exposed to various concentrations of the fusion protein products for up to 2 hours at room temperature. Chips are retrieved and evaluated for the number of viable cells quantified; adherent cells are removed by vortexing with glass beads and appropriate dilutions of the detached cells plated on nutrient agar plates for enumeration.

Raw beef or turkey is harvested from the interior of a large muscle section under sterile conditions to keep the background contamination at a minimum. This meat is then comminuted under sterile conditions to a slurry and pH adjusted. Several concentrations of the test products are added to the slurries. Following inoculation with test organisms the samples are incubated at 4° and 10°C for up to 4 weeks. Triplicate samples per variable are assayed weekly for changes in bacterial population.

C. Simulation of Packing Plant Conditions

Meat product suspensions or a range of surface presentations of bacteria are used to simulate plant equipment and facilities. SPR testing of affinity binding guides the range of conditions tested. SPR is used to measure the effect of low or high pH, high salt

5 concentrations and high temperatures on the capability of a given fusion protein to 'hang on' to its epitope. Those conditions that still allow the fusion protein to bind to its target are then tested in separate in vitro experiments for bacterial killing. For biofilms factors to be evaluated are attachment surface (*e.g.*, buna N and food grade silicone rubber, polyurethane, polyester, polyethylene, and polypropylene), presence of food residues, cleaning/sanitizing
10 agents, temperature, pH, and mixed biofilms.

Test organisms include, but are not limited to, field isolates of *E. coli* O157:H7, *Salmonella*, *Listeria monocytogenes* and *Lactobacillus plantarum*. Multiple isolates of the relevant organisms are available.

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Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention
20 as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention that are obvious to those skilled in the relevant fields are intended to be within the scope of the following claims.

SEQUENCE LISTING

<110> IOGENETICS, INC.

<120> Targeted Biocides

<130> 81344-42

<140> CA 2,525,961

<141> 2004-05-13

<150> US 60/470,841

<151> 2003-05-15

<150> US 10/844,837

<151> 2004-05-12

<160> 101

<170> PatentIn version 3.3

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<213> Bos taurus

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Ala	Gly	Ser	Gly	Phe	Thr	Gln	Gly	Val	Arg	Asn	Ser	Gln	Ser	Cys	Arg
			20				25						30		
Arg	Asn	Lys	Gly	Ile	Cys	Val	Pro	Ile	Arg	Cys	Pro	Gly	Ser	Met	Arg
		35					40					45			
Gln	Ile	Gly	Thr	Cys	Leu	Gly	Ala	Gln	Val	Lys	Cys	Cys	Arg	Arg	Lys
	50					55					60				

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<211> 24

<212> PRT

<213> Xenopus laevis

<400> 2

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Ala	Leu	Asn	Ala	Val	Leu	Lys	Gln								
				20											

<210> 3

<211> 81

<212> PRT

<213> Xenopus laevis

<400> 3

Met	Tyr	Lys	Gly	Ile	Phe	Leu	Cys	Val	Leu	Leu	Ala	Val	Ile	Cys	Ala
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Asn	Ser	Leu	Ala	Thr	Pro	Ser	Ser	Asp	Ala	Asp	Glu	Asp	Asn	Asp	Glu
			20				25						30		
Val	Glu	Arg	Tyr	Val	Arg	Gly	Trp	Ala	Ser	Lys	Ile	Gly	Gln	Thr	Leu
		35					40					45			
Gly	Lys	Ile	Ala	Lys	Val	Gly	Leu	Lys	Glu	Leu	Ile	Gln	Pro	Lys	Arg
	50					55					60				

Glu Ala Met Leu Arg Ser Ala Glu Ala Gln Gly Lys Arg Pro Trp Ile
 65 70 75 80
 Leu

<210> 4
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 <213> *Xenopus laevis*

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 20 25 30
 Arg Glu Val Arg Gly Ile Gly Lys Phe Leu His Ser Ala Gly Lys Phe
 35 40 45
 Gly Lys Ala Phe Val Gly Glu Ile Met Lys Ser Lys Arg Asp Ala Glu
 50 55 60
 Ala Val Gly Pro Glu Ala Phe Ala Asp Glu Asp Leu Asp Glu Arg Glu
 65 70 75 80
 Val Arg Gly Ile Gly Lys Phe Leu His Ser Ala Lys Lys Phe Gly Lys
 85 90 95
 Ala Phe Val Gly Glu Ile Met Asn Ser Lys Arg Asp Ala Glu Ala Val
 100 105 110
 Gly Pro Glu Ala Phe Ala Asp Glu Asp Leu Asp Glu Arg Glu Val Arg
 115 120 125
 Gly Ile Gly Lys Phe Leu His Ser Ala Lys Lys Phe Gly Lys Ala Phe
 130 135 140
 Val Gly Glu Ile Met Asn Ser Lys Arg Asp Ala Glu Ala Val Gly Pro
 145 150 155 160
 Glu Ala Phe Ala Asp Glu Asp Leu Asp Glu Arg Glu Val Arg Gly Ile
 165 170 175
 Gly Lys Phe Leu His Ser Ala Lys Lys Phe Gly Lys Ala Phe Val Gly
 180 185 190
 Glu Ile Met Asn Ser Lys Arg Asp Ala Glu Ala Val Gly Pro Glu Ala
 195 200 205
 Phe Ala Asp Glu Asp Phe Asp Glu Arg Glu Val Arg Gly Ile Gly Lys
 210 215 220
 Phe Leu His Ser Ala Lys Lys Phe Gly Lys Ala Phe Val Gly Glu Ile
 225 230 235 240
 Met Asn Ser Lys Arg Asp Ala Glu Ala Val Gly Pro Glu Ala Phe Ala
 245 250 255
 Asp Glu Asp Leu Asp Glu Arg Glu Val Arg Gly Ile Gly Lys Phe Leu
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 His Ser Ala Lys Lys Phe Gly Lys Ala Phe Val Gly Glu Ile Met Asn
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 Ser Lys Arg Asp Ala Glu Ala Val Asp Asp Arg Arg Trp Val Glu
 290 295 300

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<210> 6
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 <213> Tachypleus gigas

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 <213> Bufo gargarizans

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 Arg Leu Leu Arg Lys Gly Asn Tyr Ala Gln Arg Val Gly Ala Gly Ala
 35 40 45
 Pro Val Tyr Leu Ala Ala Val Leu Glu Tyr Leu Thr Ala Glu Ile Leu
 50 55 60
 Glu Leu Ala Gly Asn Ala Ala Arg Asp Asn Lys Lys Thr Arg Ile Ile
 65 70 75 80
 Pro Arg His Leu Gln Leu Ala Val Arg Asn Asp Glu Glu Leu Asn Lys
 85 90 95
 Leu Leu Gly Gly Val Thr Ile Ala Gln Gly Gly Val Leu Pro Asn Ile
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 Lys

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 <213> Bufo gargarizans

<400> 8
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 20

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 <211> 63
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 <213> Bombyx mori

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 Lys Ile Glu Lys Val Gly Arg Asn Val Arg Asp Gly Leu Ile Lys Ala
 35 40 45
 Gly Pro Ala Ile Ala Val Ile Gly Gln Ala Lys Ser Leu Gly Lys
 50 55 60

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 <213> Bombyx mori

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 20 25 30
 Lys Ile Glu Lys Met Gly Arg Asn Ile Arg Asp Gly Ile Val Lys Ala
 35 40 45
 Gly Pro Ala Ile Glu Val Leu Gly Ser Ala Lys Ala Ile Gly Lys
 50 55 60

<210> 11
 <211> 63
 <212> PRT
 <213> Drosophila melanogaster

<400> 11
 Met Asn Phe Tyr Lys Ile Phe Val Phe Val Ala Leu Ile Leu Ala Ile
 1 5 10 15
 Ser Ile Gly Gln Ser Glu Ala Gly Trp Leu Lys Lys Leu Gly Lys Arg
 20 25 30
 Ile Glu Arg Ile Gly Gln His Thr Arg Asp Ala Thr Ile Gln Gly Leu
 35 40 45
 Gly Ile Ala Gln Gln Ala Ala Asn Val Ala Ala Thr Ala Arg Gly
 50 55 60

<210> 12
 <211> 31
 <212> PRT
 <213> Sus scrofa

<400> 12
 Ser Trp Leu Ser Lys Thr Ala Lys Lys Leu Glu Asn Ser Ala Lys Lys
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 Arg Ile Ser Glu Gly Ile Ala Ile Ala Ile Gln Gly Gly Pro Arg
 20 25 30

<210> 13
 <211> 13
 <212> PRT
 <213> Bos taurus

<400> 13
 Ile Leu Pro Trp Lys Trp Pro Trp Trp Pro Trp Arg Arg
 1 5 10

<210> 14
 <211> 34
 <212> PRT
 <213> Lactococcus lactis

<400> 14
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 1 5 10 15
 Met Gly Cys Asn Met Lys Thr Ala Thr Cys His Cys Ser Ile His Val
 20 25 30

Ser Lys

<210> 15
 <211> 20
 <212> PRT
 <213> Rana catesbeiana

<400> 15
 Phe Leu Gly Gly Leu Ile Lys Ile Val Pro Ala Met Ile Cys Ala Val
 1 5 10 15
 Thr Lys Lys Cys
 20

<210> 16
 <211> 25
 <212> PRT
 <213> Bos taurus

<400> 16
 Phe Lys Cys Arg Arg Trp Gln Trp Arg Met Lys Lys Leu Gly Ala Pro
 1 5 10 15
 Ser Ile Thr Cys Val Arg Arg Ala Phe
 20 25

<210> 17
 <211> 19
 <212> PRT
 <213> Sus scrofa

<220>
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<400> 17
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 1 5 10 15
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<210> 18
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 <213> Sus scrofa

<400> 18
 Gly Gly Arg Leu Cys Tyr Cys Arg Arg Arg Phe Cys Ile Cys Val Gly
 1 5 10 15

<210> 19
 <211> 51
 <212> PRT
 <213> Homo sapiens

<400> 19
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 1 5 10 15
 Thr Gly Ala Asp Ser His Ala Lys Arg His His Gly Tyr Lys Arg Lys
 20 25 30

Phe His Glu Lys His His Ser His Arg Gly Tyr Arg Ser Asn Tyr Leu
 35 40 45
 Tyr Asp Asn
 50

<210> 20
 <211> 38
 <212> PRT
 <213> *Macaca fascicularis*

<400> 20
 Asp Ser His Glu Glu Arg His His Gly Arg His Gly His His Lys Tyr
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 Gly Arg Lys Phe His Glu Lys His His Ser His Arg Gly Tyr Arg Ser
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 Asn Tyr Leu Tyr Asp Asn
 35

<210> 21
 <211> 33
 <212> PRT
 <213> *Phyllomedusa sauvagei*

<400> 21
 Ala Leu Trp Lys Thr Met Leu Lys Lys Leu Gly Thr Met Ala Leu His
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 20 25 30
 Gln

<210> 22
 <211> 34
 <212> PRT
 <213> *Phyllomedusa sauvagei*

<400> 22
 Ala Leu Trp Phe Thr Met Leu Lys Lys Leu Gly Thr Met Ala Leu His
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 Ala Gly Lys Ala Ala Leu Gly Ala Ala Ala Asn Thr Ile Ser Gln Gly
 20 25 30
 Thr Gln

<210> 23
 <211> 30
 <212> PRT
 <213> *Phyllomedusa sauvagei*

<400> 23
 Ala Leu Trp Lys Asn Met Leu Lys Gly Ile Gly Lys Leu Ala Gly Lys
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 Ala Ala Leu Gly Ala Val Lys Lys Leu Val Gly Ala Glu Ser
 20 25 30

<210> 24
 <211> 21
 <212> PRT
 <213> *Misgurnus anguillicaudatus*

<400> 24

Arg Gln Arg Val Glu Glu Leu Ser Lys Phe Ser Lys Lys Gly Ala Ala
 1 5 10 15
 Ala Arg Arg Arg Lys
 20

<210> 25

<211> 27

<212> PRT

<213> *Apis mellifera*

<400> 25

Gly Ile Gly Ala Val Leu Lys Val Leu Thr Thr Gly Leu Pro Ala Leu
 1 5 10 15
 Ile Ser Trp Ile Ser Arg Lys Lys Arg Gln Gln
 20 25

<210> 26

<211> 33

<212> PRT

<213> *Pardachirus pavoninus*

<400> 26

Gly Phe Phe Ala Leu Ile Pro Lys Ile Ile Ser Ser Pro Leu Phe Lys
 1 5 10 15
 Thr Leu Leu Ser Ala Val Gly Ser Ala Leu Ser Ser Ser Gly Glu Gln
 20 25 30
 Glu

<210> 27

<211> 33

<212> PRT

<213> *Pardachirus pavoninus*

<400> 27

Gly Phe Phe Ala Leu Ile Pro Lys Ile Ile Ser Ser Pro Ile Phe Lys
 1 5 10 15
 Thr Leu Leu Ser Ala Val Gly Ser Ala Leu Ser Ser Ser Gly Gly Gln
 20 25 30
 Glu

<210> 28

<211> 176

<212> PRT

<213> *Bos taurus*

<400> 28

Met Glu Thr Gln Arg Ala Ser Leu Ser Leu Gly Arg Cys Ser Leu Trp
 1 5 10 15
 Leu Leu Leu Leu Gly Leu Val Leu Pro Ser Ala Ser Ala Gln Ala Leu
 20 25 30
 Ser Tyr Arg Glu Ala Val Leu Arg Ala Val Asp Gln Phe Asn Glu Arg
 35 40 45
 Ser Ser Glu Ala Asn Leu Tyr Arg Leu Leu Glu Leu Asp Pro Thr Pro
 50 55 60
 Asn Asp Asp Leu Asp Pro Gly Thr Arg Lys Pro Val Ser Phe Arg Val
 65 70 75 80
 Lys Glu Thr Asp Cys Pro Arg Thr Ser Gln Gln Pro Leu Glu Gln Cys
 85 90 95

Asp	Phe	Lys	Glu	Asn	Gly	Leu	Val	Lys	Gln	Cys	Val	Gly	Thr	Val	Thr
			100					105					110		
Leu	Asp	Pro	Ser	Asn	Asp	Gln	Phe	Asp	Ile	Asn	Cys	Asn	Glu	Leu	Gln
		115					120					125			
Ser	Val	Arg	Phe	Arg	Pro	Pro	Ile	Arg	Arg	Pro	Pro	Ile	Arg	Pro	Pro
		130				135					140				
Phe	Tyr	Pro	Pro	Phe	Arg	Pro	Pro	Ile	Arg	Pro	Pro	Ile	Phe	Pro	Pro
145					150					155					160
Ile	Arg	Pro	Pro	Phe	Arg	Pro	Pro	Leu	Gly	Pro	Phe	Pro	Gly	Arg	Arg
				165					170					175	

<210> 29
 <211> 155
 <212> PRT
 <213> Bos taurus

<400> 29															
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1				5					10					15	
Leu	Leu	Leu	Leu	Gly	Leu	Ala	Leu	Pro	Ser	Ala	Ser	Ala	Gln	Ala	Leu
			20					25					30		
Ser	Tyr	Arg	Glu	Ala	Val	Leu	Arg	Ala	Val	Asp	Gln	Leu	Asn	Glu	Gln
		35					40					45			
Ser	Ser	Glu	Pro	Asn	Ile	Tyr	Arg	Leu	Leu	Glu	Leu	Asp	Gln	Pro	Pro
	50				55						60				
Gln	Asp	Asp	Glu	Asp	Pro	Asp	Ser	Pro	Lys	Arg	Val	Ser	Phe	Arg	Val
65					70					75					80
Lys	Glu	Thr	Val	Cys	Ser	Arg	Thr	Thr	Gln	Gln	Pro	Pro	Glu	Gln	Cys
				85					90					95	
Asp	Phe	Lys	Glu	Asn	Gly	Leu	Leu	Lys	Arg	Cys	Glu	Gly	Thr	Val	Thr
			100					105					110		
Leu	Asp	Gln	Val	Arg	Gly	Asn	Phe	Asp	Ile	Thr	Cys	Asn	Asn	His	Gln
		115					120					125			
Ser	Ile	Arg	Ile	Thr	Lys	Gln	Pro	Trp	Ala	Pro	Pro	Gln	Ala	Ala	Arg
	130					135					140				
Leu	Cys	Arg	Ile	Val	Val	Ile	Arg	Val	Cys	Arg					
145					150					155					

<210> 30
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 <212> PRT
 <213> Ceratititis capitata

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1				5					10					15	
Gly	Lys	Ile	Ala	Leu	Pro	Ile	Ala	Lys	Ala	Ala	Leu	Pro			
			20					25							

<210> 31
 <211> 29
 <212> PRT
 <213> Ceratititis capitata

<400> 31															
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1				5					10					15	
Gly	Lys	Ala	Ala	Leu	Pro	Ile	Ala	Lys	Ala	Ala	Leu	Pro			
			20					25							

<210> 32
 <211> 170
 <212> PRT
 <213> Homo sapiens

<400> 32
 Met Lys Thr Gln Arg Asn Gly His Ser Leu Gly Arg Trp Ser Leu Val
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 20 25 30
 Leu Ser Tyr Lys Glu Ala Val Leu Arg Ala Ile Asp Gly Ile Asn Gln
 35 40 45
 Arg Ser Ser Asp Ala Asn Leu Tyr Arg Leu Leu Asp Leu Asp Pro Arg
 50 55 60
 Pro Thr Met Asp Gly Asp Pro Asp Thr Pro Lys Pro Val Ser Phe Thr
 65 70 75 80
 Val Lys Glu Thr Val Cys Pro Arg Thr Thr Gln Gln Ser Pro Glu Asp
 85 90 95
 Cys Asp Phe Lys Lys Asp Gly Leu Val Lys Arg Cys Met Gly Thr Val
 100 105 110
 Thr Leu Asn Gln Ala Arg Gly Ser Phe Asp Ile Ser Cys Asp Lys Asp
 115 120 125
 Asn Lys Arg Phe Ala Leu Leu Gly Asp Phe Phe Arg Lys Ser Lys Glu
 130 135 140
 Lys Ile Gly Lys Glu Phe Lys Arg Ile Val Gln Arg Ile Lys Asp Phe
 145 150 155 160
 Leu Arg Asn Leu Val Pro Arg Thr Glu Ser
 165 170

<210> 33
 <211> 170
 <212> PRT
 <213> Equus caballus

<400> 33
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 20 25 30
 Ser Tyr Lys Glu Ala Val Leu Arg Ala Val Asp Gly Leu Asn Gln Arg
 35 40 45
 Ser Ser Asp Glu Asn Leu Tyr Arg Leu Leu Glu Leu Asp Pro Leu Pro
 50 55 60
 Lys Gly Asp Lys Asp Ser Asp Thr Pro Lys Pro Val Ser Phe Met Val
 65 70 75 80
 Lys Glu Thr Val Cys Pro Arg Ile Met Lys Gln Thr Pro Glu Gln Cys
 85 90 95
 Asp Phe Lys Glu Asn Gly Leu Val Lys Gln Cys Val Gly Thr Val Ile
 100 105 110
 Leu Asp Pro Val Lys Asp Tyr Phe Asp Ala Ser Cys Asp Glu Pro Gln
 115 120 125
 Arg Val Lys Arg Phe His Ser Val Gly Ser Leu Ile Gln Arg His Gln
 130 135 140
 Gln Met Ile Arg Asp Lys Ser Glu Ala Thr Arg His Gly Ile Arg Ile
 145 150 155 160
 Ile Thr Arg Pro Lys Leu Leu Leu Ala Ser
 165 170

<210> 34
 <211> 159
 <212> PRT

<213> Bos taurus

<400> 34

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Met Glu Thr Gln Arg Ala Ser Leu Ser Leu Gly Arg Trp Ser Leu Trp
1          5          10          15
Leu Leu Leu Leu Gly Leu Ala Leu Pro Ser Ala Ser Ala Gln Ala Leu
          20          25          30
Ser Tyr Arg Glu Ala Val Leu Arg Ala Val Asp Gln Leu Asn Glu Lys
          35          40          45
Ser Ser Glu Ala Asn Leu Tyr Arg Leu Leu Glu Leu Asp Pro Pro Pro
          50          55          60
Lys Glu Asp Asp Glu Asn Pro Asn Ile Pro Lys Pro Val Ser Phe Arg
65          70          75          80
Val Lys Glu Thr Val Cys Pro Arg Thr Ser Gln Gln Ser Pro Glu Gln
          85          90          95
Cys Asp Phe Lys Glu Asn Gly Leu Leu Lys Glu Cys Val Gly Thr Val
          100          105          110
Thr Leu Asp Gln Val Gly Ser Asn Phe Asp Ile Thr Cys Ala Val Pro
          115          120          125
Gln Ser Val Gly Gly Leu Arg Ser Leu Gly Arg Lys Ile Leu Arg Ala
          130          135          140
Trp Lys Lys Tyr Gly Pro Ile Ile Val Pro Ile Ile Arg Ile Gly
145          150          155

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<210> 35

<211> 156

<212> PRT

<213> Equus caballus

<400> 35

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Met Glu Thr Gln Arg Asn Thr Arg Cys Leu Gly Arg Trp Ser Pro Leu
1          5          10          15
Leu Leu Leu Leu Gly Leu Val Ile Pro Pro Ala Thr Thr Gln Ala Leu
          20          25          30
Ser Tyr Lys Glu Ala Val Leu Arg Ala Val Asp Gly Leu Asn Gln Arg
          35          40          45
Ser Ser Asp Glu Asn Leu Tyr Arg Leu Leu Glu Leu Asp Pro Leu Pro
          50          55          60
Lys Gly Asp Lys Asp Ser Asp Thr Pro Lys Pro Val Ser Phe Met Val
65          70          75          80
Lys Glu Thr Val Cys Pro Arg Ile Met Lys Gln Thr Pro Glu Gln Cys
          85          90          95
Asp Phe Lys Glu Asn Gly Leu Val Lys Gln Cys Val Gly Thr Val Ile
          100          105          110
Leu Gly Pro Val Lys Asp His Phe Asp Val Ser Cys Gly Glu Pro Gln
          115          120          125
Arg Val Lys Arg Phe Gly Arg Leu Ala Lys Ser Phe Leu Arg Met Arg
          130          135          140
Ile Leu Leu Pro Arg Arg Lys Ile Leu Leu Ala Ser
145          150          155

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<210> 36

<211> 160

<212> PRT

<213> Ovis aries

<400> 36

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Met Glu Thr Gln Arg Ala Ser Leu Ser Leu Gly Arg Cys Ser Leu Trp
1          5          10          15
Leu Leu Leu Leu Gly Leu Ala Leu Pro Ser Ala Ser Ala Gln Val Leu
          20          25          30

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Ser Tyr Arg Glu Ala Val Leu Arg Ala Ala Asp Gln Leu Asn Glu Lys
 35 40 45
 Ser Ser Glu Ala Asn Leu Tyr Arg Leu Leu Glu Leu Asp Pro Pro Pro
 50 55 60
 Lys Gln Asp Asp Glu Asn Ser Asn Ile Pro Lys Pro Val Ser Phe Arg
 65 70 75 80
 Val Lys Glu Thr Val Cys Pro Arg Thr Ser Gln Gln Pro Ala Glu Gln
 85 90 95
 Cys Asp Phe Lys Glu Asn Gly Leu Leu Lys Glu Cys Val Gly Thr Val
 100 105 110
 Thr Leu Asp Gln Val Arg Asn Asn Phe Asp Ile Thr Cys Ala Glu Pro
 115 120 125
 Gln Ser Val Arg Gly Leu Arg Arg Leu Gly Arg Lys Ile Ala His Gly
 130 135 140

 Val Lys Lys Tyr Gly Pro Thr Val Leu Arg Ile Ile Arg Ile Ala Gly
 145 150 155 160

<210> 37
 <211> 12
 <212> PRT
 <213> Bos taurus

<400> 37
 Arg Leu Cys Arg Ile Val Val Ile Arg Val Cys Arg
 1 5 10

<210> 38
 <211> 30
 <212> PRT
 <213> Homo sapiens

<400> 38
 Ala Cys Tyr Cys Arg Ile Pro Ala Cys Ile Ala Gly Glu Arg Arg Tyr
 1 5 10 15
 Gly Thr Cys Ile Tyr Gln Gly Arg Leu Trp Ala Phe Cys Cys
 20 25 30

<210> 39
 <211> 29
 <212> PRT
 <213> Homo sapiens

<400> 39
 Cys Tyr Cys Arg Ile Pro Ala Cys Ile Ala Gly Glu Arg Arg Tyr Gly
 1 5 10 15
 Thr Cys Ile Tyr Gln Gly Arg Leu Trp Ala Phe Cys Cys
 20 25

<210> 40
 <211> 30
 <212> PRT
 <213> Homo sapiens

<400> 40
 Asp Cys Tyr Cys Arg Ile Pro Ala Cys Ile Ala Gly Glu Arg Arg Tyr
 1 5 10 15
 Gly Thr Cys Ile Tyr Gln Gly Arg Leu Trp Ala Phe Cys Cys
 20 25 30

<210> 41
 <211> 33
 <212> PRT
 <213> Homo sapiens

<400> 41
 Val Cys Ser Cys Arg Leu Val Phe Cys Arg Arg Thr Glu Leu Arg Val
 1 5 10 15
 Gly Asn Cys Leu Ile Gly Gly Val Ser Phe Thr Tyr Cys Cys Thr Arg
 20 25 30
 Val

<210> 42
 <211> 33
 <212> PRT
 <213> Oryctolagus cuniculus

<400> 42
 Val Val Cys Ala Cys Arg Arg Ala Leu Cys Leu Pro Arg Glu Arg Arg
 1 5 10 15
 Ala Gly Phe Cys Arg Ile Arg Gly Arg Ile His Pro Leu Cys Cys Arg
 20 25 30
 Arg

<210> 43
 <211> 33
 <212> PRT
 <213> Oryctolagus cuniculus

<400> 43
 Val Val Cys Ala Cys Arg Arg Ala Leu Cys Leu Pro Leu Glu Arg Arg
 1 5 10 15
 Ala Gly Phe Cys Arg Ile Arg Gly Arg Ile His Pro Leu Cys Cys Arg
 20 25 30
 Arg

<210> 44
 <211> 34
 <212> PRT
 <213> Oryctolagus cuniculus

<400> 44
 Gly Ile Cys Ala Cys Arg Arg Arg Phe Cys Pro Asn Ser Glu Arg Phe
 1 5 10 15
 Ser Gly Tyr Cys Arg Val Asn Gly Ala Arg Tyr Val Arg Cys Cys Ser
 20 25 30
 Arg Arg

<210> 45
 <211> 34
 <212> PRT
 <213> Oryctolagus cuniculus

<400> 45
 Gly Arg Cys Val Cys Arg Lys Gln Leu Leu Cys Ser Tyr Arg Glu Arg
 1 5 10 15

Arg Ile Gly Asp Cys Lys Ile Arg Gly Val Arg Phe Pro Phe Cys Cys
 20 25 30
 Pro Arg

<210> 46
 <211> 34
 <212> PRT
 <213> *Oryctolagus cuniculus*

<400> 46
 Val Ser Cys Thr Cys Arg Arg Phe Ser Cys Gly Phe Gly Glu Arg Ala
 1 5 10 15
 Ser Gly Ser Cys Thr Val Asn Gly Gly Val Arg His Thr Leu Cys Cys
 20 25 30
 Arg Arg

<210> 47
 <211> 33
 <212> PRT
 <213> *Oryctolagus cuniculus*

<400> 47
 Val Phe Cys Thr Cys Arg Gly Phe Leu Cys Gly Ser Gly Glu Arg Ala
 1 5 10 15
 Ser Gly Ser Cys Thr Ile Asn Gly Val Arg His Thr Leu Cys Cys Arg
 20 25 30
 Arg

<210> 48
 <211> 32
 <212> PRT
 <213> *Rattus norvegicus*

<400> 48
 Val Thr Cys Tyr Cys Arg Arg Thr Arg Cys Gly Phe Arg Glu Arg Leu
 1 5 10 15
 Ser Gly Ala Cys Gly Tyr Arg Gly Arg Ile Tyr Arg Leu Cys Cys Arg
 20 25 30

<210> 49
 <211> 30
 <212> PRT
 <213> *Rattus norvegicus*

<400> 49
 Cys Ser Cys Arg Tyr Ser Ser Cys Arg Phe Gly Glu Arg Leu Leu Ser
 1 5 10 15
 Gly Ala Cys Arg Leu Asn Gly Arg Ile Tyr Arg Leu Cys Cys
 20 25 30

<210> 50
 <211> 31
 <212> PRT
 <213> *Rattus norvegicus*

<400> 50
 Ala Cys Thr Cys Arg Ile Gly Ala Cys Val Ser Gly Glu Arg Leu Thr
 1 5 10 15

Gly Ala Cys Gly Leu Asn Gly Arg Ile Tyr Arg Leu Cys Cys Arg
 20 25 30

<210> 51
 <211> 31
 <212> PRT
 <213> Guinea pig

<400> 51
 Arg Arg Cys Ile Cys Thr Thr Arg Thr Cys Arg Phe Pro Tyr Arg Arg
 1 5 10 15
 Leu Gly Thr Cys Ile Phe Gln Asn Arg Val Tyr Thr Phe Cys Cys
 20 25 30

<210> 52
 <211> 67
 <212> PRT
 <213> Homo sapiens

<400> 52
 Met Arg Ile His Tyr Leu Leu Phe Ala Leu Leu Phe Leu Phe Leu Val
 1 5 10 15
 Pro Val Pro Gly His Gly Gly Ile Ile Asn Thr Leu Gln Lys Tyr Tyr
 20 25 30
 Cys Arg Val Arg Gly Gly Arg Cys Ala Val Leu Ser Cys Leu Pro Lys
 35 40 45
 Glu Glu Gln Ile Gly Lys Cys Ser Thr Arg Gly Arg Lys Cys Cys Arg
 50 55 60
 Arg Lys Lys
 65

<210> 53
 <211> 18
 <212> PRT
 <213> Macaca mulatta

<400> 53
 Arg Cys Ile Cys Thr Arg Gly Phe Cys Arg Cys Leu Cys Arg Arg Gly
 1 5 10 15
 Val Cys

<210> 54
 <211> 78
 <212> PRT
 <213> Helianthus annuus

<400> 54
 Met Lys Ser Ser Met Lys Met Phe Ala Ala Leu Leu Leu Val Val Met
 1 5 10 15
 Cys Leu Leu Ala Asn Glu Met Gly Gly Pro Leu Val Val Glu Ala Arg
 20 25 30
 Thr Cys Glu Ser Gln Ser His Lys Phe Lys Gly Thr Cys Leu Ser Asp
 35 40 45
 Thr Asn Cys Ala Asn Val Cys His Ser Glu Arg Phe Ser Gly Gly Lys
 50 55 60
 Cys Arg Gly Phe Arg Arg Arg Cys Phe Cys Thr Thr His Cys
 65 70 75

<210> 55
 <211> 78

<212> PRT
 <213> Helianthus annuus

<400> 55
 Met Lys Ser Ser Met Lys Met Phe Ala Ala Leu Leu Leu Val Val Met
 1 5 10 15
 Cys Leu Leu Ala Asn Glu Met Gly Gly Pro Leu Val Val Glu Ala Arg
 20 25 30
 Thr Cys Glu Ser Gln Ser His Lys Phe Lys Gly Thr Cys Leu Ser Asp
 35 40 45
 Thr Asn Cys Ala Asn Val Cys His Ser Glu Arg Phe Ser Gly Gly Lys
 50 55 60
 Cys Arg Gly Phe Arg Arg Arg Cys Phe Cys Thr Thr His Cys
 65 70 75

<210> 56
 <211> 30
 <212> PRT
 <213> Macaca mulatta

<400> 56
 Ala Cys Tyr Cys Arg Ile Pro Ala Cys Leu Ala Gly Glu Arg Arg Tyr
 1 5 10 15
 Gly Thr Cys Phe Tyr Met Gly Arg Val Trp Ala Phe Cys Cys
 20 25 30

<210> 57
 <211> 37
 <212> PRT
 <213> Androctonus australis hector

<400> 57
 Gly Phe Gly Cys Pro Phe Asn Gln Gly Ala Cys His Arg His Cys Arg
 1 5 10 15
 Ser Ile Arg Arg Arg Gly Gly Tyr Cys Ala Gly Leu Phe Lys Gln Thr
 20 25 30
 Cys Thr Cys Tyr Arg
 35

<210> 58
 <211> 38
 <212> PRT
 <213> Mytilus galloprovincialis

<220>
 <221> misc_feature
 <222> (28)..(28)
 <223> Xaa can be any naturally occurring amino acid

<400> 58
 Gly Phe Gly Cys Pro Asn Asn Tyr Gln Cys His Arg His Cys Lys Ser
 1 5 10 15
 Ile Pro Gly Arg Cys Gly Gly Tyr Cys Gly Gly Xaa His Arg Leu Arg
 20 25 30
 Cys Thr Cys Tyr Arg Cys
 35

<210> 59
 <211> 54
 <212> PRT
 <213> Heuchera sanguinea

<400> 59

Asp Gly Val Lys Leu Cys Asp Val Pro Ser Gly Thr Trp Ser Gly His
 1 5 10 15
 Cys Gly Ser Ser Ser Lys Cys Ser Gln Gln Cys Lys Asp Arg Glu His
 20 25 30
 Phe Ala Tyr Gly Gly Ala Cys His Tyr Gln Phe Pro Ser Val Lys Cys
 35 40 45
 Phe Cys Lys Arg Gln Cys
 50

<210> 60

<211> 49

<212> PRT

<213> Clitoria ternatea

<400> 60

Asn Leu Cys Glu Arg Ala Ser Leu Thr Trp Thr Gly Asn Cys Gly Asn
 1 5 10 15
 Thr Gly His Cys Asp Thr Gln Cys Arg Asn Trp Glu Ser Ala Lys His
 20 25 30
 Gly Ala Cys His Lys Arg Gly Asn Trp Lys Cys Phe Cys Tyr Phe Asn
 35 40 45
 Cys

<210> 61

<211> 91

<212> PRT

<213> Mus musculus

<400> 61

Met Lys Lys Leu Val Leu Leu Phe Ala Leu Val Leu Leu Ala Phe Gln
 1 5 10 15
 Val Gln Ala Asp Ser Ile Gln Asn Thr Asp Glu Glu Thr Lys Thr Glu
 20 25 30
 Glu Gln Pro Gly Glu Lys Asp Gln Ala Val Ser Val Ser Phe Gly Asp
 35 40 45
 Pro Gln Gly Ser Ala Leu Gln Asp Ala Ala Leu Gly Trp Gly Arg Arg
 50 55 60
 Cys Pro Gln Cys Pro Arg Cys Pro Ser Cys Pro Ser Cys Pro Arg Cys
 65 70 75 80
 Pro Arg Cys Pro Arg Cys Lys Cys Asn Pro Lys
 85 90

<210> 62

<211> 40

<212> PRT

<213> Bos taurus

<400> 62

Gln Gly Val Arg Asn Phe Val Thr Cys Arg Ile Asn Arg Gly Phe Cys
 1 5 10 15
 Val Pro Ile Arg Cys Pro Gly His Arg Arg Gln Ile Gly Thr Cys Leu
 20 25 30
 Gly Pro Gln Ile Lys Cys Cys Arg
 35 40

<210> 63

<211> 40

<212> PRT

<213> Bos taurus

<400> 63

Gln	Gly	Val	Arg	Asn	Phe	Val	Thr	Cys	Arg	Ile	Asn	Arg	Gly	Phe	Cys
1				5				10					15		
Val	Pro	Ile	Arg	Cys	Pro	Gly	His	Arg	Arg	Gln	Ile	Gly	Thr	Cys	Leu
			20					25					30		
Gly	Pro	Arg	Ile	Lys	Cys	Cys	Arg								
		35					40								

<210> 64

<211> 42

<212> PRT

<213> Bos taurus

<400> 64

Gln	Gly	Val	Arg	Asn	His	Val	Thr	Cys	Arg	Ile	Tyr	Gly	Gly	Phe	Cys
1				5				10						15	
Val	Pro	Ile	Arg	Cys	Pro	Gly	Arg	Thr	Arg	Gln	Ile	Gly	Thr	Cys	Phe
			20					25					30		
Gly	Arg	Pro	Val	Lys	Cys	Cys	Arg	Arg	Trp						
		35					40								

<210> 65

<211> 40

<212> PRT

<213> Bos taurus

<400> 65

Gln	Val	Val	Arg	Asn	Pro	Gln	Ser	Cys	Arg	Trp	Asn	Met	Gly	Val	Cys
1				5				10						15	
Ile	Pro	Ile	Ser	Cys	Pro	Gly	Asn	Met	Arg	Gln	Ile	Gly	Thr	Cys	Phe
			20					25					30		
Gly	Pro	Arg	Val	Pro	Cys	Cys	Arg								
		35					40								

<210> 66

<211> 41

<212> PRT

<213> Bos taurus

<400> 66

Gln	Arg	Val	Arg	Asn	Pro	Gln	Ser	Cys	Arg	Trp	Asn	Met	Gly	Val	Cys
1				5				10						15	
Ile	Pro	Phe	Leu	Cys	Arg	Val	Gly	Met	Arg	Gln	Ile	Gly	Thr	Cys	Phe
			20					25					30		
Gly	Pro	Arg	Val	Pro	Cys	Cys	Arg	Arg							
		35					40								

<210> 67

<211> 42

<212> PRT

<213> Bos taurus

<400> 67

Gln	Gly	Val	Arg	Asn	His	Val	Thr	Cys	Arg	Ile	Asn	Arg	Gly	Phe	Cys
1				5				10						15	
Val	Pro	Ile	Arg	Cys	Pro	Gly	Arg	Thr	Arg	Gln	Ile	Gly	Thr	Cys	Phe
			20					25					30		
Gly	Pro	Arg	Ile	Lys	Cys	Cys	Arg	Ser	Trp						
		35					40								

<210> 68
 <211> 40
 <212> PRT
 <213> Bos taurus

<400> 68
 Gln Gly Val Arg Ser Tyr Leu Ser Cys Trp Gly Asn Arg Gly Ile Cys
 1 5 10 15
 Leu Leu Asn Arg Cys Pro Gly Arg Met Arg Gln Ile Gly Thr Cys Leu
 20 25 30
 Ala Pro Arg Val Lys Cys Cys Arg
 35 40

<210> 69
 <211> 42
 <212> PRT
 <213> Bos taurus

<400> 69
 Ser Gly Ile Ser Gly Pro Leu Ser Cys Gly Arg Asn Gly Gly Val Cys
 1 5 10 15
 Ile Pro Ile Arg Cys Pro Val Pro Met Arg Gln Ile Gly Thr Cys Phe
 20 25 30
 Gly Arg Pro Val Lys Cys Cys Arg Ser Trp
 35 40

<210> 70
 <211> 38
 <212> PRT
 <213> Bos taurus

<400> 70
 Asp Phe Ala Ser Cys His Thr Asn Gly Gly Ile Cys Leu Pro Asn Arg
 1 5 10 15
 Cys Pro Gly His Met Ile Gln Ile Gly Ile Cys Phe Arg Pro Arg Val
 20 25 30
 Lys Cys Cys Arg Ser Trp
 35

<210> 71
 <211> 74
 <212> PRT
 <213> Zophobas atratus

<400> 71
 Ser Leu Gln Gly Gly Ala Pro Asn Phe Pro Gln Pro Ser Gln Gln Asn
 1 5 10 15
 Gly Gly Trp Gln Val Ser Pro Asp Leu Gly Arg Asp Asp Lys Gly Asn
 20 25 30
 Thr Arg Gly Gln Ile Glu Ile Gln Asn Lys Gly Lys Asp His Asp Phe
 35 40 45
 Asn Ala Gly Trp Gly Lys Val Ile Arg Gly Pro Asn Lys Ala Lys Pro
 50 55 60
 Thr Trp His Val Gly Gly Thr Tyr Arg Arg
 65 70

<210> 72
 <211> 67
 <212> PRT
 <213> Homo sapiens

<400> 72

Met Arg Ile His Tyr Leu Leu Phe Ala Leu Leu Phe Leu Phe Leu Val
 1 5 10 15
 Pro Val Pro Gly His Gly Gly Ile Ile Asn Thr Leu Gln Lys Tyr Tyr
 20 25 30
 Cys Arg Val Arg Gly Gly Arg Cys Ala Val Leu Ser Cys Leu Pro Lys
 35 40 45
 Glu Glu Gln Ile Gly Lys Cys Ser Thr Arg Gly Arg Lys Cys Cys Arg
 50 55 60
 Arg Lys Lys
 65

<210> 73

<211> 40

<212> PRT

<213> *Aedes aegypti*

<400> 73

Ala Thr Cys Asp Leu Leu Ser Gly Phe Gly Val Gly Asp Ser Ala Cys
 1 5 10 15
 Ala Ala His Cys Ile Ala Arg Gly Asn Arg Gly Gly Tyr Cys Asn Ser
 20 25 30
 Lys Lys Val Cys Val Cys Arg Asn
 35 40

<210> 74

<211> 35

<212> PRT

<213> *Mytilus edulis*

<220>

<221> misc_feature

<222> (28)..(28)

<223> Xaa can be any naturally occurring amino acid

<400> 74

Gly Phe Gly Cys Pro Asn Asp Tyr Pro Cys His Arg His Cys Lys Ser
 1 5 10 15
 Ile Pro Gly Arg Tyr Gly Gly Tyr Cys Gly Gly Xaa His Arg Leu Arg
 20 25 30
 Cys Thr Cys
 35

<210> 75

<211> 40

<212> PRT

<213> *Sarcophaga peregrina*

<400> 75

Ala Thr Cys Asp Leu Leu Ser Gly Ile Gly Val Gln His Ser Ala Cys
 1 5 10 15
 Ala Leu His Cys Val Phe Arg Gly Asn Arg Gly Gly Tyr Cys Thr Gly
 20 25 30
 Lys Gly Ile Cys Val Cys Arg Asn
 35 40

<210> 76

<211> 95

<212> PRT

<213> *Oryctolagus cuniculus*

<400> 76

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Met Arg Thr Leu Ala Leu Leu Ala Ala Ile Leu Leu Val Ala Leu Gln
1          5          10          15
Ala Gln Ala Glu His Val Ser Val Ser Ile Asp Glu Val Val Asp Gln
          20          25          30
Gln Pro Pro Gln Ala Glu Asp Gln Asp Val Ala Ile Tyr Val Lys Glu
          35          40          45
His Glu Ser Ser Ala Leu Glu Ala Leu Gly Val Lys Ala Gly Val Val
          50          55          60
Cys Ala Cys Arg Arg Ala Leu Cys Leu Pro Arg Glu Arg Arg Ala Gly
65          70          75          80
Phe Cys Arg Ile Arg Gly Arg Ile His Pro Leu Cys Cys Arg Arg
          85          90          95

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<210> 77

<211> 92

<212> PRT

<213> Mus musculus

<400> 77

```

Met Lys Pro Leu Val Leu Leu Ser Ala Leu Val Leu Leu Ser Phe Gln
1          5          10          15
Val Gln Ala Asp Pro Ile Gln Asn Thr Asp Glu Glu Thr Lys Thr Glu
          20          25          30
Glu Gln Ser Gly Glu Glu Asp Gln Ala Val Ser Val Ser Phe Gly Asp
          35          40          45
Arg Glu Gly Ala Ser Leu Gln Glu Glu Ser Leu Arg Asp Leu Val Cys
          50          55          60
Tyr Cys Arg Thr Arg Gly Cys Lys Arg Arg Glu Arg Met Asn Gly Thr
65          70          75          80
Cys Arg Lys Gly His Leu Met Tyr Thr Leu Cys Cys
          85          90

```

<210> 78

<211> 93

<212> PRT

<213> Mus musculus

<400> 78

```

Met Lys Thr Phe Val Leu Leu Ser Ala Leu Val Leu Leu Ala Phe Gln
1          5          10          15
Val Gln Ala Asp Pro Ile His Lys Thr Asp Glu Glu Thr Asn Thr Glu
          20          25          30
Glu Gln Pro Gly Glu Glu Asp Gln Ala Val Ser Ile Ser Phe Gly Gly
          35          40          45
Gln Glu Gly Ser Ala Leu His Glu Glu Leu Ser Lys Lys Leu Ile Cys
          50          55          60
Tyr Cys Arg Ile Arg Gly Cys Lys Arg Arg Glu Arg Val Phe Gly Thr
65          70          75          80
Cys Arg Asn Leu Phe Leu Thr Phe Val Phe Cys Cys Ser
          85          90

```

<210> 79

<211> 35

<212> PRT

<213> Mus musculus

<400> 79

```

Leu Arg Asp Leu Val Cys Tyr Cys Arg Ala Arg Gly Cys Lys Gly Arg
1          5          10          15

```

Glu Arg Met Asn Gly Thr Cys Arg Lys Gly His Leu Leu Tyr Met Leu
 20 25 30
 Cys Cys Arg
 35

<210> 80
 <211> 43
 <212> PRT
 <213> *Pyrrhocoris apterus*

<400> 80
 Ala Thr Cys Asp Ile Leu Ser Phe Gln Ser Gln Trp Val Thr Pro Asn
 1 5 10 15
 His Ala Gly Cys Ala Leu His Cys Val Ile Lys Gly Tyr Lys Gly Gly
 20 25 30
 Gln Cys Lys Ile Thr Val Cys His Cys Arg Arg
 35 40

<210> 81
 <211> 32
 <212> PRT
 <213> *Rattus norvegicus*

<400> 81
 Val Thr Cys Tyr Cys Arg Ser Thr Arg Cys Gly Phe Arg Glu Arg Leu
 1 5 10 15
 Ser Gly Ala Cys Gly Tyr Arg Gly Arg Ile Tyr Arg Leu Cys Cys Arg
 20 25 30

<210> 82
 <211> 31
 <212> PRT
 <213> *Rattus norvegicus*

<400> 82
 Val Thr Cys Ser Cys Arg Thr Ser Ser Cys Arg Phe Gly Glu Arg Leu
 1 5 10 15
 Ser Gly Ala Cys Arg Leu Asn Gly Arg Ile Tyr Arg Leu Cys Cys
 20 25 30

<210> 83
 <211> 34
 <212> PRT
 <213> *Oryctolagus cuniculus*

<400> 83
 Gly Ile Cys Ala Cys Arg Arg Arg Phe Cys Leu Asn Phe Glu Gln Phe
 1 5 10 15
 Ser Gly Tyr Cys Arg Val Asn Gly Ala Arg Tyr Val Arg Cys Cys Ser
 20 25 30
 Arg Arg

<210> 84
 <211> 64
 <212> PRT
 <213> *Pan troglodytes*

<400> 84
 Met Arg Val Leu Tyr Leu Leu Phe Ser Phe Leu Phe Ile Phe Leu Met
 1 5 10 15

Pro Leu Pro Gly Val Phe Gly Gly Ile Ser Asp Pro Val Thr Cys Leu
 20 25 30
 Lys Ser Gly Ala Ile Cys His Pro Val Phe Cys Pro Arg Arg Tyr Lys
 35 40 45
 Gln Ile Gly Thr Cys Gly Leu Pro Gly Thr Lys Cys Cys Lys Lys Pro
 50 55 60

<210> 85
 <211> 64
 <212> PRT
 <213> Homo sapiens

<400> 85
 Met Arg Val Leu Tyr Leu Leu Phe Ser Phe Leu Phe Ile Phe Leu Met
 1 5 10 15
 Pro Leu Pro Gly Val Phe Gly Gly Ile Gly Asp Pro Val Thr Cys Leu
 20 25 30
 Lys Ser Gly Ala Ile Cys His Pro Val Phe Cys Pro Arg Arg Tyr Lys
 35 40 45
 Gln Ile Gly Thr Cys Gly Leu Pro Gly Thr Lys Cys Cys Lys Lys Pro
 50 55 60

<210> 86
 <211> 68
 <212> PRT
 <213> Homo sapiens

<400> 86
 Met Arg Thr Ser Tyr Leu Leu Leu Phe Thr Leu Cys Leu Leu Leu Ser
 1 5 10 15
 Glu Met Ala Ser Gly Gly Asn Phe Leu Thr Gly Leu Gly His Arg Ser
 20 25 30
 Asp His Tyr Asn Cys Val Ser Ser Gly Gly Gln Cys Leu Tyr Ser Ala
 35 40 45
 Cys Pro Ile Phe Thr Lys Ile Gln Gly Thr Cys Tyr Arg Gly Lys Ala
 50 55 60
 Lys Cys Cys Lys
 65

<210> 87
 <211> 64
 <212> PRT
 <213> Capra hircus

<400> 87
 Met Arg Leu His His Leu Leu Leu Val Leu Phe Phe Leu Val Leu Ser
 1 5 10 15
 Ala Gly Ser Gly Phe Thr Gln Gly Ile Arg Ser Arg Arg Ser Cys His
 20 25 30
 Arg Asn Lys Gly Val Cys Ala Leu Thr Arg Cys Pro Arg Asn Met Arg
 35 40 45
 Gln Ile Gly Thr Cys Phe Gly Pro Pro Val Lys Cys Cys Arg Lys Lys
 50 55 60

<210> 88
 <211> 64
 <212> PRT
 <213> Capra hircus

<400> 88

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Met Arg Leu His His Leu Leu Leu Ala Leu Phe Phe Leu Val Leu Ser
1          5          10          15
Ala Gly Ser Gly Phe Thr Gln Gly Ile Ile Asn His Arg Ser Cys Tyr
          20          25          30
Arg Asn Lys Gly Val Cys Ala Pro Ala Arg Cys Pro Arg Asn Met Arg
          35          40          45
Gln Ile Gly Thr Cys His Gly Pro Pro Val Lys Cys Cys Arg Lys Lys
          50          55          60

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<210> 89

<211> 96

<212> PRT

<213> Macaca mulatta

<400> 89

```

Met Arg Thr Leu Val Ile Leu Ala Ala Ile Leu Leu Val Ala Leu Gln
1          5          10          15
Ala Gln Ala Glu Pro Leu Gln Ala Arg Thr Asp Glu Ala Thr Ala Ala
          20          25          30
Gln Glu Gln Ile Pro Thr Asp Asn Pro Glu Val Val Val Ser Leu Ala
          35          40          45
Trp Asp Glu Ser Leu Ala Pro Lys Asp Ser Val Pro Gly Leu Arg Lys
          50          55          60
Asn Met Ala Cys Tyr Cys Arg Ile Pro Ala Cys Leu Ala Gly Glu Arg
65          70          75          80
Arg Tyr Gly Thr Cys Phe Tyr Arg Arg Arg Val Trp Ala Phe Cys Cys
          85          90          95

```

<210> 90

<211> 96

<212> PRT

<213> Macaca mulatta

<400> 90

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Met Arg Thr Leu Val Ile Leu Ala Ala Ile Leu Leu Val Ala Leu Gln
1          5          10          15
Ala Gln Ala Glu Pro Leu Gln Ala Arg Thr Asp Glu Ala Thr Ala Ala
          20          25          30
Gln Glu Gln Ile Pro Thr Asp Asn Pro Glu Val Val Val Ser Leu Ala
          35          40          45
Trp Asp Glu Ser Leu Ala Pro Lys Asp Ser Val Pro Gly Leu Arg Lys
          50          55          60
Asn Met Ala Cys Tyr Cys Arg Ile Pro Ala Cys Leu Ala Gly Glu Arg
65          70          75          80
Arg Tyr Gly Thr Cys Phe Tyr Leu Gly Arg Val Trp Ala Phe Cys Cys
          85          90          95

```

<210> 91

<211> 33

<212> PRT

<213> Mesocricetus auratus

<400> 91

```

Val Thr Cys Phe Cys Arg Arg Arg Gly Cys Ala Ser Arg Glu Arg His
1          5          10          15
Ile Gly Tyr Cys Arg Phe Gly Asn Thr Ile Tyr Arg Leu Cys Cys Arg
          20          25          30
Arg

```

<210> 92
 <211> 31
 <212> PRT
 <213> *Mesocricetus auratus*

<400> 92
 Cys Phe Cys Lys Arg Pro Val Cys Asp Ser Gly Glu Thr Gln Ile Gly
 1 5 10 15
 Tyr Cys Arg Leu Gly Asn Thr Phe Tyr Arg Leu Cys Cys Arg Gln
 20 25 30

<210> 93
 <211> 39
 <212> PRT
 <213> *Gallus gallus*

<400> 93
 Gly Arg Lys Ser Asp Cys Phe Arg Lys Asn Gly Phe Cys Ala Phe Leu
 1 5 10 15
 Lys Cys Pro Tyr Leu Thr Leu Ile Ser Gly Lys Cys Ser Arg Phe His
 20 25 30
 Leu Cys Cys Lys Arg Ile Trp
 35

<210> 94
 <211> 43
 <212> PRT
 <213> *Allomyrina dichotoma*

<400> 94
 Val Thr Cys Asp Leu Leu Ser Phe Glu Ala Lys Gly Phe Ala Ala Asn
 1 5 10 15
 His Ser Leu Cys Ala Ala His Cys Leu Ala Ile Gly Arg Arg Gly Gly
 20 25 30
 Ser Cys Glu Arg Gly Val Cys Ile Cys Arg Arg
 35 40

<210> 95
 <211> 31
 <212> PRT
 <213> *Cavia porcellus*

<400> 95
 Arg Arg Cys Ile Cys Thr Thr Arg Thr Cys Arg Phe Pro Tyr Arg Arg
 1 5 10 15
 Leu Gly Thr Cys Ile Phe Gln Asn Arg Val Tyr Thr Phe Cys Cys
 20 25 30

<210> 96
 <211> 36
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic

<220>
 <221> MISC_FEATURE
 <222> (1)..(2)
 <223> The residue at positions 1 & 2 may represent conservatively or
 nonconservatively substituted amino acids and may be present or

absent.

<220>
 <221> MISC_FEATURE
 <222> (4)..(4)
 <223> The residue at this position may represent a conservatively or nonconservatively substituted amino acid.

<220>
 <221> MISC_FEATURE
 <222> (7)..(9)
 <223> The residue at positions 7 to 9 may represent conservatively or nonconservatively substituted amino acids.

<220>
 <221> MISC_FEATURE
 <222> (10)..(10)
 <223> The residue this position may represent conservatively or nonconservatively substituted amino acids or may be absent.

<220>
 <221> MISC_FEATURE
 <222> (12)..(14)
 <223> The residue at positions 12 to 14 may represent conservatively or nonconservatively substituted amino acids.

<220>
 <221> MISC_FEATURE
 <222> (15)..(15)
 <223> The residue at this position may represent conservatively or nonconservatively substituted amino acids or may be absent.

<220>
 <221> MISC_FEATURE
 <222> (18)..(18)
 <223> The residue at this position may represent a conservatively or nonconservatively substituted amino acid.

<220>
 <221> MISC_FEATURE
 <222> (19)..(20)
 <223> The residue at positions 19 & 20 may represent conservatively or nonconservatively substituted amino acids and may be absent.

<220>
 <221> MISC_FEATURE
 <222> (22)..(22)
 <223> The residue at this position may represent conservatively or nonconservatively substituted amino acids.

<220>
 <221> MISC_FEATURE
 <222> (24)..(28)
 <223> The residue at positions 24 to 28 may represent consecutively or nonconservatively substituted amino acids.

<220>
 <221> MISC_FEATURE
 <222> (29)..(32)
 <223> The residue at positions 29 to 32 may represent consecutively or nonconservatively substituted amino acids and may be absent.

<220>
 <221> MISC_FEATURE
 <222> (35)..(36)
 <223> The residue at positions 35 & 36 may represent conservatively or nonconservatively substituted amino acids and may be present or absent.

<400> 96
 Xaa Xaa Cys Xaa Cys Arg Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Glu
 1 5 10 15
 Arg Xaa Xaa Xaa Cys Xaa Gly Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
 20 25 30
 Cys Cys Xaa Xaa
 35

<210> 97
 <211> 2540
 <212> DNA
 <213> Homo sapiens

<400> 97
 tccggtcgac ctatggctat tggccagggt caatactatg tattggccct atgccatata 60
 gtattccata tatgggtttt cctattgacg tagatagccc ctcccaatgg gcggtcccat 120
 ataccatata tggggcttcc taataccgcc catagccact cccccattga cgtcaatggg 180
 ctctatatat ggtctttcct attgacgtca tatgggcggt cctattgacg tatatggcgc 240
 ctccccatt gacgtcaatt acggtaaatg gccgcctgg ctcaatgcc attgacgtca 300
 ataggaccac ccaccattga cgtcaatggg atggctcatt gccattcat atccgttctc 360
 acgcccccta ttgacgtcaa tgacggtaaa tggcccaactt ggcagtacat caatatctat 420
 taatagtaac ttggcaagta cattactatt ggaagtacgc cagggtacat tggcagtact 480
 cccattgacg tcaatggcgg taaatggccc gcgatggctg ccaagtacat cccattgac 540
 gtcaatgggg aggggcaatg acgcaaattg gcgttccatt gacgtaaatg ggcggtaggc 600
 gtgcctaatt ggaggtctat ataagcaatg ctcgtttagg gaaccgccat tctgcctggg 660
 gacgtcggag gagctcgaat ggagcgcgcg tcctgcttgt tgctgctgct gctgccgctg 720
 gtgcacgtct ctgcgaccac gccagaacct tgtgagctgg acgatgaaga tttccgctgc 780
 gtctgcaact tctccgaacc tcagcccgac tgggtccgaag ccttccagtg tgtgtctgca 840
 gtagaggtgg agatccatgc cggcgggtctc aacctagagc cgttttctaaa gcgcgtcgat 900
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<213> Homo sapiens

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<212> DNA

<213> Homo sapiens

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<211> 5011

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aatgtcgtga	aggaagcagt	tcctctggaa	gcttcttgaa	gacaaacaac	gtctgtagcg	3780
accctttgca	ggcagcggaa	ccccccacct	ggcgacaggt	gcctctgcgg	ccaaaagcca	3840
cgtgtataag	atacacctgc	aaaggcggca	caacccctagt	gccacgttgt	gagttggata	3900
gttgtggaaa	gagtcaaatg	gctctcctca	agcgtattca	acaaggggct	gaaggatgcc	3960
cagaagggtac	cccattgtat	gggatctgat	ctggggcctc	ggtgcacatg	ctttacatgt	4020
gtttagtcga	ggttaaaaaa	acgtctaggg	ccccgaacc	acggggagct	ggttttcctt	4080
tgaaaaacac	gatgataata	tgagtgtcta	ctcaggctcct	ggggttgctg	ctgctgtggc	4140
ttacagggtgc	cagatgtgac	atccagatga	ctcagctctc	agcctcccta	tctgcatctg	4200
tgggagaaac	tgtcaccatc	acatgtcgag	caagtgagaa	catttacagt	tatttagcat	4260
ggtatcagca	gaaacaggga	aaatctcctc	agttcctggt	ctataatgca	gaaaccttag	4320
cagaagggtgt	gccatcaagg	ttcagtggca	gtggatcagg	caaacagttt	tctctgaaga	4380
tcaacagcct	gcagcctgaa	gattttggga	gttattactg	tcaacatcat	tatgggtactc	4440
atccgacgtt	cggtggaggc	accaagctgg	aaatcaaacg	ggctgatgct	gcaccaactg	4500
tatccatctt	cccaccatcc	agtgagcagt	taacatctgg	aggtgcctca	gtcgtgtgct	4560
tcttgaacaa	cttctacccc	aaagacatca	atgtcaagtg	gaagattgat	ggcagtgaac	4620
gacaaaatgg	cgtcctgaac	agttggactg	atcaggacag	caaagacagc	acctacagca	4680
tgagcagcac	cctcacgttg	accaaggacg	agtatgaacg	acataacagc	tatacctgtg	4740
aggccactca	caagacatca	acttcaccca	ttgtcaagag	cttcaacagg	aatgagtgtt	4800
agagacaaag	gtcctgagac	gccaccacca	gctccccagc	tccatcctat	cttcccttct	4860
aaggctcttg	aggcttcccc	acaagcgacc	taccactgtt	gcggtgctcc	aaacctcctc	4920
cccacctcct	tctcctcctc	ctccctttcc	ttggctttta	tcactgctaat	atttgcagaa	4980
aatattcaat	aaagtgagtc	tttgcacttg	a			5011

CLAIMS:

1. A non-therapeutic method of treating a non-human object, comprising:
 - a) providing
 - i) a recombinant fusion protein, wherein said protein comprises a microorganism targeting molecule joined to at least a portion of a protein biocide molecule, wherein said microorganism targeting molecule binds to a bacteria, and wherein said microorganism targeting molecule is CD14 and said protein biocide is phospholipase A2; and
 - ii) a non-human object suspected of being contaminated with a bacteria;
 - and
 - b) applying said recombinant fusion protein to said non-human object under conditions such that said recombinant fusion protein neutralizes said bacteria suspected of contaminating said non-human object.
2. The method of claim 1, wherein said non-human object is food processing equipment, military equipment, personal protective gear, a medical device, a domestic object, or a building structure.
3. The method of claim 2, wherein said domestic object is an appliance or a surface.
4. The method of claim 2, wherein said building structure is heating and ventilation equipment, a wall or wall cavity, or a plumbing system.
5. A method of treating an animal carcass or part thereof comprising:
 - a) providing
 - i) a recombinant fusion protein comprising a microorganism targeting molecule joined to at least a portion of a protein biocide molecule, wherein said microorganism targeting molecule binds to a bacteria, and wherein said microorganism targeting molecule is CD14 and said protein biocide is phospholipase A2; and

ii) an animal carcass suspected of being contaminated or infected with a bacteria; and

b) applying said recombinant fusion protein to said animal carcass or part thereof under conditions such that said recombinant fusion protein neutralizes said bacteria suspected of contaminating or infecting said animal carcass.

6. The method of claim 5, wherein said animal carcass comprises a bovine carcass or part thereof.

7. The method of claim 5, wherein said animal carcass comprises a porcine carcass or part thereof.

8. The method of claim 5, wherein said animal carcass comprises an avian carcass or part thereof.

9. The method of claim 5, wherein said animal carcass comprises an aquatic animal carcass or part thereof.

10. A method of treating a food product comprising:

a) providing

i) a recombinant fusion protein comprising a microorganism targeting molecule joined to at least a portion of a protein biocide molecule, wherein said microorganism targeting molecule binds to a bacteria, and wherein said microorganism targeting molecule is

CD14 and said protein biocide is phospholipase A2; and

ii) a food product suspected of being contaminated with a bacteria; and

b) applying said recombinant fusion protein to said food product under conditions such that said recombinant fusion protein neutralizes said bacteria suspected of contaminating said food product.

11. The method of claim 10, wherein said food product is a meat product, a processed meat product, vegetable, leaf, stem, seed, fruit, root, beer, wine, a dairy product, or animal feed.

12. The method of claim 10, wherein said food product is in the process of being produced.

13. Use of a recombinant fusion protein for the treatment of a subject suspected of being contaminated or infected with a bacteria, wherein said recombinant fusion protein comprises a microorganism targeting molecule joined to at least a portion of a protein biocide molecule, wherein said microorganism targeting molecule binds to said bacteria, and wherein said microorganism targeting molecule is CD14 and said protein biocide is phospholipase A2; and wherein said recombinant fusion protein is for application to said subject under conditions such that said recombinant fusion protein neutralizes said bacteria.

14. The use of claim 13, wherein said subject is a mammal.

15. The use of claim 14, wherein said mammal is a ruminant.

16. The use of claim 15, wherein said ruminant is a bovine.

17. The use of claim 14, wherein said mammal is a human.

18. The use of claim 13, wherein said subject is an avian species.

19. The use of any one of claims 13 to 18, wherein said subject is suspected of being contaminated or infected with an antibiotic resistant bacteria.

20. The use of any one of claims 13 to 19, wherein said subject is suspected of being contaminated or infected with an artificially engineered bacteria.

21. The use of any one of claims 13 to 20, wherein said subject is deceased.

22. A method of supplementing a food ration comprising:
- a) providing
 - i) a recombinant fusion protein comprising a microorganism targeting molecule joined to at least a portion of a protein biocide molecule, wherein said microorganism targeting molecule binds to a bacteria, and wherein said microorganism targeting molecule is CD14 and said protein biocide is phospholipase A2, and wherein said fusion protein neutralizes said bacteria; and
 - ii) a food ration; and
 - b) supplementing said food ration with said recombinant fusion protein.

23. A food comprising:
 a recombinant fusion protein comprising a microorganism targeting molecule joined to at least a portion of a protein biocide molecule, wherein said microorganism targeting molecule binds to a bacteria, and wherein said microorganism targeting molecule is CD14 and said protein biocide is phospholipase A2, and wherein said fusion protein neutralizes said bacteria; and at least one foodstuff.

24. The food of claim 23, wherein said food is for a human.

25. The food of claim 23, wherein said food is for a farm animal.

26. The food of claim 23, wherein said food is for a companion animal.

27. A composition comprising:

a milk protein; and

a recombinant fusion protein wherein said recombinant fusion protein comprises a microorganism targeting molecule joined to at least a portion of a protein biocide molecule, wherein said microorganism targeting molecule binds to a bacteria, and wherein said

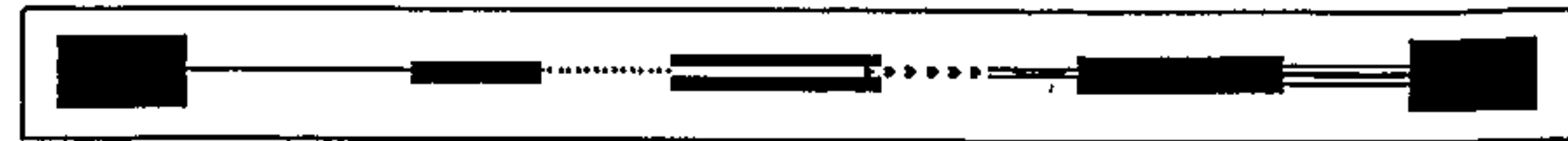
microorganism targeting molecule is CD14 and said protein biocide is phospholipase A2, and wherein said fusion protein neutralizes said bacteria.

28. A method of treating a plant or a part of a plant, comprising:
- a) providing
 - i) a recombinant fusion protein, wherein said protein comprises a microorganism targeting molecule joined to at least a portion of a protein biocide molecule, wherein said microorganism targeting molecule binds to a bacteria, and wherein said microorganism targeting molecule is CD14 and said protein biocide is phospholipase A2; and
 - ii) a plant suspected of being infected or contaminated by a bacteria;
 - and
 - b) applying said recombinant fusion protein to said plant or plant part under conditions such that said recombinant fusion protein neutralizes said bacteria.

29. The method of claim 28, wherein said plant or plant part is a seed, a vegetable, a stem, a leaf, a root, a growing plant, or a fruit.

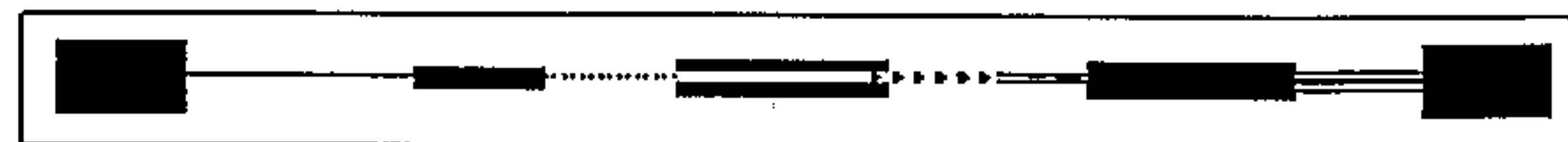
Figure 1

A



- Moloney Murine Leukemia Virus 5'LTR
- Moloney Murine Leukemia Virus extended packaging region
- bovine alpha-lactalbumin Promoter **
- α -Lactalbumin signal peptide
- == Murine IgG2a heavy chain gene (cDNA)
- Encephalomyocarditis virus internal ribosome entry site
- α -Lactalbumin signal peptide
- Murine antibody κ light chain gene (cDNA)
- === RNA transport element
- Moloney Murine Leukemia Virus 3' LTR

B



- Moloney Murine Leukemia Virus 5'LTR
- Moloney Murine Leukemia Virus extended packaging region
- bovine alpha-lactalbumin Promoter **
- α -Lactalbumin signal peptide
- == Murine IgG2a heavy chain gene (cDNA)
- Encephalomyocarditis virus internal ribosome entry site
- α -Lactalbumin signal peptide
- Murine antibody κ light chain gene (cDNA)
- === RNA transport element
- Moloney Murine Leukemia Virus 3' LTR

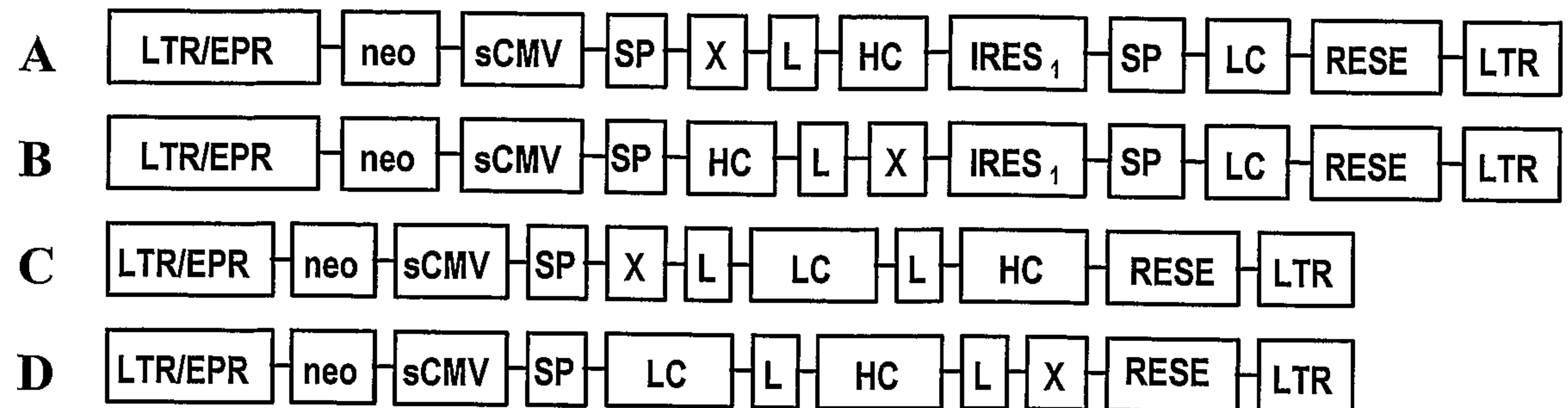
Figure 2

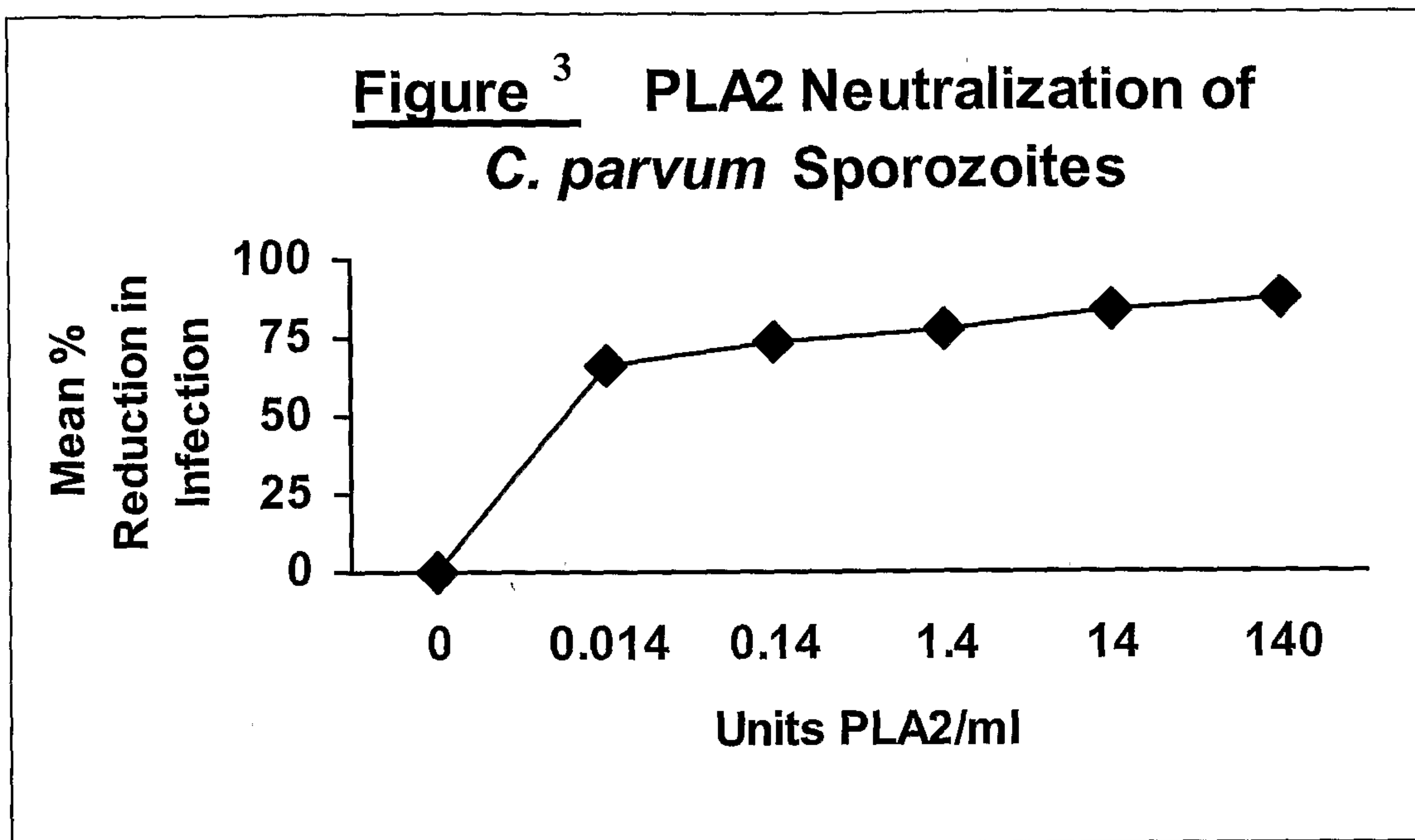
Figure 3

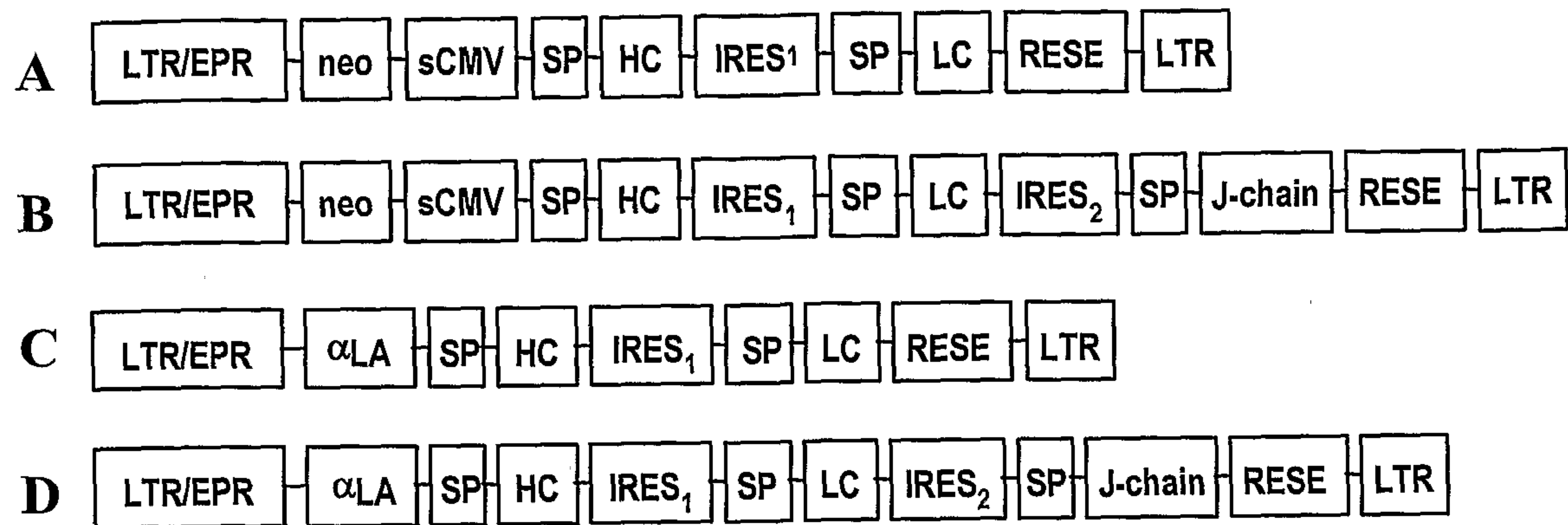
Figure 4

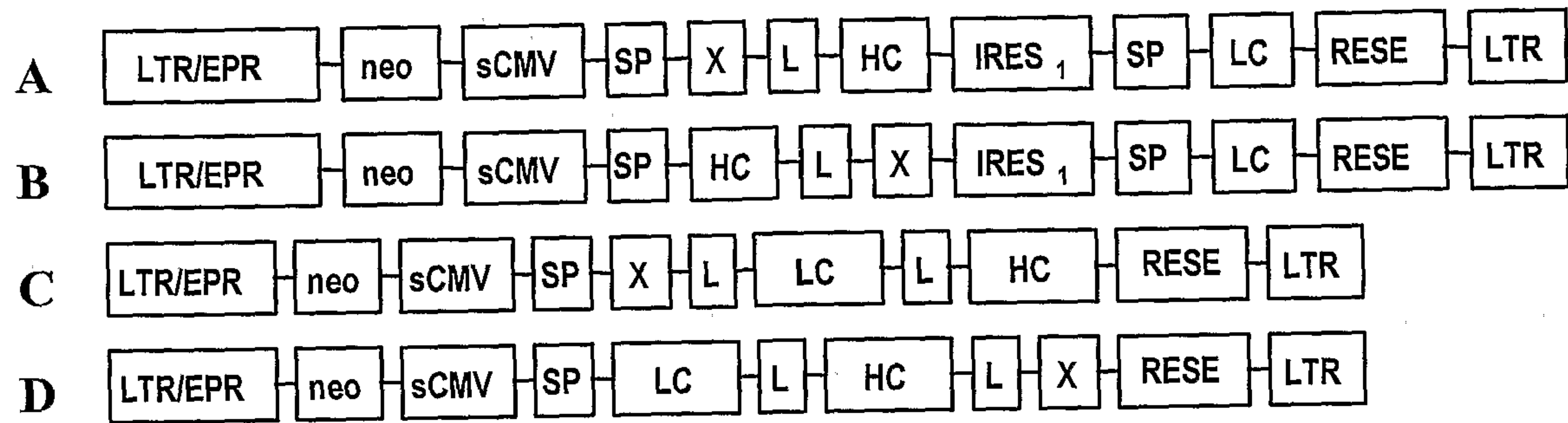
Figure 5

Figure 6

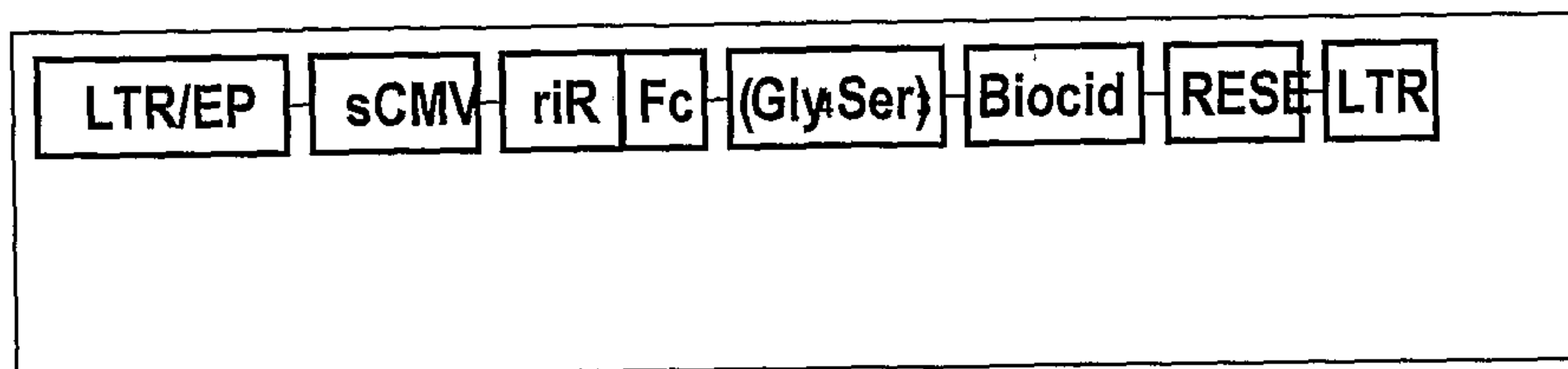


Figure 7

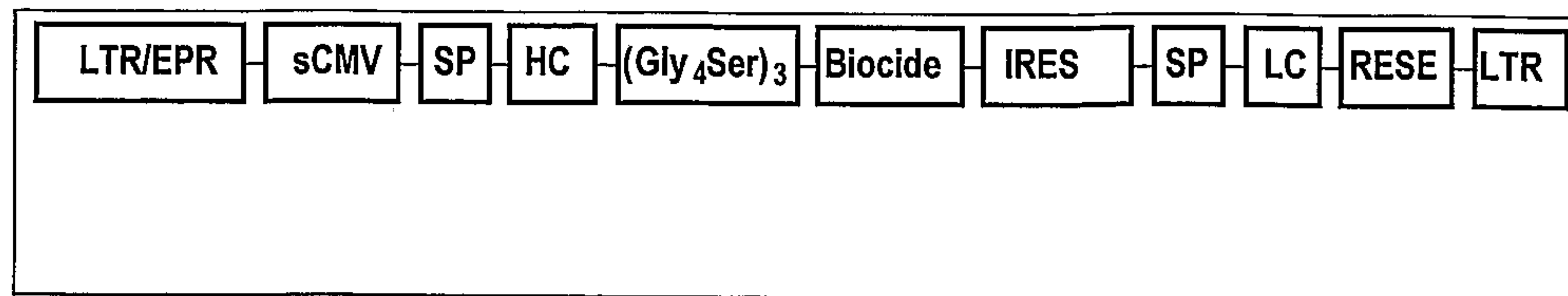


Figure 8

Human CD14-PLA2 constuct (SEQ ID NO:97)

TCCGGTCGACCTATGGCTATTGGCCAGGTTCAATACTATGTATTGGCCCTATGCC
ATATAGTATTCCATATATGGGTTTTCTTATTGACGTAGATAGCCCCTCCCAATGG
GCGGTCCCATATACCATATATGGGGCTTCCTAATAACCGCCCATAGCCACTCCCC
CATTGACGTCAATGGTCTCTATATATGGTCTTTCCTATTGACGTCATATGGGCGG
TCCTATTGACGTATATGGCGCCTCCCCCATTGACGTCAATTACGGTAAATGGCC
CGCCTGGCTCAATGCCCATTGACGTCAATAGGACCACCCACCATTGACGTCAAT
GGGATGGCTCATTGCCCATTTCATATCCGTTCTCACGCCCCCTATTGACGTCAATG
ACGGTAAATGGCCCCACTTGGCAGTACATCAATATCTATTAATAGTAACTTGGCA
AGTACATTACTATTGGAAGTACGCCAGGGTACATTGGCAGTACTCCCATTGACG
TCAATGGCGGTAAATGGCCCCGCGATGGCTGCCAAGTACATCCCCATTGACGTCA
ATGGGGAGGGGCAATGACGCAAATGGGCGTTCCATTGACGTAAATGGGCGGTA
GGCGTGCCTAATGGGAGGTCTATATAAGCAATGCTCGTTTAGGGAACCGCCATT
CTGCCTGGGGACGTCGGAGGAGCTCGAATGGAGCGCGCGTCCTGCTTGTTGCTG
CTGCTGCTGCCGCTGGTGCACGTCTCTGCGACCACGCCAGAACCTTGTGAGCTG
GACGATGAAGATTTCCGCTGCGTCTGCAACTTCTCCGAACCTCAGCCCCGACTGG
TCCGAAGCCTTCCAGTGTGTGTCTGCAGTAGAGGTGGAGATCCATGCCGGCGGT
CTCAACCTAGAGCCGTTTCTAAAGCGCGTCGATGCGGACGCCGACCCGCGGCA
GTATGCTGACACGGTCAAGGCTCTCCGCGTGCGGGCGGCTCACAGTGGGAGCCG
CACAGGTTCTGCTCAGCTACTGGTAGGCGCCCTGCGTGTGCTAGCGTACTCCC
GCCTCAAGGAACTGACGCTCGAGGACCTAAAGATAACCGGCACCATGCCTCCG
CTGCCTCTGGAAGCCACAGGACTTGCACTTTCCAGCTTGCGCCTACGCAACGTG
TCGTGGGCGACAGGGCGTTCTTGGCTCGCCGAGCTGCAGCAGTGGCTCAAGCCA
GGCCTCAAGGTACTGAGCATTGCCCAAGCACACTCGCCTGCCTTTTCCTGCGAA
CAGGTTTCGCGCCTTCCCGGGCCCTTACCAGCCTAGACCTGTCTGACAATCCTGGA
CTGGGCGAACGCGGACTGATGGCGGCTCTCTGTCCCCACAAGTTCCCGGGCCATC
CAGAATCTAGCGCTGCGCAACACAGGAATGGAGACGCCACAGGCGTGTGCGC
CGCACTGGCGGGCGGCAGGTGTGCAGCCCCACAGCCTAGACCTCAGCCACAACCT
CGCTGCGCGCCACCGTAAACCCTAGCGCTCCGAGATGCATGTGGTCCAGCGCCC

TGAACTCCCTCAATCTGTCGTTTCGCTGGGCTGGAACAGGTGCCTAAAGGACTGC
CAGCCAAGCTCAGAGTGCTCGATCTCAGCTGCAACAGACTGAACAGGGGCGCCG
CAGCCTGACGAGCTGCCCCGAGGTGGATAACCTGACACTGGACGGGAATCCCTT
CCTGGTCCCTGGAAGTGCCCTCCCCACGAGGGCTCAATGAACTCCGGCGTGGT
CCCAGCCTGTGCACGTTTCGACCCTGTCGGTGGGGGTGTCGGGAACCCTGGTGCT
GCTCCAAGGGGCCCCGGGGCTTTGCCGGTGGAGGCGGTTTCAGGCGGAGGTGGCT
CTGGCGGTGGCGGATCGAAGACCCTCCTACTGTTGGCAGTGATCATGATCTTTG
GCCTACTGCAGGCCCATGGGAATTTGGTGAATTTCCACAGAATGATCAAGTTGA
CGACAGGAAAGGAAGCCGCACTCAGTTATGGCTTCTACGGCTGCCACTGTGGC
GTGGGTGGCAGAGGATCCCCCAAGGATGCAACGGATCGCTGCTGTGTCACTCAT
GACTGTTGCTACAAACGTCTGGAGAAACGTGGATGTGGCACCAAATTTCTGAGC
TACAAGTTTAGCAACTCGGGGAGCAGAATCACCTGTGCAAAACAGGACTCCTG
CAGAAGTCAACTGTGTGAGTGTGATAAGGCTGCTGCCACCTGTTTTGCTAGAAA
CAAGACGACCTACAATAAAAAGTACCAGTACTATTCCAATAAACACTGCAGAG
GGAGCACCCCTCGTTGCTGAGTCCCCTCTTCCCTGGAAACCTTCCACCCAGTGCT
GAATTTCCCTCTCTCATACCCTCCCTCCCTACCCTAACCAAGTTCCTTGGCCATG
CAGAAAGCATCCCTCACCCATCCTAGAGGCCAGGCAGGAGCCCTTCTATACCCA
CCCAGAATGAGACATCCAGCAGATTTCCAGCCTTCTACTGCTCTCCTCCACCTC
AACTCCGTGCTTAACCAAAGAAGCTGTACTCCGGGGGGTCTCTTCTGAATAAAG
CAATTAGC

1-678 Simian CMV promoter

679-1803 Coding sequence for human CD14

1804-1848 Codon-optimized Glycine serine linker

1849-2280 Coding sequence for human PLA2 group IIA

2281-2541 3'UTR with poly-A site

Figure 9

Human LBP-PLA2 constuct (SEQ ID NO:98)

TCCGGTCGACCTATGGCTATTGGCCAGGTTCAATACTATGTATTGGCCCTATGCC
ATATAGTATTCCATATATGGGTTTTCTATTGACGTAGATAGCCCCCTCCCAATGG
GCGGTCCCATATACCATATATGGGGCTTCCTAATAACCGCCCATAGCCACTCCCC
CATTGACGTCAATGGTCTCTATATATGGTCTTTCCTATTGACGTCATATGGGCGG
TCCTATTGACGTATATGGCGCCTCCCCCATTGACGTCAATTACGGTAAATGGCC
CGCCTGGCTCAATGCCCATTGACGTCAATAGGACCACCCACCATTGACGTCAAT
GGGATGGCTCATTGCCCATTTCATATCCGTTCTCACGCCCCCTATTGACGTCAATG
ACGGTAAATGGCCCACTTGGCAGTACATCAATATCTATTAATAGTAACTTGGCA
AGTACATTACTATTGGAAGTACGCCAGGGTACATTGGCAGTACTCCCATTGACG
TCAATGGCGGTAAATGGCCCGCGATGGCTGCCAAGTACATCCCCATTGACGTCA
ATGGGGAGGGGCAATGACGCAAATGGGCGTTCCATTGACGTAAATGGGCGGTA
GGCGTGCCTAATGGGAGGTCTATATAAGCAATGCTCGTTTAGGGAACCGCCATT
CTGCCTGGGGACGTTCGGAGGAGCTCGAATGGGGGCCTTGGCAAGAGCCCTGCC
GTCCATACTGCTGGCATTGCTGCTTACGTCCACCCCAGAGGCTCTGGGTGCCAA
CCCCGGCTTGGTCGCCAGGATCACCGACAAGGGACTGCAGTATGCGGGCCCAGG
AGGGGCTATTGGCTCTGCAGAGTGAGCTGCTCAGGATCACGCTGCCTGACTTCA
CCGGGGACTTGAGGATCCCCCACGTTCGGCCGTGGGCGCTATGAGTTCCACAGCC
TGAACATCCACAGCTGTGAGCTGCTTCACTCTGCGCTGAGGCCTGTCCCCGGCC
AGGGCCTGAGTCTCAGCATCTCCGACTCCTCCATCCGGGTCCAGGGCAGGTGGA
AGGTGCGCAAGTCATTCTTCAAACCTACAGGGCTCCTTTGATGTCAGTGTCAAGG
GCATCAGCATTTTCGGTCAACCTCCTGTTGGGCAGCGAGTCCTCCGGGAGGCCCA
CAGGTTACTGCCTCAGCTGCAGCAGTGACATCGCTGACGTGGAGGTGGACATGT
CGGGAGATTCGGGGTGGCTCTTGAACCTCTTCCACAACCAGATTGAGTCCAAGT
TCCAGAAAGTACTGGAGAGCAGGGGTGGAGGCGGTTCAGGCGGAGGTGGCTCT
GGCGGTGGCGGATCGAAGACCCTCCTACTGTTGGCAGTGATCATGATCTTTGGC
CTACTGCAGGCCCATGGGAATTTGGTGAATTTCCACAGAATGATCAAGTTGACG
ACAGGAAAGGAAGCCGCACTCAGTTATGGCTTCTACGGCTGCCACTGTGGCGTG
GGTGGCAGAGGATCCCCCAAGGATGCAACGGATCGCTGCTGTGTCACCTCATGA

CTGTTGCTACAAACGTCTGGAGAAACGTGGATGTGGCACCAAATTTCTGAGCTA
CAAGTTTAGCAACTCGGGGAGCAGAATCACCTGTGCAAAACAGGACTCCTGCA
GAAGTCAACTGTGTGAGTGTGATAAGGCTGCTGCCACCTGTTTTGCTAGAAACA
AGACGACCTACAATAAAAAAGTACCAGTACTATTCCAATAAACACTGCAGAGGG
AGCACCCCTCGTTGCTGAGTCCCCTCTTCCCTGGAAACCTTCCACCCAGTGCTGA
ATTTCCCTCTCTCATACCCTCCCTCCCTACCCTAACCAAGTTCCTTGGCCATGCA
GAAAGCATCCCTCACCCATCCTAGAGGCCAGGCAGGAGCCCTTCTATACCCACC
CAGAATGAGACATCCAGCAGATTTCAGCCTTCTACTGCTCTCCTCCACCTCAA
CTCCGTGCTTAACCAAAGAAGCTGTACTCCGGGGGGGTCTCTTCTGAATAAAGCA
ATTAGC

1-678 Simian CMV promoter

679-1267 Coding sequence for human LPB

1268-1311 Codon-optimized Glycine serine linker

1312-1743 Coding sequence for human PLA2 group IIA

1744-2003 3'UTR with poly-A initiation site

Figure 10

Human MBL-PLA2 constuct (SEQ ID NO:99)

TCCGGTCGACCTATGGCTATTGGCCAGGTTCAATACTATGTATTGGCCCTATGCC
ATATAGTATTCCATATATGGGTTTTCTTATTGACGTAGATAGCCCCTCCCAATGG
GCGGTCCCATATACCATATATGGGGCTTCCTAATAACCGCCCATAGCCACTCCCC
CATTGACGTCAATGGTCTCTATATATGGTCTTTCCTATTGACGTCATATGGGCGG
TCCTATTGACGTATATGGCGCCTCCCCCATTGACGTCAATTACGGTAAATGGCC
CGCCTGGCTCAATGCCCATTGACGTCAATAGGACCACCCACCATTGACGTCAAT
GGGATGGCTCATTGCCCATTTCATATCCGTTCTCACGCCCCCTATTGACGTCAATG
ACGGTAAATGGCCCACCTTGGCAGTACATCAATATCTATTAATAGTAACTTGGCA
AGTACATTACTATTGGAAGTACGCCAGGGTACATTGGCAGTACTCCCATTGACG
TCAATGGCGGTAAATGGCCCCGCGATGGCTGCCAAGTACATCCCCATTGACGTCA
ATGGGGAGGGGCAATGACGCAAATGGGCGTTCCATTGACGTAAATGGGCGGTA
GGCGTGCCTAATGGGAGGTCTATATAAGCAATGCTCGTTTAGGGAACCGCCATT
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CTTCTCCTGAGTATGGTGGCAGCGTCTTACTCAGAACTGTGGCCTGTGAGGAT
GCCCAAAGACCTGCCCTGCAGTGATTGCCTGTAGCTCTCCAGGCATCAACGGC
TTCCCAGGCAAAGATGGGCGTGATGGCACCAAGGGAGAAAAGGGGGGAACCAG
GCCAAGGGCTCAGAGGCTTACAGGGCCCCCCTGGAAAGTTGGGGCCTCCAGGA
AATCCAGGGCCTTCTGGGTCACCAGGACCAAAGGGGCCAAAAAGGAGACCCTGG
AAAAAGTCCGGATGGTGATAGTAGCCTGGCTGCCTCAGAAAGAAAAGCTCTGC
AAACAGAAATGGCACGTATCAAAAAGTGGCTGACCTTCTCTCTGGGCAAACAA
GTTGGGAACAAGTTCTTCCTGACCAATGGTGAAATAATGACCTTTGAAAAAGTG
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GAGAATGGAGCCATTCAGAATCTCATCAAGGAGGAAGCCTTCCTGGGTATCACT
GATGAGAAGACAGAAGGGCAGTTTGTGGATCTGACAGGAAATAGACTGACCTA
CACAACTGGAACGAGGGTGAACCCAACAATGCTGGTTCTGATGAAGATTGTG
TATTGCTACTGAAAAATGGCCAGTGGAATGACGTCCCCTGCTCCACCTCCCATC
TGGCCGTCTGTGAGTTCCCTATCGGTGGAGGCGGTTTCAGGCGGAGGTGGCTCTG
GCGGTGGCGGATCGAAGACCCTCCTACTGTTGGCAGTGATCATGATCTTTGGCC

TACTGCAGGCCCATGGGAATTTGGTGAATTTCCACAGAATGATCAAGTTGACGA
CAGGAAAGGAAGCCGCACTCAGTTATGGCTTCTACGGCTGCCACTGTGGCGTGG
GTGGCAGAGGATCCCCCAAGGATGCAACGGATCGCTGCTGTGTCACTCATGACT
GTTGCTACAAACGTCTGGAGAAACGTGGATGTGGCACCAAATTTCTGAGCTACA
AGTTTAGCAACTCGGGGAGCAGAATCACCTGTGCAAAAACAGGACTCCTGCAGA
AGTCAACTGTGTGAGTGTGATAAGGCTGCTGCCACCTGTTTTGCTAGAAACAAG
ACGACCTACAATAAAAAGTACCAGTACTATTCCAATAAACACTGCAGAGGGAG
CACCCCTCGTTGCTGAGTCCCCTCTTCCCTGGAAACCTTCCACCCAGTGCTGAAT
TCCCTCTCTCATACCCTCCCTCCCTACCCTAACCAAGTTCCTTGGCCATGCAGA
AAGCATCCCTCACCCATCCTAGAGGCCAGGCAGGAGCCCTTCTATACCCACCCA
GAATGAGACATCCAGCAGATTTCCAGCCTTCTACTGCTCTCCTCCACCTCAACTC
CGTGCTTAACCAAAGAAGCTGTACTCCGGGGGGGTCTCTTCTGAATAAAGCAATT
AGC

1-678 Simian CMV promoter

679-1425 Coding sequence for human MBL

1423-1467 Codon-optimized Glycine serine linker

1468-1899 Coding sequence for human PLA2 group IIA

1900-2159 3'UTR with poly-A initiation site

Figure 11

Human SP-D-PLA2 constuct (SEQ ID NO:100)

TCCGGTCGACCTATGGCTATTGGCCAGGTTCAATACTATGTATTGGCCCTATGCC
ATATAGTATTCCATATATGGGTTTTCTTATTGACGTAGATAGCCCCTCCCAATGG
GCGGTCCCATATACCATATATGGGGCTTCCTAATACCGCCCATAGCCACTCCCC
CATTGACGTCAATGGTCTCTATATATGGTCTTTCCTATTGACGTCATATGGGCGG
TCCTATTGACGTATATGGCGCCTCCCCCATTGACGTCAATTACGGTAAATGGCC
CGCCTGGCTCAATGCCCATTGACGTCAATAGGACCACCCACCATTGACGTCAAT
GGGATGGCTCATTGCCCATTATATCCGTTCTCACGCCCCCTATTGACGTCAATG
ACGGTAAATGGCCCACTTGGCAGTACATCAATATCTATTAATAGTAACTTGGCA
AGTACATTACTATTGGAAGTACGCCAGGGTACATTGGCAGTACTCCCATTGACG
TCAATGGCGGTAAATGGCCCGCGATGGCTGCCAAGTACATCCCCATTGACGTCA
ATGGGGAGGGGCAATGACGCAAATGGGCGTTCCATTGACGTAAATGGGCGGTA
GGCGTGCCTAATGGGAGGTCTATATAAGCAATGCTCGTTTAGGGGAACCGCCATT
CTGCCTGGGGACGTCGGAGGAGCTCGAATGCTGCTCTTCCTCCTCTCTGCACTG
GTCCTACTCACACAGCCCCTGGGCTACCTGGAAGCAGAAATGAAGACCTACTCC
CACAGAACAAACGCCCAGTGCTTGCACCCTGGTCATGTGTAGCTCAGTGGAGAGT
GGCCTGCCTGGTCGCGATGGACGGGATGGGAGAGAGGGGCCCTCGGGGCGAGAA
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AGCTGGAAGAGAAGGTCCCCTGGGGGAAGCAGGGGAACATAGGACCTCAGGGC
AAGCCAGGCCCAAAGGAGAAGCTGGGCCCCAAAGGAGAAGTAGGTGCCCCAG
GCATGCAGGGCTCGGCAGGGGCAAGAGGCCTCGCAGGCCCTAAGGGAGAGCG
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CTGGAGCCATGGGTCCCCAGGGAAGTCCAGGTGCCAGGGGACCCCCGGGATTG
AAGGGGGACAAAGGCATTCCTGGAGACAAAGGAGCAAAGGGAGAAAGTGGGC
TTCCAGATGTTGCTTCTCTGAGGCAGCAGGTTGAGGCCTTACAGGGACAAGTAC
AGCACCTCCAGGCTGCTTTCTCTCAGTATAAGAAAGTTGAGCTCTTCCCAAATG
GCCAAAGTGTCGGGGGAGAAGATTTTCAAGACAGCAGGCTTTGTAAAACCATTT
ACGGAGGCACAGCTGCTGTGCACACAGGCTGGTGGACAGTTGGCCTCTCCACG

CTCTGCCGCTGAGAATGCCGCCTTGCAACAGCTGGTCGTAGCTAAGAACGAGGC
TGCTTTCCTGAGCATGACTGATTCCAAGACAGAGGGCAAGTTCACCTACCCAC
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GCGGGTCAGAGGACTGTGTGGAGATCTTCACCAATGGCAAGTGGAATGACAGG
GCTTGTGGAGAAAAGCGTCTTGTGGTCTGCGAGTTCGGTGGAGGCGGTTCAGGC
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ATCAAGTTGACGACAGGAAAGGAAGCCGCACTCAGTTATGGCTTCTACGGCTG
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GTGTCATCATGACTGTTGCTACAAACGTCTGGAGAAACGTGGATGTGGCACCA
AATTTCTGAGCTACAAGTTTAGCAACTCGGGGAGCAGAATCACCTGTGCAAAAC
AGGACTCCTGCAGAAGTCAACTGTGTGAGTGTGATAAGGCTGCTGCCACCTGTT
TTGCTAGAAACAAGACGACCTACAATAAAAAGTACCAGTACTATTCCAATAAA
CACTGCAGAGGGAGCACCCCTCGTTGCTGAGTCCCCTCTTCCCTGGAAACCTTC
CACCCAGTGCTGAATTTCCCTCTCTCATACCCTCCCTCCCTACCCTAACCAAGTT
CCTTGGCCATGCAGAAAGCATCCCTCACCCATCCTAGAGGCCAGGCAGGAGCC
CTTCTATACCCACCCAGAATGAGACATCCAGCAGATTTCCAGCCTTCTACTGCT
CTCCTCCACCTCAACTCCGTGCTTAACCAAAGAAGCTGTACTCCGGGGGGGTCTC
TTCTGAATAAAGCAATTAGC

1-678 Simian CMV promoter

679-1803 Coding sequence for human SP-D

1804-1848 Codon-optimized Glycine serine linker

1849-2280 Coding sequence for human PLA2 group IIA

2281-2540 3'UTR with poly-A initiation site

Figure 12

Mouse IgM-PLA2 constuct (SEQ ID NO:101)

TCCGGTCGACCTATGGCTATTGGCCAGGTTCAATACTATGTATTGGCCCTATGCC
ATATAGTATTCCATATATGGGTTTTCTTATTGACGTAGATAGCCCCTCCCAATGG
GCGGTCCCATATACCATATATGGGGCTTCCTAATAACCGCCCATAGCCACTCCCC
CATTGACGTCAATGGTCTCTATATATGGTCTTTCCTATTGACGTCATATGGGCGG
TCCTATTGACGTATATGGCGCCTCCCCCATTGACGTCAATTACGGTAAATGGCC
CGCCTGGCTCAATGCCCATTGACGTCAATAGGACCACCCACCATTGACGTCAAT
GGGATGGCTCATTGCCCATTTCATATCCGTTCTCACGCCCCCTATTGACGTCAATG
ACGGTAAATGGCCCACTTGGCAGTACATCAATATCTATTAATAGTAACTTGGCA
AGTACATTACTATTGGAAGTACGCCAGGGTACATTGGCAGTACTCCCATTGACG
TCAATGGCGGTAAATGGCCCGCGATGGCTGCCAAGTACATCCCCATTGACGTCA
ATGGGGAGGGGCAATGACGCAAATGGGGCGTTCCATTGACGTAAATGGGGCGGTA
GGCGTGCCTAATGGGAGGTCTATATAAGCAATGCTCGTTTAGGGAACCGCCATT
CTGCCTGGGGACGTTCGGAGGAGCTCGAATGGCTGTCCTGGTGCTGTTCTCTGC
CTGGTTGCATTTCCAAGCTGTGTCCTGTCCCAGGTGCAGCTGAAGGAGTCAGGA
CCTGGCCTGGTGGCGCCCTCACAGAGCCTGTCCATCACTTGCACTGTCTCTGGG
TTTTCATTAACCAACTATGGTGTACATTGGGTTCGCCAGCCTCCAGGAAAGGGT
CTGGAGTGGCTGGGAGTAATATGGGCTGGTGGAAACACAAATTATAATTCGGC
TTTTATGTCCAGACTGAGCATCACCAAAGACAACCTCCAAGAGCCAAGTTTTTCAT
AAAAATGAACAGTCTGCAAACCTGATGACACAGCCATGTACTACTGTGCCAGAG
AATATAGGCACGGGGCTTACTATGCTATGGACTACTGGGGTCAAGGAACCTCA
GTCACCGTCTCCTCAGAGAGTCAGTCCTTCCCAAATGTCTTCCCCCTCGTCTCCT
GCGAGAGCCCCCTGTCTGATAAGAATCTGGTGGCCATGGGCTGCCTGGCCCGGG
ACTTCCTGCCCAGCACCATTTCTTTCACCTGGAACCTACCAGAACAACACTGAAG
TCATCCAGGGTATCAGAACCTTCCCAACACTGAGGACAGGGGGCAAGTACCTA
GCCACCTCGCAGGTGTTGCTGTCTCCCAAGAGCATCCTTGAAGGTTTCAGATGAA
TACCTGGTATGCAAAATCCACTACGGAGGCAAAAACAGAGATCTGCATGTGCC
CATTCCAGCTGTTCGCAGAGATGAACCCCAATGTAAATGTGTTTCGTCCCACCACG
GGATGGCTTCTCTGGCCCTGCACCACGCAAGTCTAAACTCATCTGCGAGGCCAC
GAACTTCACTCCAAAACCGATCACAGTATCCTGGCTAAAGGATGGGAAGCTCGT

GGAATCTGGCTTCACCACAGATCCGGTGACCATCGAGAACAAAGGATCCACAC
CCCAAACCTACAAGGTCATAAGCACACTTACCATCTCTGAAATCGACTGGCTGA
ACCTGAATGTGTACACCTGCCGTGTGGATCACAGGGGTCTCACCTTCTTGAAGA
ACGTGTCCTCCACATGTGCTGCCAGTCCCTCCACAGACATCCTGACCTTCACCAT
CCCCCCTCCTTTGCCGACATCTTCCTCAGCAAGTCCGCTAACCTGACCTGTCTG
GTCTCAAACCTGGCAACCTATGAAACCCTGAATATCTCCTGGGCTTCTCAAAGT
GGTGAACCACTGGAAACCAAAATTAAAATCATGGAAAGCCATCCCAATGGCAC
CTTCAGTGCTAAGGGTGTGGCTAGTGTTTGTGTGGAAGACTGGAATAACAGGAA
GGAATTTGTGTGTACTGTGACTCACAGGGATCTGCCTTCACCACAGAAGAAATT
CATCTCAAAACCCAATGAGGTGCACAAACATCCACCTGCTGTGTACCTGCTGCC
ACCAGCTCGTGAGCAACTGAACCTGAGGGAGTCAGCCACAGTCACCTGCCTGG
TGAAGGGCTTCTCTCCTGCAGACATCAGTGTGCAGTGGCTTCAGAGAGGGCAAC
TCTTGCCCCAAGAGAAGTATGTGACCAGTGCCCCGATGCCAGAGCCTGGGGCCC
CAGGCTTCTACTTTACCCACAGCATCCTGACTGTGACAGAGGAGGAATGGAACCT
CCGGAGAGACCTATACCTGTGTTGTAGGCCACGAGGCCCTGCCACACCTGGTGA
CCGAGAGGACCGTGGACAAGTCCACTGGTAAACCCACACTGTACAATGTCTCCC
TGATCATGTCTGACACAGGCGGCACCTGCTATTGAAATTCGCCCCCTCTCCCTCCC
CCCCCCTAACGTTACTGGCCGAAGCCGCTTGGAATAAGGCCGGTGTGCGTTTG
TCTATATGTTATTTTCCACCATATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAA
ACCTGGCCCTGTCTTCTTGACGAGCATTCTAGGGGTCTTTCCCCTCTCGCCAAA
GGAATGCAAGGTCTGTTGAATGTTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTCT
TGAAGACAAACAACGTCTGTAGCGACCCTTTGCAGGCAGCGGAACCCCCCACC
TGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTGTATAAGATACACCTGCAA
AGGCGGCACAACCCAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAAGAGTC
AAATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCAGAAGGT
ACCCCATTTGTATGGGATCTGATCTGGGGCCTCGGTGCACATGCTTTACATGTGTT
TAGTCGAGGTTAAAAAAACGTCTAGGCCCCCGAACCACGGGGACGTGGTTTTTC
CTTTGAAAAACACGATGATGGTGGAGGCGGTTCAGGCGGAGGTGGCTCTGGCG
GTGGCGGATCGAAGACCCTCCTACTGTTGGCAGTGATCATGATCTTTGGCCTAC
TGCAGGCCCATGGGAATTTGGTGAATTTCCACAGAATGATCAAGTTGACGACAG
GAAAGGAAGCCGCACTCAGTTATGGCTTCTACGGCTGCCACTGTGGCGTGGGTG
GCAGAGGATCCCCCAAGGATGCAACGGATCGCTGCTGTGTCACTCATGACTGTT

GCTACAAACGTCTGGAGAAACGTGGATGTGGCACCAAATTTCTGAGCTACAAG
TTTAGCAACTCGGGGAGCAGAATCACCTGTGCAAAACAGGACTCCTGCAGAAG
TCAACTGTGTGAGTGTGATAAGGCTGCTGCCACCTGTTTTGCTAGAAACAAGAC
GACCTACAATAAAAAGTACCAGTACTATTCCAATAAACACTGCAGAGGGAGCA
CCCCTCGTTGCTGAAATTCGCCCCCTCTCCCTCCCCCCCCCCTAACGTTACTGGCC
GAAGCCGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCAT
ATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTGAC
GAGCATTCTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAA
TGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACAACGTCTGT
AGCGACCCTTTGCAGGCAGCGGAACCCCCCACCTGGCGACAGGTGCCTCTGCG
GCCAAAAGCCACGTGTATAAGATACACCTGCAAAGGCGGCACAACCCCAAGTGC
CACGTTGTGAGTTGGATAGTTGTGGAAAGAGTCAAATGGCTCTCCTCAAGCGTA
TTCAACAAGGGGCTGAAGGATGCCCAGAAGGTACCCCATTTGTATGGGATCTGA
TCTGGGGCCTCGGTGCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAACG
TCTAGGCCCCCGAACCACGGGGACGTGGTTTTCTTTGAAAAACACGATGATA
ATATGAGTGTvCTvACTCAGGTCCTGGGGTTGCTGCTGCTGTGGCTTACAGGTGC
CAGATGTGACATCCAGATGACTCAGTCTCCAGCCTCCCTATCTGCATCTGTGGG
AGAAACTGTCACCATCACATGTCGAGCAAGTGAGAACATTTACAGTTATTTAGC
ATGGTATCAGCAGAAACAGGGAAAATCTCCTCAGTTCCTGGTCTATAATGCAGA
AACCTTAGCAGAAGGTGTGCCATCAAGGTTCAAGTGGCAGTGGATCAGGCAAAC
AGTTTTCTCTGAAGATCAACAGCCTGCAGCCTGAAGATTTTGGGAGTTATTACT
GTCAACATCATTATGGTACTCATCCGACGTTTCGGTGGAGGCACCAAGCTGGAAA
TCAAACGGGCTGATGCTGCACCAACTGTATCCATCTTCCCACCATCCAGTGAGC
AGTTAACATCTGGAGGTGCCTCAGTCGTGTGCTTCTTGAACAACCTTCTACCCCA
AAGACATCAATGTCAAGTGGAAGATTGATGGCAGTGAACGACAAAATGGCGTC
CTGAACAGTTGGACTGATCAGGACAGCAAAGACAGCACCTACAGCATGAGCAG
CACCTCACGTTGACCAAGGACGAGTATGAACGACATAACAGCTATACCTGTG
AGGCCACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATG
AGTGTTAGAGACAAAGGTCCTGAGACGCCACCACCAGCTCCCCAGCTCCATCCT
ATCTTCCCTTCTAAGGTCTTGGAGGCTTCCCCACAAGCGACCTACCACTGTTGCG
GTGCTCCAAACCTCCTCCCCACCTCCTTCTCCTCCTCCTCCTTTCTTGGCTTTT
ATCATGCTAATATTTGCAGAAAATATTCAATAAAGTGAGTCTTTGCACTTGA

1-678 Simian CMV promoter

679-3041 Coding sequence for mouse IgM

3042-3086 Codon-optimized Glycine serine linker

3087-3518 Coding sequence for human PLA2 group IIA

3519-4099 ECMV IRES element

4100-4804 Coding sequence for mouse kappa light chain

4805-5013 3'UTR with poly-A site