BACTERIAL DELIVERY SYSTEM

FIELD OF THE INVENTION

The present invention relates to the delivery of biologically active agents *in vivo*. In particular, the present invention relates to treating, palliating or preventing diseases using a bacterial delivery system for delivering biologically active agents directly to an anatomical site *in vivo*. In one embodiment, the bacterial delivery system comprises *Helicobacter* or bacterium exhibiting *Helicobacter* features for delivering heterologous nucleic acid into an animal or animal cell, wherein the heterologous nucleic acid is expressed. In a further embodiment the *Helicobacter* has been engineered to contain a DNA vector that encodes heterologous nucleic acid, wherein upon infection the nucleic acid vector is expressed such that the biologically active agent is delivered to the animal body, especially at the mucosa.

BACKGROUND TO THE INVENTION

There is a continuing need for long-term delivery of pharmacologic and immunologic agents to individuals with both congenital and acquired diseases. In most cases, this involves repeated administration of therapeutic compounds several times per day, daily or at intervals. However, it is appreciated that any long-term therapeutic or preventive regime has problems such as compliance, side effects and drug resistance. Consequently, there is a constant need to identify treatments, which will be usually 100% effective, free from side effects and cheap.

One form of delivery that has been investigated in recent times for various therapeutic and prophylactic agents has been the use of microorganisms. Genes of interest from various organisms including bacteria, viruses, parasites as

well as mammals have, for example, been cloned into a variety of bacteria, viruses and mycobacteria for the purpose of directing these micro-organisms to express foreign protein or impart certain desired properties. These microorganisms have been used in vaccination programs, gene-replacement therapies and therapeutic composition delivery.

Microorganisms have also been used to transform animal (host) cells *in vivo*. Host cell transformation can be accomplished using gene-delivery vectors comprising replication incompetent viruses (see for example U.S. Pat. No. 5,824,544), naked DNA, (see for example U.S. Pat. No. 6,261,834), liposomes containing recombinant expression cassettes (see for example U.S. Pat. No. 6,271,207). Other molecular-based therapeutic composition delivery approaches include using replication incompetent recombinant viruses designed to express heterologous surface proteins (see, for example, U.S. Pat. No. 6,376,236).

In recent times, pharmaceutical researchers have also attempted to develop methods for *in vivo* therapeutic composition expression using recombinant organism-based vectors, inanimate vectors and naked DNA. Examples of recombinant organism-based vectors include recombinant bacteria (see for example U.S. Pat. No. 5,547,664) and viruses such as alphaviruses (see for example U.S. Pat. No. 6,391,632), vaccinia viruses (see for example U.S. Pat. No. 6,267,965), adenoviruses (see for example U.S. Pat. No. 5,698,202) and adenovirus associated virus (AAV) (see for example U.S. Pat. No. 6,171,597). Inanimate vectors include lipidic gene delivery vector constructs such as DNA/cationic liposome complexes, DNA encapsulated in neutral or anionic liposomes, and liposome-entrapped, polycation-condensed DNA (LPDI and LPDII). (see Ropert, 1999, *Braz J Med Biol Res*, 32(2):163-9).

Examples of other genes that have been delivered by various bacteria include cloning the invasion genes of *Shigella* into the normally non-invasive *E. coli* rendering the *E. coli* invasive and therefore more suitable for use as a vaccine strain, or cloning of *Plasmodium falciparum* malaria genes into Salmonella typhimurium which subsequently express these malaria proteins and, following oral administration of the bacteria, induce specific cytotoxic T cell immunity and protection in mice against malaria challenge (see, for example, Hone *et al.*, 1991, Vaccine, 9:810-816; Tacket *et al.*, 1992, *Infect. Immun.*, 60:536-541; Hone *et al.*, 1992, *J. Clin. Invest.*, 90:412-420; Chatfield *et al.*, 1992, *Vaccine*, 10:8-11; Tacket *et al.*, 1992, *Vaccine*, 10:443-446; and Mims *et al.*, 1993, *In*: Medical Microbiology, Eds., Mosby-Year Book Europe Ltd., London; Sadoff *et al.*, 1988, *Science*, 240:336-338; Aggrawal *et al.*, 1990, *J. Exp. Med.*, 172:1083-1090).

Attenuated or less virulent *Shigella* (see, for example, Noriega *et al.*, 1994, *Infect. Immun.*, 62:5168-5172; US Pat. Appl. No. 20020176848), *Salmonella* (see, for example, US Pat. No. 6,531,313; US Pat. Appl. No. 20030170211), *Listeria* (see, for example, Schafer *et al.*, 1992, *J. Immunol.*, 149:53-59; US Pat. Appl. No. 20030008839), and other bacteria have been given orally to immunise against subsequent infection with more virulent forms of these bacteria. Likewise, attenuated bacterial and mycobacterial organisms such as *Bacille Calmette-Guerin* (BCG) (Lagranderie *et al.*, 1993, *Vaccine*, 11:1283-1290; Flynn, 1994, *Cell. Molec. Biol.*, 40(Suppl. 1):31-36) have been administered parenterally to protect against related organisms such as M. tuberculosis.

Some of the other bacterial species that have been used as vectors include *Yersinia enterocolitica* (van Damme *et al.*, 1992, *Gastroenterol.*, 103:520-531) and Vibrio cholerae (Levine *et al.*, 1994, *In*: *Vibrio cholerae*, Molecular to

Global Perspective's, Wachsmuth *et al.*, Eds, ASM Press, Washington, D.C., pages 395-414).

Despite all of the research all of the above bacterial delivery systems have technical difficulties, which need to be overcome before these can be used *in vivo*. Indeed, most of the bacterial systems require the bacteria themselves to produce functional molecules and are dependent on a bacterium which is sufficiently attenuated to be safe for use in humans, but still able to produce biologically active agents. However, all of the attenuated strains of bacteria used previously are not capable of surviving lengthy periods *in vivo*, without causing side effects. More importantly, many of these bacterial species are not capable of delivering therapeutic or prophylactic agents to the mucosal epithelial cells of an animal. However, many of the most important therapeutic agents are taken up via the mucosa; therefore there is a need for a bacterial delivery system capable of delivering biologically active agents directly to the mucosa.

U.S. Pat. No. 5,877,159 to Powell *et al.*, describes live bacteria that can invade mucosal cells without establishing a productive infection or causing disease to thereby introduce a eukaryotic expression cassette encoding an antigen capable of being expressed by the animal cells. While this method allows delivery of the DNA vaccine to mucosal surfaces, including easy administration, a concern for vaccine delivery in developing countries, it does not have the advantage of providing amplifiable mRNA encoding the gene of interest. Moreover, the bacterium disclosed in Powell *et al.* is not capable of sustained delivery.

Non-pathogenic, non-colonising, non-invasive food-grade bacterium Lactococcus lactis has been used previously in the past to deliver agents to the mucosa (see, for example, UK patent GB-2278358B). However, while *Lactococcus lactis*

is non-invasive it is not capable of establishing a chronic infection, which is capable of delivering continuous therapeutic agents directly into mucosal epithelial cells.

Consequently, there is a continuing need for a delivery system, which is capable of establishing a non-invasive, chronic infection in close proximity to the mucosal epithelial cells of an animal such that biologically active agents can be delivered thereto. Moreover, there is a continuing need for improved delivery mechanisms for pharmacologically active molecules at the mucosal surface sufficient to elicit a useful and beneficial immunogenic response. Such would provide an effective *in vivo* delivery system for pharmacological active agents, as well as an effective method for immunization, i.e., antigen exposure at mucosal surface sufficient to elicit a general humoral and mucosal immune response.

SUMMARY OF THE INVENTION

The present invention is directed to overcoming the above-mentioned challenges and is exemplified in a number of implementations and applications, some of which are summarized below.

The inventors have now surprisingly found that *Helicobacter* and in particular *Helicobacter* pylori, which is capable of forming a chronic infection, can be used to produce continuous drug delivery. Moreover, *Helicobacter* of the present invention can be activated and inactivated at various times and because of its chronicity, the *Helicobacter* can be used to deliver drugs through the gastric mucosa.

Helicobacter pylori is a gram-negative spiral shaped bacterium found almost exclusively in the human gastric mucosa. The acidity of the human stomach is an effective

barrier to colonization by essentially all bacteria, with the exception of *Helicobacter* species.

H. pylori has the unique ability to colonize and persist for decades within the human gastric mucosa, despite development of a mucosal inflammatory and immune response. This characteristic renders *H. pylori* an interesting candidate for the delivery of selected agents though the mucosa.

In accordance with some aspects, compositions, methods and systems are provided for preparing and using a *Helicobacter*-based construct comprising a *Helicobacter* sequence having a promoter region and a non-*Helicobacter* sequence encoding a non-*Helicobacter* pharmacologically active molecule. This construct in some embodiments is described as a vector or a plasmid vector, wherein the promoter sequence is capable of controlling the expression of the non-*Helicobacter* pharmacologically active molecule of interest.

Accordingly, in a first aspect the present invention provides a method of delivering one or more biologically active agents to a subject comprising the step of administering to a subject an effective amount, preferably a therapeutically or prophylactically effective amount of, *Helicobacter* cells or bacterial cells having the features of *Helicobacter*, which cells express said one or more biologically active agents.

In a second aspect, the present invention provides a non-invasive or non-pathogenic *Helicobacter* cell expressing one or more heterologous biologically active agents.

Preferably, the *Helicobacter* is of the species *H. pylori*.

The biologically active agent can be either homologous to

the *Helicobacter* genus or heterologous to cells of the genus or species of the *Helicobacter* cells used for delivery. Heterologous agents may be derived from either eukaryotic or prokaryotic sources.

In some aspects, the *Helicobacter*-based vector and vector plasmid constructs comprise biologically active agents such as an antigen, organic or inorganic molecule or substance, a pharmacological agent eg a therapeutic agent or prophylactic agent, such as a gene product or gene sequence (isolated nucleic acid). By way of example, such agents may include an immunoregulatory agent, hormone, ligand, an enzyme or an anti-sense RNA, a catalytic RNA, a protein, a peptide or any other molecule which can be present on or released from the bacterial cells so that it may be delivered to an animal or to an animal cell.

In some embodiments, the biologically active agent is encoded by a nucleic acid molecule, which is preferably obtained in isolated form, and subsequently inserted into the bacterial delivery vehicle of this invention. It will be appreciated by those skilled in the art that the isolated nucleic acid molecule of the present invention may be cDNA, genomic DNA, RNA, or a hybrid molecule thereof. Preferably, the nucleic acid is cDNA.

By way of example, a protein and/or peptide of interest may comprise ghrelin, amylin, insulin, motilin, β-glucosidase, a chemical chaperone, or other molecule useful in the treatment of Gauchers disease, cell wasting, human immunodeficiency disease (AIDS), appetite suppression, preparations useful in the treatment of diabetes, etc.

The isolated nucleic acid is preferably incorporated into an expression vector, which can be transformed into and maintained in the *Helicobacter* cells.

Accordingly, in a third aspect, the present invention provides a recombinant vector for delivering biologically active agents directly to an anatomical site in need thereof. The *Helicobacter* cell of the present invention has preferably been adapted to secrete and/or express on its surface the biologically active agents.

In a fourth aspect, the present invention provides a vector or construct for delivering *in vivo* an effective amount of a biologically active agent, comprising:

a) a nucleotide sequence encoding a biologically active agent;

b) operatively linked thereto, a control or regulatory sequence capable of controlling the expression of the nucleotide sequence of (a) such that the biologically active agent is produced *in vivo* when the delivery vehicle comprising the vector is delivered to a subject.

The vector maybe modified chemically by means of chemical or enzymatic treatment, or *in vivo* by means of recombinant DNA technology to produce a modified or variant vector.

Such a construct may differ from those disclosed, for example, by virtue of one or more nucleotide substitutions, deletions or insertions, but substantially retain a desired biological activity of the construct or nucleic acid molecule, or its encoded product, in accordance with this invention.

In another embodiment of the present invention, the *Helicobacter* vector or construct is provided with a reporter gene(s) expressed from a constitutive promoter cloned into the expression vector and used as a screening tool. Non-limiting examples of reporter genes suitable for use herein include green fluorescent protein (GFP), β-galactosidase, amylase, and chloramphenicol acetyl

transferase (CAT).

In another embodiment of the present invention the *Helicobacter* cell is used to deliver a heterologous gene of interest to a subject in need thereof. The gene of interest may encode a therapeutic product (a transgene product), including, but not limited to a peptide hormone (such as, but not limited to α-melanocyte-stimulating hormone (α-MSH), insulin, growth hormone, and parathyroid hormone), a cytokine including, but not limited to an interferon, interleukin (IL)-2, IL-4, IL-10, IL-12, granulocyte colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF) and erythropoietin (EPO).

In still another embodiment, the present invention provides methods for treating, palliating or preventing a disease in a subject. These methods are facilitated with the use of a therapeutic or prophylactic composition. Accordingly, in a fifth aspect, the present invention provides a pharmaceutical composition comprising (a) live non-pathogenic *Helicobacter* cells expressing and/or secreting a biologically active agent, together with (b) a therapeutically effective carrier.

In a sixth aspect the present invention provides a method of treating, preventing or palliating a disease comprising administering to a subject in need thereof an effective amount of a *Helicobacter* cells of the present invention that express the biologically active agent.

Non-limiting examples of subjects in which the present invention may be used, include mammals such as primates, equines, bovines, porcines, ovines and rodents. Also intended are fish and birds.

In one embodiment of the present invention the disease being treated, prevented or palliated is cancer, a disease

or condition of the immune/hematopoietic system, a disease or condition of the reproductive system, a disease or condition of the musculoskeletal system, a disease or condition of the cardiovascular system, a disease or a condition described as mixed fetal, a disease or a condition of the excretory system, a disease or a condition of the neural/sensory system, a disease or a condition of the endocrine system, a disease or condition of the respiratory system, a disease or condition of the digestive system and a disease or condition associated with connective/epithelial tissue or disease or conditions caused by bacterial, viral or parasitic infection.

The present invention provides a variety of pharmaceutically acceptable preparations formulated for delivery to a patient, such as, gastrically, orally, or intranasaly. In particular embodiments, the composition are suitable for delivery at a mucosal surface. In particular embodiments, the composition is suitable for delivery to the mucosal surface of the gut.

By way of example, the mucosa may be that of the gastric, vaginal, nasal, oral, or ocular surface, or any other of the body characterized by the presence of a penetrable mucosal surface or lining. In some embodiments, the mucosal surface is the gastric mucosal surface.

The various delivery forms of the compositions are readily prepared for use in the practice of the present invention given the specific types and ratios of specific *Helicobacter*, *Helicobacter* constructs and other delivery vehicles described herein, and those formulation techniques known to those in the formulary arts, such as are described in Remington's Pharmaceutical Sciences, 20th edition, Mack Publishing Company, which text is specifically incorporated herein by reference.

It is envisioned that the delivery system may be employed in animals, particularly primates, including humans, equines, bovines, ovines, and rodents, fish and birds. It is also anticipated that the preparations may be used on both infants and adults, as well as parentally or for administration to pregnant or lactating animals. The preparations and methods may be further described as suitable for both male and female animals.

In yet another aspect, a method is provided for vaccinating an animal. In some embodiments, the method comprises administering a composition comprising a vaccine comprising cells transformed with the *Helicobacter*- based plasmid vector and/or plasmid vectors as described herein. In other embodiments, the method provides for the delivery of an effective amount of the pharmacologically active molecule of interest sufficient to eliminate or inhibit a disease or physiological condition in the animal, or sufficient to elicit an immune response specific for the pharmacologically active molecule of interest.

By way of example, the non-*Helicobacter* pharmacologically active molecule of interest useful in the vaccine may comprise a mammalian protein, peptide, enzyme, hormone, or any combination of these. In particular embodiments, the pharmacologically active molecule of interest is further defined as a human pharmacologically active molecule of interest. In some embodiments the pharmacologically active molecule of interest is a human pathogen molecule/antigen, human protein antigen, such as amylin or an analog or derivative thereof, or ghrelin, or an analog or derivative thereof.

In particular embodiments, the vaccine conveys immunity against the human pathogen, Ebola virus, HIV virus, Marburg virus, influenza virus, and the like. Replication competent vaccines based on attenuated recombinant vesicular

stomatitis virus vectors have been described by Jones *et al.* (2005, *Nat. Med.* 11: 786-90) that include Ebola glycoprotein and Marburg glycoprotein. Hence, it is envisioned that constructs using the *Helicobacter*-based vector systems and plasmid vector systems with these and other glycoprotein's associated with human pathogens may also be provided according to the present invention together with the disclosure provided herein.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a schematic diagram of the plasmid construct pHPA1 (2.8kb).

Figure 2 is a schematic diagram of the plasmid construct pHP3 (3.4kb).

Figure 3 is a schematic diagram of the plasmid vector pTMI03-8.

Figure 4 shows the structure of sulfasalazine (SSN).

Figure 5 is a schematic diagram showing the use of ion exchange resin (Amberlite XE-96) conjugated with a dye (Azure-A).

BRIEF DESCRIPTION OF THE SEQUENCES

The following nucleic acid and amino acid sequences are referenced throughout the description of the present invention:

SEQ ID NO: 1 - Nucleotide sequence of plasmid pHP1 (2796 nucleotides);

SEQ ID NO: 2 - Nucleotide sequence of pHP1 (2796 nucleotides);

SEQ ID NO: 3 - Nucleotide sequence of plasmid pHP3 (3444 nucleotides);

SEQ ID NO: 4 - Hepatitis C virus are antigen (HCV) nucleotide Sequence;

SEQ ID NO: 5 - Nucleotide sequence 135 bp (45 amino acids) immunogenic coding sequence from the Hepatitis C virus (HCV) core antigen;

SEQ ID NO: 6 - Nucleotide sequence of the surface exposed loop of the *HopE* gene (at nt504, aa position 168) of *H. pylori*;

SEQ ID NO: 7 - Upstream primer (29 nucleotides);

SEQ ID NO: 8 - Downstream Primer (28 nucleotides);

SEQ ID NO: 9 - Oligonucleotide Primer (15 nucleotides).

DEFINITION OF TERMS

Prior to setting forth the invention, it may be helpful to an understanding thereof to set forth definitions of certain terms that will be used hereinafter.

An "antibiotic resistance gene" as defined herein includes heterologous nucleic acid sequences purposely provided to a vector and used as a selection system. The term "antibiotic resistance gene" does not include other mechanisms or genes that impart antibiotic resistance to naturally occurring micro-flora organisms.

The term "attenuated" as used herein for example to describe a bacterial strain, particularly an *E. coli* or a *Helicobacter* strain such as *Helicobacter pylori*, is defined

as a strain that is less virulent and/or toxic (invasive) that a native, wild type bacterial strain.

The term "biologically active" as used herein refers to ability to perform a biological function and with reference to a polypeptide implies that the polypeptide adopts a stable conformation ("folded form") which is the same or closely analogous to its native conformation. When folded correctly or substantially correctly, for example with formation of proper folded units α-helices, β-sheets, domains, disulphide bridges etc., a polypeptide should have the ability to perform its natural function. Generally, the unit of function in a polypeptide is a domain.

Mere ability to be bound by an antibody or other receptor, either with or without elicitation of an immune response, is passive or does not constitute "biological activity". Any antigen has the ability to be bound by an antibody but is not necessarily biologically active.

"Clinical grade vector" as used herein means a plasmid or other expression vector that is capable of being expressed in *Helicobacter* or a non-pathogenic bacterium engineered to have features of *Helicobacter*. The clinical grade vectors of the present invention do not use antibiotic resistance markers for selection and/or have been modified to prevent replication outside the host e.g. such as a suicide vector.

An example of suicide system in *H. pylori* has been described by Panthel *et al.* 2003 (*Infection & Immunity*, 71: 109-116). This system introduces a plasmid into *H. pylori* which contains the PhiX174 lysis gene E. To eradicate the strain, incubation at 42°C for 5 hours was used. *In vivo* this would mean that the animal would consume a drink at 45-50°C to raise the temperature of the gastric environment above 42°C.

A second example is the *L-Dap* selection system, commonly used to allow survival of bacterial mutants on supplemented plates (see, for example, Kirata *et al.* 1997 (*Infection & Immunity*, 65: 4158-4164). In this system the animal subject must supplement their diet with a missing substrate i.e. diamino-pimelic-acid (DAP), in order for the *DapE* deficient *H. pylori* mutant to survive. In order to eradicate the then DAP consumption is ceased.

A third possible system relates to metronidazole sensitivity of *H. pylori* because of its rdxA gene. Excessive replication of the rdxA gene is harmful to mammalian cells and *E. coli*. However, duplication may be tolerated by the bacterium. Therefore a *Helicobacter* species of the present invention can be engineered to contain two copies of rdxA which prevent the normal mutation-dependant rdxA loss. The introduction of at least two functional rdxA genes into the *Helicobacter* genome should result in a *Helicobacter* strain, which is permanently sensitive to metronidazole. Jeong *et al.* 2000 (*J. Bacteriol.*, 182: 5082-5090) showed that the nitroreductase produced by a functional rdxA gene converts metronidazole from a prodrug to a bactericidal compound. The mode of action of the active compound is to cause DNA breaks of the *Helicobacter* genome.

"Detectable immune response" as used herein is either an antibody (humoral) or cytotoxic (cellular) response formed in an animal in response to an antigen that can be measured using routine laboratory methods including, but not limited to enzyme-linked immunosorbant assays (ELISA), radio-immune assays (RIA), Enzyme-linked ImmunoSPOT (ELISPOT), immunofluorescent assays (IFA), complement fixation assays (CF), Western Blot (WB) or an equivalent thereto.

"Gene of interest" as used herein refers to any nucleic acid sequence encoding for a polypeptide or protein whose

expression is desired. The gene of interest may or may not include the promoter or other regulatory components. The gene of interest also includes constructs capable of producing anti-sense RNA.

"Gene therapy" as used herein is defined as the delivery of a gene of interest to an animal in need thereof using a recombinant vector. The gene of interest can be a transgene encoding for a therapeutic or prophylactic protein or polypeptide including, but not limited to cytokines, anti-inflammatories, anti-proliferatives, antibiotics, metabolic inhibitors/activators and immunologically active antigens and fragments thereof. Furthermore, "gene therapy" as used herein also includes gene replacement technologies directed at both inherited and non-inherited disorders.

The term *Helicobacter* includes all bacteria of the genus *Helicobacter* including *H. pylori* and *H. mustelae*. The term also includes bacteria that have similar biology to *H. pylori* in that they are capable of residing on the gastric mucosa of primates and/or capable of establishing a chronic, but isolated infection of the mucosa. The term also encompasses bacteria that have been modified so that the bacterium has *H. pylori* features such as the ability to reside on the gastric mucosa.

A "heterologous" polypeptide is one not native to *Helicobacter*, i.e., not expressed by *Helicobacter* in nature or prior to introduction into *Helicobacter*, or an ancestor thereof, of encoding nucleic acid for the biologically active agent.

"Host" as used herein defines the intended recipient of a therapeutic composition of the present invention. Host includes all animals. Specifically, hosts include, but are not limited to, primates (including man), bovine, equine, canine, feline, porcine, ovine, rabbits, rodents, birds and

fish.

"Immunologically inert" as used herein shall mean any substance, including microorganisms such as microflora that does not provoke a significant immune response in its host. Examples of immunologically inert materials as used herein include stainless steel, biocompatible polymers such as poly-L-lactide, medical grade plastics and the microflora organisms of the present invention. A "significant immune" response is any immune response that would limit or restrict the *in vivo* utility of a material or organism used in accordance with the teachings of the present invention. A detectable immune response is not necessarily a "significant immune response."

An "isolated nucleic acid" is a nucleic acid the structure of which is not identical to that of any naturally occurring nucleic acid or to that of any fragment of a naturally occurring genomic nucleic acid spanning more than three separate genes. The term therefore covers, for example, (a) a DNA molecule which has the sequence of part of a naturally occurring genomic DNA molecule but is not flanked by both of the coding sequences that flank that part of the molecule in the genome of the organism in which it naturally occurs; (b) a nucleic acid incorporated into a vector or into the genomic DNA of a prokaryote or eukaryote in a manner such that the resulting molecule is not identical to any naturally occurring vector or genomic DNA; (c) a separate molecule such as a cDNA, a genomic fragment, a fragment produced by polymerase chain reaction (PCR), or a restriction fragment; and (d) a recombinant nucleotide sequence that is part of a hybrid gene, i.e., a gene encoding a fusion protein. Specifically excluded from this definition are nucleic acids present in mixtures of (i) DNA molecules, (ii) transfected cells, and (iii) cell clones, e.g., as these occur in a DNA library such as a cDNA or genomic DNA library.

"Percent identity (homology)" of two amino acid sequences or of two nucleic acids is determined using the algorithm of Karlin and Altschul, 1990, *Proc. Natl. Acad. Sci. USA*, 87:2264-2268, 1990, modified as in Karlin and Altschul (*Proc. Natl. Acad. Sci. USA* 90:5873-5877, 1993). Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.* (*J. Mol. Biol.* 215:403-410, 1990). BLAST nucleotide searches are performed with the NBLAST program, score=100, wordlength=12, to obtain nucleotide sequences homologous to a nucleic acid molecule of the invention. BLAST protein searches are performed with the XBLAST program, score=50, wordlength=3, to obtain amino acid sequences homologous to a reference polypeptide (eg., SEQ ID NO: 2). To obtain gapped alignments for comparison purposes, Gapped BLAST is utilised as described in Altschul *et al.* (*Nucleic Acids Res.* 25:3389-3402, 1997). When utilising BLAST and Gapped BLAST programs, the default parameters of the respective programs (eg., XBLAST and NBLAST) are used. These maybe found on the World Wide Web at the URL "ncbi.nim.nih.gov."

The term "reporter gene" as used herein is a nucleic acid sequence incorporated into (or adjacent to) the heterologous nucleic acid encoding for the gene of interest that provides the transformed vector expressing the gene of interest an identifiable phenotype. Non-limiting examples of reporter genes include GFP, β-galactosidase, amylase, and CAT.

"Screening marker" as used herein refers to an identifying characteristic (phenotype) provided to a transformed vector made in accordance with the teachings of the present invention. In one embodiment of the present invention the screening marker is a reporter gene.

"Selectable marker," "selectable gene," "reporter gene" and

"reporter marker" (referred to hereinafter as a "selectable marker") as used herein refer to nucleic acid sequences encoding for phenotypic traits that permit the rapid identification and isolation of a transformed bacterial vector. Generally, bacterial vectors deemed "clinical grade" and made in accordance with the teachings of the present invention are those vectors having selectable markers that do not encode for antibiotic resistance.

"Transgene" as used herein refers to a gene that is inserted, using cDNA technology, into a cell in a manner that ensures its function, replication and transmission as a normal gene.

"Transforming nucleic acid sequence" as used herein means a plasmid, or other expression cassette containing a nucleic acid sequence encoding a gene of interest. In some embodiments of the present invention the nucleic acid sequence can encode for one or more therapeutic agents. "Transforming nucleic acid sequence" can also be used to mean a "transgene" in accordance with certain embodiments of the present invention. In another embodiment of the present invention the transforming nucleic acid sequence includes nucleic acid sequence encoding for a promoter and/or other regulatory elements.

The term "cancer" as used herein refers to neoplastic diseases (e.g., leukemia, cancers and "hyperproliferative disorders"). The neoplasm may be located in a tissue selected from the group consisting of: colon, abdomen, bone, breast, digestive system, liver, pancreas, prostate, peritoneum, lung, blood (e.g., leukemia), endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), uterus, eye, head and neck, nervous (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic, and urogenital.

In one embodiment the term "cancer" also encompasses pre-neoplastic conditions selected from the group consisting of hyperplasia (e.g., endometrial hyperplasia), metaplasia (e.g., connective tissue metaplasia and/or dysplasia (e.g., cervical dysplasia, and bronchopulmonary dysplasia).

In another embodiment, the term "cancer" also encompasses benign dysproliferative disorder selected from the group consisting of: benign tumors, fibrocystic conditions, and tissue hypertrophy.

The term "a disease or condition of the immune/haematopoietic system" as used herein refers to a disease or condition selected from the group consisting of: anaemia, pancytopenia, leukopenia, thrombocytopenia, leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, acute lymphocytic anaemia (ALL), plasmacytomas, multiple myeloma, Burkitt's lymphoma, arthritis, asthma, AIDS, autoimmune disease, rheumatoid arthritis, granulomatous disease, immune deficiency, inflammatory bowel disease, sepsis, neutropenia, neutrophilia, psoriasis, immune reactions to transplanted organs and tissues, systemic lupus erythematosis, haemophilia, hypercoagulation, diabetes mellitus, endocarditis, meningitis, Lyme Disease, Celiac disease and allergies.

The term "a disease or condition of the reproductive system" as used herein refers to a disease or condition selected from the group consisting of: cryptorchism, prostatitis, inguinal hernia, varicocele, leydig cell tumours, verrucous carcinoma, prostatitis, malacoplakia, Peyronie's disease, penile carcinoma, squamous cell hyperplasia, dysmenorrhea, ovarian adenocarcinoma, Turner's syndrome, mucopurulent cervicitis, Sertoli-leydig tumours, ovarian cancer, uterine cancer, pelvic inflammatory disease, testicular cancer, prostate cancer, Klinefelter's syndrome, Young's syndrome, premature ejaculation, diabetes

mellitus, cystic fibrosis, Kartagener's syndrome, testicular atrophy, testicular feminization, anorchia, ectopic testis, epididymitis, orchitis, gonorrhoea, syphilis, testicular torsion, vasitis nodosa, germ cell tumours, stromal tumours, dysmenorrhea, retroverted uterus, endometriosis, fibroids, adenomyosis, anovulatory bleeding, amenorrhoea, Cushing's syndrome, hydatidiform moles, Asherman's syndrome, premature menopause, precocious puberty, uterine polyps, dysfunctional uterine bleeding, cervicitis, chronic cervicitis, mucopurulent cervicitis, cervical dysplasia, cervical polyps, Nabothian cysts, cervical erosion, cervical incompetence, cervical neoplasms, pseudohermaphroditism, and premenstrual syndrome.

The term "a disease or condition of the musculoskeletal system" as used herein refers to a disease or condition selected from the group consisting of: bone cancers (e.g., osteochondromas, benign chondromas, chondroblastoma, chondromyxoid fibromas, osteoid osteomas, giant cell tumours, multiple myeloma, osteosarcomas), Paget's Disease, rheumatoid arthritis, systemic lupus erythematosus, osteomyelitis, Lyme Disease, gout, bursitis, tendonitis, osteoporosis, osteoarthritis, muscular dystrophy, mitochondrial myopathy, cachexia, and multiple sclerosis.

The term "a disease or condition of the cardiovascular system" as used herein refers to a disease or condition selected from the group consisting of: myxomas, fibromas, rhabdomyomas, cardiovascular abnormalities (e.g., congenital heart defects, cerebral arteriovenous malformations, septal defects), heart disease (e.g., heart failure, congestive heart disease, arrhythmia, tachycardia, fibrillation, pericardial Disease, endocarditis), cardiac arrest, heart valve disease (e.g., stenosis, regurgitation, prolapse), vascular disease (e.g., hypertension, coronary artery disease, angina, aneurism, arteriosclerosis,

peripheral vascular disease), hyponatremia, hypematremia, hypokalemia, and hyperkalemia.

The term "a disease or condition described as mixed fetal" as used herein refers to a disease or condition selected from the group consisting of: spina bifida, hydranencephaly, neurofibromatosis, fetal alcohol syndrome, diabetes mellitus, PKU, Down's syndrome, Patau syndrome, Edwards syndrome, Turner syndrome, Apert syndrome, Carpenter syndrome, Conradi syndrome, Crouzon syndrome, cutis laxa, Cornelia de Lange syndrome, Ellis-van Creveld syndrome, Holt-Oram syndrome, Kartagener syndrome, Meckel-Gruber syndrome, Noonan syndrome, Pallister-Hall syndrome, Rubinstein-Taybi syndrome, Scimitar syndrome, Smith-Lemli-Opitz syndrome, thromocytopenia-absent radius (TAR) syndrome, Treacher Collins syndrome, Williams syndrome, Hirschsprung's disease, Meckel's diverticulum, polycystic kidney disease, Turner's syndrome, and gonadal dysgenesis, Klippel-Feil syndrome, Ostogenesis imperfecta, muscular dystrophy, Tay-Sachs disease, Wilm's tumour, neuroblastoma, and retinoblastoma.

The term "a disease or condition of the excretory system" as used herein refers to a disease or condition selected from the group consisting of: bladder cancer, prostate cancer, benign prostatic hyperplasia, bladder disorders (e.g., urinary incontinence, urinary retention, urinary obstruction, urinary tract Infections, interstitial cystitis, prostatitis, neurogenic bladder, hematuria), renal disorders (e.g., hydronephrosis, proteinuria, renal failure, pyelonephritis, urolithiasis, reflux nephropathy, and unilateral obstructive uropathy).

The term "a disease or condition of the neural/sensory system" as used herein refers to a disease or condition selected from the group consisting of: brain cancer (e.g., brain stem glioma, brain tumours, central nervous system

(Primary) lymphoma, central nervous system lymphoma, cerebellar astrocytoma, and cerebral astrocytoma, neurodegenerative disorders (e.g., Alzheimer's Disease, Creutzfeldt-Jakob Disease, Parkinson's Disease, and Idiopathic Presenile Dementia), encephalomyelitis, cerebral malaria, meningitis, metabolic brain diseases (e.g., phenylketonuria and pyruvate carboxylase deficiency), cerebellar ataxia, ataxia telangiectasia, and AIDS Dementia Complex, schizophrenia, attention deficit disorder, hyperactive attention deficit disorder, autism, and obsessive compulsive disorders.

The term "a disease or condition of the respiratory system" as used herein refers to a disease or disorder selected from the group consisting of: cancers of the respiratory system such as larynx cancer, pharynx cancer, trachea cancer, epiglottis cancer, lung cancer, squamous cell carcinomas, small cell (oat cell) carcinomas, large cell carcinomas, and adenocarcinomas. Allergic reactions, cystic fibrosis, sarcoidosis, histiocytosis X, infiltrative lung diseases (e.g., pulmonary fibrosis and lymphoid interstitial pneumonia), obstructive airway diseases (e.g., asthma, emphysema, chronic or acute bronchitis), occupational lung diseases (e.g., silicosis and asbestosis), pneumonia, and pleurisy.

The term "a disease or condition of the endocrine system" as used herein refers to a disease or condition selected from the group consisting of: cancers of endocrine tissues and organs (e.g., cancers of the hypothalamus, pituitary gland, thyroid gland, parathyroid glands, pancreas, adrenal glands, ovaries, and testes), diabetes (e.g., diabetes insipidus, type I and type II diabetes mellitus), obesity, disorders related to pituitary glands (e.g., hyperpituitarism, hypopituitarism, and pituitary dwarfism), hypothyroidism, hyperthyroidism, goiter, reproductive disorders (e.g., male and female infertility), disorders

related to adrenal glands (e.g., Addison's Disease, corticosteroid deficiency, and Cushing's Syndrome), kidney cancer (e.g., hypemephroma, transitional cell cancer, and Wilm's tumour), diabetic nephropathy, interstitial nephritis, polycystic kidney disease, glomerulonephritis (e.g., IgM mesangial proliferative glomerulonephritis and glomerulonephritis caused by autoimmune disorders; such as Goodpasture's syndrome), and nephrocalcinosis.

The term "a disease or condition of the digestive system" as used herein refers to a disease or condition selected from the group consisting of: ulcerative colitis, appendicitis, Crohn's disease, hepatitis, hepatic encephalopathy, portal hypertension, cholelithiasis, cancer of the digestive system (e.g., biliary tract cancer, stomach cancer, colon cancer, gastric cancer, pancreatic cancer, cancer of the bile duct, tumours of the colon (e.g., polyps or cancers), and cirrhosis), pancreatitis, ulcerative disease, pyloric stenosis, gastroenteritis, gastritis, gastric atropy, benign tumours of the duodenum, distension, irritable bowel syndrome, malabsorption, congenital disorders of the small intestine, bacterial and parasitic infection, megacolon, Hirschsprung's disease, aganglionic megacolon, acquired megacolon, colitis, anorectal disorders (e.g., anal fistulas, haemorrhoids), congenital disorders of the liver (e.g., Wilson's disease, hemochromatosis, cystic fibrosis, biliary atresia, and alpha 1-antitrypsin deficiency), portal hypertension, cholelithiasis, and jaundice.

The term "a disease or condition of the connective / epithelial" as used herein refers to a disease or condition selected from the group consisting of: connective tissue metaplasia, mixed connective tissue disease, focal epithelial hyperplasia, epithelial metaplasia, mucoepithelial dysplasia, graft v. host disease, polymyositis, cystic hyperplasia, cerebral dysplasia,

tissue hypertrophy, Alzheimer's disease, lymphoproliferative disorder, Waldenstron's macroglobulinemia, Crohn's disease, pernicious anaemia, idiopathic Addison's disease, glomerulonephritis, bullous pemphigoid, Sjogren's syndrome, diabetes mellitus, cystic fibrosis, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, osteoporosis, osteocarthritis, periodontal disease, wound healing, relapsing polychondritis, vasculitis, polyarteritis nodosa, Wegener's granulomatosis, cellulitis, rheumatoid arthritis, psoriatic arthritis, discoid lupus erythematosus, systemic lupus erythematosus, scleroderma, CREST syndrome, Sjogren's syndrome, polymyositis, dermatomyositis, mixed connective tissue disease, relapsing polychondritis, vasculitis, Henoch-Schonlein syndrome, erythema nodosum, polyarteritis nodosa, temporal (giant cell) arteritis, Takayasu's arteritis, Wegener's granulomatosis, Reiter's syndrome, Behcet's syndrome, ankylosing spondylitis, cellulitis, keloids, Ehler Danlos syndrome, Marfan syndrome, pseudoxantoma elasticum, osteogenese imperfecta, chondrodysplasias, epidermolysis bullosa, Alport syndrome, and cutis laxa.

The term "a" and "the" as used in the present descriptive is intended to include both one (the singular) and more than one (plural).

A "therapeutically effective amount" of an active agent or combination of agents as described herein is understood to comprise an amount effective to elicit the desired response but insufficient to cause a toxic reaction. A desired response, for example, may constitute the formation of a sufficient and/or acceptable detectable antibody titer level in a blood sample. The dosage and duration of treatment of the preparation to be administered to a subject will be determined by the health professional attending the subject in need of treatment, and will consider the age, sex, weight, extent of existing diseased

state and/or tissue damage of the subject, and specific formulation of *Helicobacter* and the gene of interest product being used as the treatment for the subject.

The phrase, "effective level" refers to the level of the desired activity of the molecules and not necessarily limited to the number of molecules. For example, the effective level of amylin may be decreased to stimulate ghrelin secretion by using amylin antagonists, without a necessary concomitant decrease in the amount of free amylin present in a subject.

The phrase "ghrelin-associated diseases and disorders" refers to any condition that can be treated prevented or ameliorated through the modulation of ghrelin activity. These include conditions that are enhanced, exacerbated or stimulated by ghrelin, for example, growth hormone release or drive to eat. The physiological actions of ghrelin are considered to include, by way of example, the stimulation of growth hormone release, the stimulation of hormone secretion from lactotrophs and corticotropes, orexigenic and cardiovascular actions, anti-proliferative effects on thyroid and breast tumors and regulation of gastric motility and acid secretion through vagal mediation. (See WO 2005021026).

Throughout the specification, unless the context requires otherwise, the word "comprise" or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

Where the definition of terms departs from the commonly used meaning of the term, applicant intends to utilize the definitions provided herein, unless specifically indicated.

DETAILED DESCRIPTION OF THE INVENTION

Before describing the present invention in detail, it is to be understood that this invention is not limited to particularly exemplified methods and may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting which will be limited only by the appended claims.

All publications, patents and patent applications cited herein, whether supra or infra, are hereby incorporated by reference in their entirety. However, publications mentioned herein are cited for the purpose of describing and disclosing the protocols, reagents and vectors which are reported in the publications and which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

Furthermore, the practice of the present invention employs, unless otherwise indicated, conventional immunological and molecular biological techniques and pharmacology within the skill of the art. Such techniques are well known to the skilled worker, and are explained fully in the literature. See, eg., Coligan *et al.* "Current protocols in Protein Science" (1999) Volume I and II (John Wiley & Sons Inc.); Sambrook *et al.*, (Molecular Cloning: A Laboratory Manual, 2nd & 3rd Editions, Cold Spring Harbor Laboratory press (1989) (2001); and Bailey, J.E. and Ollis, D.F., Biochemical Engineering Fundamentals, McGraw-Hill Book Company, NY, 1986.

It must be noted that as used herein and in the appended

claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a nucleic acid" includes a plurality of such nucleic acids, and a reference to "an isolated peptide" is a reference to one or more peptides, and so forth. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any materials and methods similar or equivalent to those described herein can be used to practice or test the present invention, the preferred materials and methods are now described.

Delivery of therapeutic compositions and nucleic acids to specific target sites within the animal body is an ongoing challenge faced by the drug development industry. The present inventor has developed a *Helicobacter*-based bacterial delivery system capable of carrying vectors encoding biologically active agents, wherein these agents are expressed on the surface of the bacterium, or secreted therefrom. In one embodiment, the bacterium is a species of *Helicobacter*. More preferably, *H. pylori*. In some embodiments the strain of *H. pylori* can be any strain known in the field. In some embodiments, the *H. pylori* strain is a non-pathogenic strain such as genomic strain 26695.

In another embodiment, a bacterium, other than *Helicobacter*, is utilised wherein the bacterium has been genetically altered such that it has *Helicobacter* or *H. pylori* features including the ability to chronically colonise the gastric mucosa or other areas of gastrointestinal tract, urinary tract, bronchial epithelium or other mucosal organ, without significant toxicity to the host.

In one embodiment, the *H. pylori* has been manipulated so

that some of the pathogenic features have been removed and/or attenuated. For example, the vacuolating cytotoxin and the *cag* pathogenicity island genes can be removed so that the *H. pylori* is less pathogenic. In one embodiment, the *H. pylori* has been manipulated so that some of the pathogenic features have been removed and/or attenuated. For example, the vacuolating cytotoxin and the *cag* pathogenicity island genes can be removed so that the *H. pylori* are less pathogenic. Attenuating mutations can be introduced into *Helicobacter* using non-specific mutagenesis either chemically, using N-methyl-N-nitro-N-nitrosoquanidine, or using recombinant DNA technologies.

The skilled person will appreciate that the methods of the present invention could be used to deliver a range of biologically active agents. Examples of suitable agents include ones which are capable of functioning locally or systemically, eg an agent capable of exerting endocrine activities affecting local or whole-body metabolism and/or an agent which is capable of regulating the activities of cells belonging to the immuno/haemopoeitic system and/or an agent which is capable of affecting the viability, growth and differentiation of a variety of normal or neoplastic cells in the body or affecting the immune regulation or induction of acute phase inflammatory responses to injury and infection and/or an agent which is capable of enhancing or inducing resistance to infection of cells and tissues mediated by chemokines acting on their target cell receptors, or the proliferation of epithelial cells or the promotion of wound healing and/or an agent which modulates the expression or production of substances by cells in the body.

Specific examples of such biologically active agents include insulin, growth hormone, prolactin, calcitonin, luteinising hormone, parathyroid hormone, somatostatin, thyroid stimulating hormone, vasoactive intestinal

polypeptide, a structural group 1 cytokine adopting an antiparallel 4 α helical bundle structure such as IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-11, IL-12, IL-13, GM-CSF, M-CSF, SCF, IFN-γ, EPO, G-CSF, LIF, OSM, CNTF, GH, PRL or IFN α/β, a structural group 2 cytokine which are often cell-surface associated, form symmetric homotrimers and the subunits take up the conformation of β-jelly roll described for certain viral coat proteins such as the tumor necrosis factor (TNF) family of cytokines, eg TNF α, TNF β, CD40, CD27 or FAS ligands, the IL-1 family of cytokines, the fibroblast growth factor family, the platelet derived growth factors, transforming growth factor β and nerve growth factors, a structural group 3 cytokine comprising short chain α/β molecules, which are produced as large transmembrane pre-cursor molecules which each contain at least one epidermal growth factor (EGF) domain in the extracellular region, e.g., the EGF family of cytokines, the chemokines characterised by their possession of amino acid sequences grouped around conserved cysteine residues (the C--C or C--X--C chemokine subgroups) or the insulin related cytokines, a structural group 4 cytokine which exhibit mosaic structures such as the heregulins or neuregulins composed of different domains, e.g., EGF, immunoglobulin-like and kringle domains.

Alternatively, the biologically active agent can be a receptor or antagonist for biologically active agent as defined above.

In some embodiments, the *H. pylori*-based vector and/or vector plasmid construct is employed to create transformed cells (such as an *E. coli* or *Helicobacter* cell) that permits the expression and/or secretion of a biologically active agent from an isolated nucleic acid molecule contained within it at the mucosa surface of a host to which the transformed cell preparation is administered. The isolated nucleic acid contained within the transformed cell

(or vector) may comprise one or more nucleic acid constructs in which nucleic acid encoding the biologically active agent is under control of appropriate regulatory sequences for expression in the *H. pylori*.

Suitable vectors and shuttle vector sequences comprising nucleic acid for introduction into *H. pylori* can be chosen or constructed, to contain appropriate regulatory sequences, including promoter sequences, terminator fragments, enhancer sequences, marker genes and other sequences as appropriate. Vectors may be plasmids, viral eg. phage or phagemid, as appropriate. For further details see, for example, Sambrook *et al.*, *supra*. Many known techniques and protocols for manipulation of nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are described in detail in Short Protocols in Molecular Biology, Second Edition, Ausubel *et al.* eds., John Wiley & Sons, 1992. The disclosures of Sambrook *et al. supra* and Ausubel *et al.* are incorporated herein by reference.

In some embodiments, the coding sequence for the biologically active agent is contained in an operon, i.e., a nucleic acid construct for multi-cistronic expression. In an operon, transcription from the promoter results in a mRNA which comprises more than one coding sequence, each with its own suitably positioned ribosome binding site upstream. Thus, more than one agent can be translated from a single mRNA. Use of an operon enables expression of the biologically active agent to be coordinated.

A nucleic acid construct or vector comprising a coding sequence for a biologically active agent of the present invention is preferably under the control of a promoter for expression in *H. pylori*.

In one embodiment, the promoter employed in accordance with the present invention is expressed constitutively in the *H. pylori*. Use of a constitutive promoter avoids the need to supply an inducer or other regulatory signal for expression to take place. Preferably, the promoter directs expression at a level at which the *H. pylori* host cell remains viable, i.e., retains some metabolic activity, even if growth is not reduced. Advantageously then, such expression may be at a low level. For example, where the expression product accumulates intracellularly, the level of expression may lead to accumulation of the expression product at less than about 10% of cellular protein, preferably about or less than about 5%, for example about 1-3%.

The promoter may be homologous to the *H. pylori* strain employed, i.e., one found in that strain of *H. pylori* in nature. In some embodiments the promoter is arabinose inducible promoter. Other promoters include FlaB sigma 54 promoter (Josenhans *et al.*, 1998, *FEMS Microbiol Lett*, 161(2): 263-73), T7 promoter, and nir B promoter of salmonella (Chatfield *et al.*, 1992, *Biotechnology*, 10(8): 888-92).

In another embodiment the promoter is inducible. Inducible promoters that may be used with the clinical grade vectors include, but are not limited to, a pH inducible promoter as described in U.S. Pat. No. 6,242,194 issued to Kullen *et al.*, a lactose inducible promoter such as that used in *E. coli* plasmids (e.g., pBluescript™ from Stratagene) or the endogenous lactose promoter in *Lactobacillus*; promoters induced during anaerobic growth such as the promoter for alcohol dehydrogenase (adhE), as described in Aristarkhov *et al.*, 1999, *J. Bacteriology*, Vol. 178(14), 4327-4332.

In one embodiment, the constructs of the present invention also include a toxic gene. These toxic genes are preferably under the control of inducible promoters so

that, on completion of treatment, the *Helicobacter* of the present invention can be readily eliminated by inducing the expression of the toxic gene. Non-limiting examples of toxic genes include bacterial autolysins under the control of an inducible promoter. The autolysing gene may then be triggered at the appropriate time and place in the gastrointestinal tract through the use of one or more of the inducible promoters described immediately above.

In some embodiment, the engineered *Helicobacter* vector and plasmid vector constructs are sensitive to oxygen. This oxygen sensitivity is another method for limiting dissemination of the clinical grade vectors of the present invention. The environment of the human gut is very low in oxygen, suitable for growth of anaerobic and microaerophilic microorganisms, including *Helicobacter*. Thus, an efficient means of eliminating *Helicobacter* delivery vehicles once they have exited the human body upon discharge of intestinal waste into the oxygen-rich outside environment, is to engineer genes into the transformed micro-organisms that confer oxygen sensitivity.

The nucleic acid construct or constructs of the present invention may comprise a secretory signal sequence. Thus, in some embodiments the nucleic acid encoding the biologically active agent eg non-*Helicobacter* polypeptide, may provide for secretion of the agent at a cell membrane by appropriately coupling a nucleic acid sequence encoding a secretory signal sequence to the nucleic acid sequence encoding the molecule (polypeptide). The ability of *Helicobacter* harbouring the nucleic acid to secrete the polypeptide may be tested *in vitro* in culture conditions, which maintain viability of the *Helicobacter*.

Suitable secretory signal sequences include any of those with activity in Gram negative organisms such as *Escherichia*, *Klebsiella* and *Salmonella*. Secretory signal

sequences may include the secretion leader of the Staphylokinase enzyme secreted by some strains of Staphylococcus, which is known to function in both Gram-positive and Gram-negative hosts (see "Gene Expression Using *Bacillus*", Rapoport (1990) *Curr Opin Biotech* 1:21-27).

Other secretory signal sequences that can be used include, for example, the β-lactamase gene (Talmadge *et al.*, 1980, *Proc. Natl. Acad. Sci. USA* 77:3369-3373) or the enteroinvasive *E. coli* hemolysin A (hlyA) (Su *et al.*, 1992, *Microbial Pathogen*, 13:465-476). An illustrative list of secretory signal sequences is presented in Pugsley, 1988, Protein secretion across the outer membrane of gram-negative bacteria. *In*: Protein Transfer and organelle Biogenesis, R. C. Dand and P. W. Robbins (eds), Academic Press, Inc., San Diego, pp 607-652.

Selectable markers provide researchers and technicians a convenient means for distinguishing transformed microorganisms from non-transformed ones in a mixed population. One means of identifying transformed organism is to incorporate a selectable marker nucleic acid sequence into the plasmid containing the gene of interest. The selectable marker sequence is generally inserted downstream of the gene of interest and is driven off the same promoter. As a result, cells successfully transformed with the gene of interest will also be transformed with selectable marker nucleic acid sequence. When antibiotic resistance is used as the selectable marker, only transformed cells will survive and/or grow in media containing the antibiotic.

Thus, antibiotic resistance is a convenient and much used phenotype when developing transformants. However, vectors having antibiotic resistant genes as selective markers are capable of horizontal gene transfer that can endow other

organisms with antibiotic-resistant phenotypes. This risk is especially acute when *Helicobacter* is used as part of a therapeutic vector.

In order to use *Helicobacter* as a gene delivery system to animals, the present disclosure presents, in some embodiments, a clinical grade vector system that does not use an antibiotic selection marker. One of the alternatives to using antibiotic resistance genes provided by the present delivery systems includes clinical grade vectors having chromosomal deletions or lethal mutations in an essential "house-keeping" gene. Next, a functional analogous house-keeping gene is inserted into a plasmid encoding for a gene of interest. Consequently, the "house-keeping" gene becomes the selectable marker allowing for the rapid isolation and identification of transformants.

Examples of essential "house-keeping" genes include genes that encode for any number of metabolic regulators and/or enzymes including, but not limited to kinases, proteases, synthetases, dehydrogenases and others. Another alternative to antibiotic resistance genes provided by the present invention includes clinical grade vectors having reporter genes incorporated into the plasmid containing the gene of interest. Other non-limiting examples of reporter genes used in accordance with the teachings of the present invention include green fluorescent Protein (GFP), β-galactosidase and amylase.

In one embodiment, the biologically active polypeptide preferably has cytokine activity. Cytokines are discussed in "The Cytokine Facts Book", Callard and Gearing (1994), Academic Press. Preferred polypeptides with cytokine activity are interleukins, including Interleukin-2 (IL-2) and Interleukin 6 (IL-6).

In some embodiments, the *Helicobacter* delivery system,

vector or vector plasmid system comprises a nucleic acid construct as described above is introduced into a *Helicobacter* or other suitable host cell, to provide transformed cells. Thus, a further aspect of the present invention provides a method comprising introducing nucleic acid as disclosed into a non-pathogenic *Helicobacter*. The transformation of a culture of host cells, such as *Helicobacter*, may employ any available technique. For *H. pylori* cells, suitable techniques may include calcium chloride transformation, electroporation and transfection using bacteriophage.

The introduction of the vector plasmid into a *Helicobacter* cell may be followed by causing or allowing expression from the nucleic acid, e.g., by culturing *H. pylori* under conditions for expression of the gene. Growing the *Helicobacter* in culture under conditions for expression of the biologically active polypeptide may be employed to verify that the *Helicobacter* contain the encoding nucleic acid and is able to produce the encoded material.

In a further aspect, the present invention provides a method of delivering a therapeutic or prophylactic dose of a biologically active agent *in vivo*, the method comprising administering to a subject an effective amount of the non-pathogenic preparation of the *H. pylori* compositions and vaccines of the present invention.

It will be appreciated that the methods of the present invention and the use of a non-invasive or non-pathogenic *Helicobacter* as described herein provide a wide range of therapeutic methods which would enable the skilled person to manipulate, for instance, the immume response of a subject. Thus, in one aspect the present invention provides a method of regulating the survival, growth, differentiation, effector functions or susceptibility to infection of cells or tissues which comprises administering

to a subject a non-invasive or non-pathogenic *Helicobacter* as defined herein.

In another aspect, a method of boosting an immune response against tumour cells or an infection colonising a mucosal surface or adjacent or distant tissue is provided which comprises administering to a subject a non-invasive or non-pathogenic *Helicobacter* as defined herein.

In yet another aspect, a method of modulating the type of immune response (antibody versus cell-mediated) against a pathogenic infectious agent is provided which comprises administering to a subject a non-invasive or non-pathogenic *Helicobacter* as defined herein.

In another aspect, a method of modulating the infiltration of normal tissues with inflammatory or tumour cells is provided which comprises administering to a subject a non-invasive or non-pathogenic *Helicobacter* as defined herein.

In some aspects a method of controlling the rate of growth, rate of invasion or survival of tumour cells is provided which comprises administering to a subject a non-invasive or non-pathogenic *Helicobacter* as defined herein.

In yet another aspect a method of inducing apoptosis in tumour cells is provided which comprises administering to a subject a non-invasive or non-pathogenic *Helicobacter* as defined herein.

Other aspects provided for a method of down-regulating an immune response is provided which comprises administering to a subject a non-invasive or non-pathogenic bacterium which expresses a biologically active agent.

In another aspect a method of treating an allergic autoimmune or other immune dysregulative disease state is

provided, which comprises administering to a subject a non-invasive or non-pathogenic *Helicobacter* which expresses a biologically active agent.

The subject can be any primate, equine, bovine, porcine, ovine, rodent, fish, or bird. In one embodiment, the subject is human. Administration may conveniently be nasal or oral.

In a therapeutic context, i.e., where the biological effect of delivery of the biologically active agent to a subject is beneficial to that subject, administration is preferably in a "therapeutically effective amount", this being sufficient to show benefit to a subject. Such benefit may be at least amelioration or reduce the severity or occurrence of at least one symptom. In a prophylactic context, the amount may be sufficient to reduce the deleterious effect on the subject of a subsequent pathogenic challenge, for instance by enhancing the immune response. The actual amount administered, and rate and time-course of administration will depend on the aim of the administration, e.g. the biological effect sought in view of the nature and severity of the challenge, and is the subject of routine optimisation. Prescription of treatment, including prophylactic vaccination, for example decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors.

A composition comprising *Helicobacter* may be administered in accordance with the present invention alone or in combination with other treatments, either simultaneously or sequentially.

The present invention also provides a pharmaceutical composition comprising a *Helicobacter* as disclosed. Such a pharmaceutical composition is in one embodiment preferably suitable for application to a mucosal membrane.

Pharmaceutical compositions according to the present invention, and for use in accordance with the present invention, may comprise, in addition to the *Helicobacter*, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material may depend on the route of administration. For oral administration a parenterally acceptable aqueous solution may be employed which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required. As discussed, a pharmaceutical comprising a *Helicobacter* for administration in accordance with the present invention may comprise one or more nutrient substances, e.g., an energy source such as glucose, amino acids and so on.

In another aspect, the present invention provides a method of manufacture of pharmaceutical formulations comprising formulating *Helicobacter* as disclosed with a suitable carrier medium suitable for administration to an individual. In one embodiment, the pharmaceutical is suitable for application to a mucosal membrane of an individual.

In another aspect, the present invention provides a non-pathogenic *Helicobacter* expressing a heterologous biologically active polypeptide for pharmaceutical use, e.g., for use in a method of treatment of the human or animal body by surgery or therapy, including prophylaxis ("vaccination").

In one embodiment the methods of the present invention can

be used to treat, prevent or palliate a disease such as cancer. The methods and deliver system can also be used to treat or prevent a disease or condition of the immune/haematopoietic system, a disease or condition of the reproductive system, a disease or condition of the musculoskeletal system, a disease or condition of the cardiovascular system, a disease or condition described as mixed fetal, a disease or condition of the excretory system, a disease or condition of the neural/sensory system, a disease or condition of the endocrine system, a disease or condition of the respiratory system, a disease or condition of the digestive system and a disease or condition associated with connective/epithelial tissue or a disease or condition caused by bacterial, viral or parasitic infection.

In another embodiment, the *Helicobacter* delivery system described herein is capable of concomitant or sequential delivery of a number of different nucleic acid molecules, which encode products capable of treating a number of conditions or diseases as described herein. Moreover, preferred delivery systems would also deliver compositions capable of producing additional desirable physiological effects such as appetite suppression or enhancement.

An example of suicide system in *H. pylori* has been described by Panthel *et al.* 2003 (*Infection & Immunity*, 71: 109-116). This system introduces a plasmid into *H. pylori* which contains the PhiX174 lysis gene E. To eradicate the strain, incubation at 42°C for 5 hours was used. *In vivo* this would mean that the animal would consume a drink at 45-50°C to raise the temperature of the gastric environment above 42°C.

A second example is the L-Dap selection system, commonly used to allow survival of bacterial mutants on supplemented plates (see, for example, Kirata *et al.* 1997 (Infection &

Immunity, 65: 4158-4164). In this system the animal subject must supplement their diet with a missing substrate i.e., diamino-pimelic-acid (DAP), in order for the DapE deficient *H. pylori* mutant to survive. In order to eradicate the mutants, DAP consumption is ceased.

A third possible system relates to metronidazole sensitivity of *H. pylori* because of its rdxA gene. Excessive replication of the rdxA gene is harmful to mammalian cells and *E. coli*. However, duplication may be tolerated by the bacterium. Therefore a *Helicobacter* species of the present invention can be engineered to contain two copies of rdxA which prevent the normal mutation-dependant rdxA loss. The introduction of at least two functional rdxA genes into the *Helicobacter* genome will result in a *Helicobacter* strain that is permanently sensitive to metronidazole. Jeong *et al.* 2000 (*J. Bacteriol.*, 182: 5082-5090) showed that the nitroreductase produced by a functional rdxA gene converts metronidazole from a prodrug to a bactericidal compound. The mode of action of the active compound is to cause DNA breaks of the *Helicobacter* genome.

The invention will now be further described by reference only to the following non-limiting examples. It should be understood, however, that the examples following are illustrative, and should not be taken in any way as a restriction on the generality of the invention described herein. In particular, while the invention is described in detail in relation to the use of a specific *H. pylori* strain, it will be clearly understood that the findings herein are not limited to this strain.

EXAMPLE 1 VECTORS AND TRANSGENIC *H. pylori* ORGANISMS FOR STABLE EXPRESSION OF FOREIGN PROTEINS

The genetic manipulation of *H. pylori* is uncommon. The present example demonstrates the utility of the invention for providing a genetically transformed *Helicobacter*, particularly transformed *H. pylori*. The transformed bacterium are prepared using plasmids and plasmid vectors derived from *Helicobacter*, which have had been subject to prior manipulation in a non-*Helicobacter* organism, such as *E. coli*.

Several *H. pylori* plasmids described in the literature can be successfully converted to *H. pylori/E. coli* shuttle vectors. Many strains of *E. coli* have been reported to be naturally competent for DNA uptake. Resistance markers for streptomycin, rifampin and metronidazole have also been successfully transformed into most strains of *H. pylori*. However, while plasmid DNA from *E. coli* and other organisms can be introduced into *H. pylori* these plasmids cannot be stably maintained. Moreover, *H. pylori* plasmids cannot be transformed into *E. coli* or *Helicobacter* species. Accordingly, *H. pylori* shuttle vectors must be constructed.

Two plasmids from *H. pylori* are illustrated in the schematics shown in Figures 1 and 2. Vectors pHPA1 (2.8kb) (Fig. 1) and pHP3 (3.4kb) (Fig. 2) have been sequenced and it has been revealed that pHPA1 replicated via the theta mode of plasmid replication. In contrast to rolling-circle replicating plasmids, theta plasmids do not generate single-stranded DNA intermediates during replication and are thus more stable vector candidates because they are less prone to undergo illegitimate recombination. Furthermore, the pHPA1 origin of replication (*ori*) contains a series of direct repeat sequences (termed "iterons") that are involved in replication control and maintaining stable copy number. Vector pHP623 shares many of these features.

The nucleotide sequences for these two vectors are shown below.

(1) Plasmid pHP1 shown in double stranded form (top strand is SEQ ID NO:1; bottom strand is SEQ ID NO:2)

```
GTCATGCGCGTTGTTTTTAATTACATTTTAAACAACTTGTTGTTGTTTTTACATGTTTTACTCGC      65
CAGTACGCGCAACAAAAATTAATGTAAAATTTGTTGAACAACAACAAAAATGTACAAAATGAGCG

ATGCGCGCGCGTGAGGGATTGGGGGTTGCAACCCCCTAAATAACGAAGCTGTAGGGTTTCTCATT     130
TACGCGCGCGCACTCCCTAACCCCCAACGTTGGGGGATTTATTGCTTCGACATCCCAAAGAGTAA

TTTGTGGTGAAAATGAATAAAACAGAACTTCTTGCCAACACTAACAGAACTTCTTGCCAACACTA     195
AAACACCACTTTTACTTATTTTGTCTTGAAGAACGGTTGTGATTGTCTTGAAGAACGGTTGTGAT

ACAGAACTTCTTGCCAACACTAACAGAACTTCTTGCCAACACTAACAGAACTTCTTTATTTTAAA     260
TGTCTTGAAGAACGGTTGTGATTGTCTTGAAGAACGGTTGTGATTGTCTTGAAGAAATAAAATTT

GTTATGATTATTAACAATTTTTAGACATAATAACAGCGTGTGAAGATACTTTTGTAGCGGTATTT     325
CAATACTAATAATTGTTAAAAATCTGTATTATTGTCGCACACTTCTATGAAAACATCGCCATAAA

CCTATGTGCGGCAAAATTTGGAGCAATTAGCTTGACTTGGTTGAGTTAGTGGGTTGGAGGATAGA     390
GGATACACGCCGTTTTAAACCTCGTTAATCGAACTGAACCAACTCAATCACCCAACCTCCTATCT

GAGGGCGACACCTCGTTAGGAGGTATCAATGTGAAAGTATTTGTCGTATTAGTTCTAGTATTAGT     455
CTCCCGCTGTGGAGCAATCCTCCATAGTTACACTTTCATAAACAGCATAATCAAGATCATAATCA

AATTCTCGCACAATTGCTATATTAGGCTTATTCGTGGTCTAACCCCTTGTTTATGGGGGTTGGCT     520
TTAAGAGCGTGTTAACGATATAATCCGAATAAGCACCAGATTGGGGAACAAATACCCCCAACCGA

CGTTATAAGCATACTGATACGATCACACTTATTATACACCAAAAGATAAGGAGTATAGAGTGGAA     585
GCAATATTCGTATGACTATGCTAGTGTGAATAATATGTGGTTTTCTATTCCTCATATCTCACCTT

TTTGATCAATCAGATTTACAAAAAGCGTTGAAAATATTAGATACACTCCCACAAACCCCACAAAT     650
AAACTAGTTAGTCTAAATGTTTTTCGCAACTTTTATAATCTATGTGAGGGTGTTTGGGGTGTTTA

TGAGCTACAAAAACAAGAAATACAAAACCGCATCAACAAAATAACAGAGACAATCATTAAAGAAT     715
ACTCGATGTTTTTGTTCTTTATGTTTTGGCGTAGTTGTTTTATTGTCTCTGTTAGTAATTTCTTA

TACTATCAAAGCATGAAATCAAGAAAGAAGAACTAGAACCCACTCTAACCCCAAAACCCACACCA     780
ATGATAGTTTCGTACTTTAGTTCTTTCTTCTTGATCTTGGGTGAGATTGGGGTTTTGGGTGTGGT

CTCAAAGAGCCACAAACCACCCCAACACCATGCAAAGATTTAGTGGTTAGCACCCCTAAAGATAA     845
GAGTTTCTCGGTGTTTGGTGGGGTTGTGGTACGTTTCTAAATCACCAATCGTGGGGATTTCTATT

AACCTAATATCACCTACCACAATAACGCTAATAAGGTCAATCTAGGGAAATTGAGCGAAAGGGAA     910
TTGGATTATAGTGGATGGTGTTATTGCGATTATTCCAGTTAGATCCCTTTAACTCGCTTTCCCTT

GCCAATCTTTTATTCGCTATTTTTCAAAAACTCAAAGCCCAAGGGAATACCCTCATTCGTTTTGA     975
CGGTTAGAAAATAAGCGATAAAAAGTTTTTGAGTTTCGGGTTCCCTTATGGGAGTAAGCAAAACT

ACCGCAAGATTTGAAACGCATGCTAAACATAGATATTTCTAATGAGCGCTTATCAGAAGTCGTTA    1040
TGGCGTTCTAAACTTTGCGTACGATTTGTATCTATAAAGATTACTCGCGAATAGTCTTCAGCAAT

TTAAGCTGTGGGATAGCATTAAAACCGCTGATTTTTGGAAAATTAGCGAAACCGAAACTTCAATC    1105
AATTCGACACCCTATCGTAATTTTGGCGACTAAAAACCTTTTAATCGCTTTGGCTTTGAAGTTAG

ATTCAAGAAAATTACATGCTTTTTAGTCGGTGTAAAATTGAATTGAACAAACCGAGTAAAGATTT    1170
TAAGTTCTTTTAATGTACGAAAAATCAGCCACATTTTAACTTAACTTGTTTGGCTCATTTCTAAA

GAAGTATTTAGAAATCCAACTCAACGATAACTATCAAGACTTACTCAACAATCTGGGCATGGGTC    1235
CTTCATAAATCTTTAGGTTGAGTTGCTATTGATAGTTCTGAATGAGTTGTTAGACCCGTACCCAG

AATACACTTCTTTCAATCTGTTAGAATTTCAAAGAGTGAGGGGTAAATACGCTAAAACGCTCTAT    1300
TTATGTGAAGAAAGTTAGACAATCTTAAAGTTTCTCACTCCCCATTTATGCGATTTTGCGAGATA

CGCTTGCTCAAGCAATACAAAAGCACAGGGATTTTGAGCGTGGAATGGACTCAATTCAGGGAGCT    1365
GCGAACGAGTTCGTTATGTTTTCGTGTCCCTAAAACTCGCACCTTACCTGAGTTAAGTCCCTCGA

TTTAGACATTCCAAAAGACTACAAAATGGAAAACATCGATCAAAAAGTCTTAACCCCCTCTCTCA    1430
```

AAATCTGTAAGGTTTTCTGATGTTTTACCTTTTGTAGCTAGTTTTTCAGAATTGGGGGAGAGAGT

AAGAACTCAGAAAAATCTACCCTTTTGAACACTTGAGCTATAAAAAAGAACGCAAAAGCCATTAC 1495
TTCTTGAGTCTTTTTAGATGGGAAAACTTGTGAACTCGATATTTTTTCTTGCGTTTTCGGTAATG

AAGCGCAAAGTAACCCACATTGATTTTTATTTTGAGCAATTTCCTTAAGGCGAAAATAAGAAACA 1560
TTCGCGTTTCATTGGGTGTAACTAAAAATAAAACTCGTTAAAGGAATTCCGCTTTTATTCTTTGT

AAACAAAGCCGACAAGCAACGCGCTCAAAGGGACATCAAGCTTGTAGCATGGGATATTCACAACC 1625
TTTGTTTCGGCTGTTCGTTGCGCGAGTTTCCCTGTAGTTCGAACATCGTACCCTATAAGTGTTGG

AAATCGCTAAAAGAAACGCAAAAGCCACTATGGAAGCTAGGTTTCTTGAATTGAAAACTTTGATC 1690
TTTAGCGATTTTCTTTGCGTTTTCGGTGATACCTTCGATCCAAAGAACTTAACTTTTGAAACTAG

GGCTATCAGTTCAGGAACAATGACAGTAGGAACAAATTAAAGATTGACAACACCACTTTTGAAAG 1755
CCGATAGTCAAGTCCTTGTTACTGTCATCCTTGTTTAATTTCTAACTGTTGTGGTGAAAACTTTC

AATCAAATGTATTTACATGTATCTTAACCCTAAAAATAAGCATAACCCCCAAAAATTCCTTGTAT 1820
TTAGTTTACATAAATGTACATAGAATTGGGATTTTTATTCGTATTGGGGGTTTTTAAGGAACATA

CCAACAAGACATTCGCATTGGAACTACTATATATCAATAGATACAGCCTAAAAAAAAGACAACTT 1885
GGTTGTTCTGTAAGCGTAACCTTGATGATATATAGTTATCTATGTCGGATTTTTTTTCTGTTGAA

GCTAGAAGAATTTAACCCCCCAAAATCCACCCTATCACCAACGAACCTATCAAGGAATTTGCAGA 1950
CGATCTTCTTAAATTGGGGGGTTTTAGGTGGGATAGTGGTTGCTTGGATAGTTCCTTAAACGTCT

ATACATCGGCAAAACGATTAACATCACCAACTTCAATGTGGATCAATGCCATGAGGGAATCAGCA 2015
TATGTAGCCGTTTTGCTAATTGTAGTGGTTGAAGTTACACCTAGTTACGGTACTCCCTTAGTCGT

ACTACCTGACAATCACTAGGATCGTGAACTGGACGTAATCGGATCTGTATTTGGTCCAGATGTGG 2080
TGATGGACTGTTAGTGATCCTAGCACTTGACCTGCATTAGCCTAGACATAAACCAGGTCTACACC

ATAAGCCTGGGACTTCTCAAGCCTTTCATTGCTAAAGTGAGAAAATTTGGGGATTGGTTCAAGAA 2145
TATTCGGACCCTGAAGAGTTCGGAAAGTAACGATTTCACTCTTTTAAACCCCTAACCAAGTTCTT

CACTACAGGTGAAAAGACAGATGCATGCTGACTAAACTCATAGAAAAACTGAATCACGAAAGAAA 2210
GTGATGTCCACTTTTCTGTCTACGTACGACTGATTTGAGTATCTTTTTGACTTAGTGCTTTCTTT

GAATGCAAGCAGAAAACAAACACCTAAAAGAACAAGGACTAGAAAAAATCTACACTCAAAAAGAC 2275
CTTACGTTCGTCTTTTGTTTGTGGATTTTCTTGTTCCTGATCTTTTTTAGATGTGAGTTTTTCTG

TACGAGCAGTTAAAAGAACAGCATTTGAAAGAAATTGAAGCACTCAAAAAAGAAATCCAAAAAAC 2340
ATGCTCGTCAATTTTCTTGTCGTAAACTTTCTTTAACTTCGTGAGTTTTTTCTTTAGGTTTTTTG

CAAGCAAGAAACATACACGCAACCAAAAGAATGTAGCCATTTAGCGCATTCTTTTAGCCCTAATT 2405
GTTCGTTCTTTGTATGTGCGTTGGTTTTCTTACATCGGTAAATCGCGTAAGAAAATCGGGATTAA

CATTCTTTCAATCAAAATCCGACTAATTCATCGGCTAAACGCTAAAAATCGCTTAAAACGAAAAA 2470
GTAAGAAAGTTAGTTTTAGGCTGATTAAGTAGCCGATTTGCGATTTTTAGCGAATTTTGCTTTTT

TACAAAGCAAAAAACTTCATTCCCCTTTTAGTCGTTAACCATTTAGCCAATCTAACTAGTTTAGC 2535
ATGTTTCGTTTTTTGAAGTAAGGGGAAAATCAGCAATTGGTAAATCGGTTAGATTGATCAAATCG

ATCTAAAGGCGAATCTATCTTGTGTTAGACATCCAACCTTACCAAAACCGCAGAGCGAGCTTAAG 2600
TAGATTTCCGCTTAGATAGAACACAATCTGTAGGTTGGAATGGTTTTGGCGTCTCGCTCGAATTC

AGAGATTCAAGCGGTTTTGCACGATTGTTTGCTGCCAAGAAAACCAACAAGCGAAGTAAGGCGCA 2665
TCTCTAAGTTCGCCAAAACGTGCTAACAAACGACGGTTCTTTTGGTTGTTCGCTTCATTCCGCGT

TAGACAAAAGCGCATCGCAGTTTGAAAGCGTAGGCGTCAGAAGTGGTTTGCGTTAGAATCAAACA 2730
ATCTGTTTTCGCGTAGCGTCAAACTTTCGCATCCGCAGTCTTCACCAAACGCAATCTTAGTTTGT

AGATAGCGCAAACCTGGCGTTAGGCTAAAAAACCCCTAAAAACTAAAACCCCAAAATATGTAGTGC 2796
TCTATCGCGTTTGGACCGCAATCCGATTTTTTGGGGATTTTTGATTTTGGGGTTTTATACATCACG

(

2) Plasmid pHP3 shown in single stranded form (SEQ ID NO:3):

TCTACACAATTAACAATCTTTAGCTACAATAACAGCGTGTGAAGATGCTTTCACAGCGGT 60
ATTTCCTATGTGCGGCAAAATTTGGAGCAATTAACTTGACTTGGTTGGGTTAGTGGGTTG 120

GAGGATAGAGAGGGCGACACCTCGTTAGGAGGTATCAATGTGAAAGTATTTGTCGTATTA 180
GTTCTAGTATTAGTAATTCTCGCACAATTGCTATATTAGGCTTATTTGTGGTCTAACCCC 240
TTGTTTATGGGGGTTAGATCCTTATAAGCATACTGATACGATCACACTTATTATACACCA 300
AAAGATAAGGAGTATAGAGTGGAATTTGATCAATTAGAATCACAAAGATCAGACTTACAA 360
AAAGTGTTAAAAGAATTAGATACACTCCCAAAAACCCCACAAATTGAGCTACAAAAACAA 420
GAAATACAAAACCGCATCAACAAAATAACAGACACAATCATTAAAGAATTACTATCAAAA 480
CATGAAATCAAAAAAGAAGAACTAGAACCCACTCTAACCCCAAAACCCACACCAACAAAA 540
GAGCCACAAACCACCCCCACACCATGCAAAAATTTAGTGGTTAGCACCCCTAAAGATAAA 600
ACCTATATCACCTACCACAATAACGCTAATAAGGTCAATCTAGGGAAATTGAGCGAAAGG 660
GAAGCCAATCTTTTATTCGCTATTTTTCAAAGGCTTAAAGATCAAGGGAATACCCTCATT 720
CGTTTTGAACCGCAAGATTTAAAACGCATGATCATGGTCAAATCCAACTTAACCAACAGG 780
CAATTATTGCAAGTCTTAAAAAATTTGCTTGACAACATTAGCGGTGCTAATTTTTGGATC 840
AATTAGAGAGCATGTTGAAAATGGCGAAATCTATGAAGATCACACTAGCTACATGCTTTT 900
CAAACAATTTGAAATCCGCATCCATAAGCCAACACAAACTATAGAATACTTAGATGTCCA 960
ACTCAATGATAGCTATCAATACTTGCTCAACAATCTAGGAATGGGCGGTCAATACACTTC 1020
TTTCAATCTCTTAGAATTTCAAAGGGTGAGGGGCAAATAGTGAGAGCGTTAAATTTCCCC 1080
CCCCTATTCCCCTTAAAAAGGACCCTTATCCCAGGGAATTTTTGGCCCCAATACAATTAG 1140
GGCCAAAAACCCGGTCCCTTCCATGGCTTAACCAACCCAATTGGGGGATTCCAATTTCCC 1200
CTGGATGGGAATAACCCAAGGCTTTTTTTGAAAATTCCACCTACCATTTGGTCCAAAATT 1260
GGATGGACAATTCCAAATTCCAAATCTTCTTTTCCAAGAATGGGGGCCAACCCTTGACAA 1320
ACTCCTTAAACCTTTTCATTCGGCTAAAAGGTTGAAAAACATTTGGAAGATTTGGTTTAA 1380
GGAAATATTTATCGGGTGAAAAGACCAGATGCATGGCTAACTTAAACTCCATAGAAAAAC 1440
TGAATCACGAAAGAAAGAATGCTATCAAAAATGGCATTTACCACTTGATCCAAATCAAAT 1500
TTTCTTACAACTCCAATCGCATTGAAGGAAGCGGTTTGACTTATGAACAAACCGCTCATA 1560
TTTTTGACAAATCCGTTCTCATAACTGAAAAAAACACCAATATCAAACTTGATGATATTT 1620
TTGAAACTATCAATCATTTTGAATGCGTGAATTACTTGCTTGAAAGCTATAAAGAACCTT 1680
TGAGTTTAGAATACTTTAAGAATTTACACAAAATCTTGAAAAAGAATTGTTCTGATGAAG 1740
TTATTGGTGATTTTAAAAAACGCCCTAATTTTGTAGGCAATAGCGCCACAACAAGACCCA 1800
AATTAGTTGAAAGCGAATTGACAAATCTTGTGAAAAATTATCAACGCAACCTTGAAGTGA 1860
GTTTGAAAAACAATATCATGCCTTTCATCATAGAAAACGAACACAAAGCCTTTTACTACA 1920
GGGGCATCAAAGAATATGACAACACAAAAGGCTACTTGAAAGACACCATTTTGCAAAGTC 1980
AAGACAATTTCAATGAAATGGTTAGCTATTTCTTTTCTTGAGTGAAACCGCTTATTTTTG 2040
CTTGTGTGCTTTTGTTTTTTGCGTTTTTAGTTGTAGGTGGTAAGAAATATCGGTTTTTTG 2100
CTTTTCGTTGGTTGTAGGCGATTTTAGATAGCAAAAAACAGCTAAAAAATCCAAGCAACC 2160
TAATTGATTTCAAACCAACTTCATTTCCCTTTTAGTCGTTAGCCATTTAGCCAATCTAAC 2220
TAGTTTAGCATCTAAAAGCGCATATAACTTGAGTTAGCAATCCAACCAATACTAAAACCG 2280
CCTAGCGAAGCGTTAGCGAGCAAAATAAGCGGTTTTAGACCGATTGTTTGCTGACAAGCA 2340
AACACCAATAAGCGAGCGTTAGCGAGCATGGACAAAAGCGCATCGCAGTTTGAAAGCGTA 2400
GGCGTTAGCCGAAGCTGTTTTGCGTAAGCAAATCAAACAAGATAGCGCAAGCCGAGGTGC 2460
AGCCCAAGAATTTGAATTAATCCATGCGGTGTTTAGGGCGTTTTAGCGTGATCGCTTTAT 2520
TACATGTTTTAAACAGCATGCTGTTTTTTACATGTTTTACTCGCATGCGCGCGCGCTAGG 2580
TATTGGTGGTTGGAATAGCCTAAATAACGCAGCTGTATGGTTTCTCATTTTTCGGTGACA 2640
ATGAATAAGGGGTAGTTCTTGCGAGTCATAAGTGTAGTTCTTGCGAGTCATAAGTGTAGT 2700
TCTTGCGAGTCATAAGTGTAGTTCTTGCGAGTCATAAGTGTAGTTCTCTTCACAATATCT 2760
ACACAATTCACAATCTCTAGCTACAATAACAGCGTGTGAAGATGCTTTCACAGCGGTATT 2820
TCCTATGTGCGGCAAAATTTGGAGCAATTAGCTTTAAAAGCTAGTGGGTTGGGAGTTTGT 2880
AGCGGGTATGCACTCCGTTAGGAGGCACACCATGAAAGCATTTTTGATAGTAGTGATTTT 2940
AGTGGTAATCTTGACACAGCCACTATATTAAAACCTTAGCGTTTTAATAACCCTTATAAG 3000
TCCGCCAAGACTTCTTAAGGGTTTCACTCCTGTTATTATATCGTCTTTTGAAAAATAAGC 3060
ATTAAAAGGCGCTTAAATGCCCATGAATACGAATTTTGAACAGCTTAGAAAACAAGAATT 3120
GGAATTACGAAAATTATTAGAAGAATTAGAAACGCTCCCACAAACCCCACAAATTAAACT 3180
GCAAAAACAAAAAATACAAACTTACATAGACAAGATAACACCAAGTATTTTGAGCGGTTT 3240
TGATCAAAAATTCAAAGAAATTATAGAAAATCTATCAAATGAATTTGAAAAAGAAAAATC 3300
CACACCACTCAAAGAGCCACAAACCACCCCCACACCATGCAAAGATTTAGTGGTTAGCAC 3360
CCCTAAAGATAACACCTATACCACCTACCACAATAACGCTAATAAGGTCAATCTAGGGAA 3420
ATTGAGCGAAAGGGAAGCCAATCT 3444

An additional nucleotide sequence that was cloned is provided at SEQ ID NO: 4, which includes a 135 bp segment

that encodes a peptide of 45 amino acids (SEQ ID NO: 5). This smaller 45 amino acid peptide is an immunogenic polypeptide of the Hepatitis C virus (HCV) core antigen. The nucleic acid sequence encoding the 45 amino acid peptide is shown below with the indicated 135 nucleotides underscored. (SEQ ID NO: 5)

CATGAGCACG AATCCTAAAC CTCAAAGAAA AACCAAACGT AACACCAACC GTCGCCCACA
GGACGTCAAG TTCCCGGGTG GCGGTCAGAT CGTTGGTGGA GTTTACTTGT TGCCGCGCAG
GGGCCCTAGA TTGGGTGTGC GCGCGACGAG GAAGACTTCC GAGCGGTCGC AACCTCGAGG
TAGACGTCAG CCTATCCCCA AGGCACGTCG GCCCGAGGGC AGGACCTGGG CTCAGCCCGG
GTACCCTTGG CCCCTCTATG GCAATGAGGG TTGCGGGTGG GCGGGATGGC TCCTGTCTCC
CCGTGGCTCT CGGCCTAGCT GGGGCCCCAC AGACCCCCGG CGTAGGTCGC GCAATTTGGG
TAAGGTCATC GATACCCTTA CGTGCGGCTT CGCCGACCTC ATGGGGTACA TACCGCTCGT
CGGCGCCCCT CTTGGAGGCG CTGCCAGGGC CCTGGCGCAT GGCGTCCGGG TTCTGGAAGA
CGGCGTGAAC TATGCAACAG GGAACCTTCC TGGTTGCTCT TTCTCTATCT TCCTTCTGGC
CCTGCTCTCT TGCCTGACTG TGCCCGCTTC AGCCTACCAA
SEQ ID NO:4

AATCCTAAAC CTCAAAGAAA AACCAAACGT AACACCAACC GTCGCCCACA GGACGTCAAG
TTCCCGGGTG GCGGTCAGAT CGTTGGTGGA GTTTACTTGT TGCCGCGCAG GGGCCCTAGA
TTGGGTGTGC GCGCG
SEQ ID NO:5

The nucleic acid of SEQ ID NO: 4 was cloned into the *hop*E gene (SEQ ID NO: 6, shown below), of *H. pylori* 26695 at nt504 of SEQ ID NO: 4 (noted in bold/underscore; corresponding to amino acid residue 168 of the protein product) so that the expressed product would be located as part of the surface exposed loop of the HopE gene product. This construct, designated as vector pTMI03-8 (Figure 6) was expressed on the surface of *E. coli*.

ATGCCATAGC ATTTTTATCC ATAAGATTAG CGGATCCTAC CTGACGCTTT
TTATCGCAAC TCTCTACTGT TTCTCCATAC CCGTTTTTTG GGCTAACAGG
AGGAATTAAC C

1 ATGGAATTTA TGAAAAAGTT TGTAGCTTTA GGGCTTCTAT CCGCAGTTTT
51 AAGCTCTTCG TTGTTAGCCG AAGGTGATGG TGTTTATATA GGGACTAATT
101 ATCAGCTTGG ACAAGCCCGT TTGAATAGTA ATATTTATAA TACAGGGGAT
151 TGCACAGGGA GTGTTGTAGG TTGCCCCCCA GGTCTTACCG CTAATAAGCA
201 TAATCCAGGA GGCACCAATA TCAATTGGCA TGCTAAATAC GCTAATGGGG
251 CTTTGAATGG TCTTGGGTTG AATGTGGGTT ATAAGAAGTT CTTCCAGTTC
301 AAGTCTTTTG ATATGACAAG CAAGTGGTTT GGTTTTAGAG TGTATGGGCT
351 TTTTGATTAT GGGCATGCCA CTTTAGGCAA GCAAGTTTAT GCACCTAATA
401 AAATCCAGTT GGATATGGTC TCTTGGGGTG TGGGGAGCGA TTTGTTAGCT
451 GATATTATTG ATAACGATAA CGCTTCTTTT GGTATTTTTG GTGGGGTCGC
501 TATCGGCGGT AACACTTGGA AAAGCTCAGC GGCAAACTAT TGGAAAGAGC
551 AAATCATTGA AGCTAAGGGT CCTGATGTTT GTACCCCTAC TTATTGTAAC
601 CCTAACGCTC CTTATAGCAC CAAAACTTCA ACCGTCGCTT TTCAGGTATG
651 GTTGAATTTT GGGGTGAGAG CCAATATTTA CAAGCATAAT GGCGTAGAGT
701 TTGGCGTGAG AGTGCCGCTA CTCATCAACA AGTTTTTGAG TGCGGGTCCT
751 AACGCTACTA ATCTTTATTA CCATTTGAAA CGGGATTATT CGCTTTATTT
801 AGGGTATAAC TACACTTTTT

CTCGAGATCT GCAGCTGGTA CGATATGGGA ATTCGAAGCT TTCTAGAACA

AAAACTCATC TCAGAAGAGG ATCTGAATAG CGCCGTCGAC CATCATCATC
ATCATTGAGT TTAACGGTCT CCAGCTTGGC TGTTTTGGCG GATGAGAGAA
GATTTTCAGC CTGATACAGA TTAAATC **(SEQ ID NO:6)**

Briefly, one method of accomplishing the isolation of *hopE* gene is amplification from *H. pylori* 22695 by using *Taq* DNA polymerase. The upstream primer 5'AAGGATCCGATAGGAATGTAAAGGAATGG-3' (SEQ ID NO:7) containing a *Bam*HI site and the downstream primer 5'CCGAATTCTAAAGGCATGAACGCTTGCA-3' (SEQ ID NO:8) containing a *Eco*RI site can be constructed by using a DNA synthesizer, such as the Perkin-Elmer Applied Biosystems, Inc. model 332 (ABI; Mississauga, Ontario, Canada). The resulting PCR fragment can be blunt-end cloned into the *Eco*RV site in pBluescript II KS(+) in the same orientation as the *lac* promoter.

PCR primers can then be designed to insert two unique restriction enzyme sites into the *hopE* gene for insertion of the 135bp immunogenic coding sequence from the Hepatitis C virus (HCV) core antigen. The PCR amplification using *Taq* DNA polymerase can be performed using a touchdown amplification procedure as follows. The PCR thermocycler is programmed for an initial denaturation step of 96°C for 4 min, followed by 18 cycles at an initial annealing temperature of 65°C (for 90 s), which is decreased by 0.5°C for each successive cycle, an extension step at 72°C for 6 min, and denaturation at 96°C for 1 min. Subsequent to completion of the first 18 cycles, an additional 14 amplification cycles can be performed by using 72°C extension and 96°C denaturation steps with a constant 55°C annealing temperature. The resulting amplicon is then purified by column, precipitated with ethanol, and made blunt by digestion with the Klenow fragment of DNA polymerase. The PCR products can be digested with restriction enzyme to remove the template DNA, and

religated into an appropriate vector such as pTM103.8 under the control of the arabinose inducible promoter, and transformed into *E. coli* JM105.

Recombinant clones can be identified by using oligonucleotide primer 5'-AGATCTAAGGACGTC-3' (SEQ ID NO:9) plus the reverse sequencing primer in PCR amplification reactions. Identified clones can be sequenced to verify that the inserted restriction endonuclease sites are in frame and that no errors had been introduced into the *hopE* gene. The 135bp immunogenic coding sequence from the Hepatitis C virus (HCV) core antigen can then be inserted using standard techniques.

Once vector pTMI03-8 had been created it was transformed into *E. coli*, which was grown at 37°C. Cells were then harvested and the expression of the HCV insert was confirmed by Western Blot.

Briefly, a procedure for this is as follows. Cells are harvested from about 20 plates and resuspended in 20% sucrose with 50mg of DNase I (Boehringer Mannheim) in 10mM Tris-HCl (pH8.0). The cells are then disrupted with a French pressure cell at 15,000 lb/in^2. Broken cells are overlaid on a sucrose step gradient of 1ml of 70% and 6ml of 70% sucrose in 10mM Tris-HCl (pH8.0). The outer membrane fraction is collected and pelleted at 150,000 × *g*, and the pellet is resuspended in 100µl of distilled water.

Alternatively, outer membranes from 500 ml of log-phase culture can be solubilized in 10mM Tris-HCl (pH 8.0)-3% *n*-octyl-polyoxyethylene incubated at 23°C for 1h and centrifuged for 30min at 173,000 × *g*. The pellet is resuspended in 10mM Tris-HCl-3% *n*-octyl-polyoxyethylene-5mM EDTA (pH8.0), incubated at 23°C for 1h, and centrifuged for 30min at 173,000 × *g*, and the supernatant collected. A Western immunoblot indicated the presence of HCV/*hop*E in

the supernatant of the second solubilization step. The supernatant containing HCV/*hopE* is mixed with an equal volume of 0.125 M Tris-HCl (pH 6.8), 4% (wt/vol) sodium dodecyl sulfate (SDS), and 20% (vol/vol) glycerol and subjected to SDS-12% polyacrylamide gel electrophoresis (PAGE). If required the HCV/*hopE* band can be excised from an unstained portion of the gel and eluted overnight at 4°C into 10mM Tris-HCl (pH8.0), 1mM EDTA (pH8.0), and 100mM NaCl. The elution supernatant can then be run on an SDS-PAGE gel to check for purity, and a Western immunoblot using standard techniques undertaken. For example, isolated outer membranes can be loaded at a concentration of 15µg/lane. Electrophoresis is then carried out by SDS-PAGE on a discontinuous 12% polyacrylamide gel. Proteins are then stained with Coomassie brilliant blue. For Western immunoblotting, unstained gels can be electroblotted onto Immobilon-P membranes (Millipore, Bedford, Mass.). After blocking for 2h at 23°C with 3% bovine serum albumin (BSA; Boehringer Mannheim)-0.1% Tween 20 (Sigma) in phosphate-buffered saline (PBS), the membranes are then incubated with a 1/10,000 dilution of anti-HCV rabbit antiserum in 1% BSA-0.05% Tween 20 in PBS for 1 h at 37°C. The membranes are then washed with PBS and incubated with a 1/5,000 dilution of an alkaline phosphatase-conjugated secondary antibody (Bio-Rad, Richmond, Calif.) for 1h at 37°C. The bound antibodies are detected with 5-bromo-4-chloro-3-indolylphosphate (BCIP, Calbiochem, La Jolla, Calif.) and nitroblue tetrazolium (NBT, Sigma).

EXAMPLE 2 EXPRESSION VECTORS AND SELECTION OF ANTIGENS FOR STABLE EXPRESSION

Because *HopE* is a native protein of *H. pylori* and is tolerated by this organism and can thus form a construct useful for expressing foreign antigens or other heterologous gene products in *H. pylori*. Other *H. pylori/E. coli* shuttle vectors that can be readily

developed as described above include, for example, vectors comprising two plasmid *ori* sites and markers which are suitable for each host. Markers might include genes for chloramphenicol/kanamycin resistance as well as promoters that can be recognised by both the *E. coli* and *H. pylori* transcriptional systems.

The requirements for replication in *E. coli* can be achieved by using any number of known *E. coli* plasmids (e.g. pBR322).

Constructed shuttle vectors can be tested for replication in both *E. coli* and *H. pylori in vitro* and compared to existing shuttle plasmids described in the literature.

The choice of antigen or other heterologous gene product to be expressed would ideally be one that is not toxic to *H. pylori* and *E. coli* and that is highly immunogenic (or possesses another desirable property) when delivered to a selected site in a mammal. In the case of an expressed antigen for immunization of the mammal, such a site might be a mucosal site.

As described in Example 1, *hopE*/HCV core antigen fusion protein can be expressed at the *E. coli* surface. The product of pTMI03-8 (Figure 3) is preferably targeted to the *H. pylori* outer membrane, so that it would display the HCV core antigen in a mucosal environment.

Tetanus toxoid (TT) has been studied extensively as an antigen in humans, and immune responses to it are well characterized. TT elicits good mucosal immune responses when administered orally or intranasally when displayed on the surface of bacterial spores (Duc & Cutting, 2003, *Expert Opinion Biol Ther*, 3(8), 1263-70). The tetanus toxin C fragment can be fused to the *hopE* gene product as described above or engineered to contain a membrane anchor

and cell surface target sequence. The advantage of this system is the existence of well-characterized murine models for assessment of the effectiveness of the vaccination procedure, whereas; in the case of HCV where the known murine models typically rely on immune-deficient mice. The gB protein of cytomegalovirus (CMV) has been shown to immunize mice against a lethal dose of an engineered *Vaccinia* virus expressing the gB antigen. Accordingly, shuttle vectors such as those described herein, comprising gB antigen in place of an HCV antigen can be constructed using the protocol described in Example 1.

A number of promoters can be used in the shuttle vectors. Ideally the promoter used is inducible either by a natural *in vivo* colonization mechanism of *Helicobacter* or by induction with an innocuous foodstuff or chemical that can be consumed. For example, the promoter from the *H. pylori* histidine kinase HP165 that is reported to be induced by acidic pH and may be a virulence factor related to gastric mucosa colonization is one such promoter. The benefit of this promoter is that a construct can be made *in vitro* with the foreign antigen only becoming expressed when exposed to the acidic environment of the gastric mucosa.

Other promoters include the arabinose inducible promoter used in pTMI03.8 and FlaB sigma 54 promoter (*Josenhans et al.*, 1998, *FEMS Microbial Lett.*, 161(2), 263-73), the extensively studied T7 promoter used in many constitutive and inducible *E. coli* systems and the *nir B* promoter of *Salmonella* which is induced in anaerobic environments (Chatfield *et al.*, 1992, *Biotechnology*, 10(8): 888-92). The ability of any of these promoters to function in *H. pylori* can be tested using the system developed by Angelini *et al.* (2004) (*Plasmid*, 51:101-107) that uses CAT and GFP reporters as a readout of promoter activity in a *H. pylori* plasmid vector.

The target sites of expression will depend on the antigen or other gene product used. Initial studies focused on the *Hop*E proteins and fusion polypeptides which target the expressed polypeptide (e.g., antigen) to the cell surface of *H. pylori*.

Plasmid stability is also very important and, while the use of antibiotic resistance genes as a selective determinant for plasmid maintenance is useful *in vitro*, is less practical *in vivo*. An alternative is a balanced-lethal system, for example, the *asd* gene that is used inactivated in *Salmonella*. The *asd* gene, which exists natively in *H. pylori*, encodes aspartate-β-semialdehyde dehydrogenase (an enzyme in biosynthetic pathway for diaminopimelic acid (DAP), an essential component of the cell wall peptidoglycan of gram-negative bacteria). In the absence of DAP, *asd* mutants undergo lysis. Since DAP is not present in mammalian tissues, this balanced-lethal system imposes a requirement for all living *H. pylori* to carry the recombinant *asd* gene-containing plasmid.

In order to use the *asd* gene system the genomic copy of the *asd* gene is inactivated using standard gene knockout protocols. This strain of *H. pylori* will then only grow with the supply of DAP or with a plasmid that contains the *asd* gene.

Other systems that can be used for similar purposes include *E. coli* enterotoxin or Cholera toxin (CT) as mucosal adjuvants. Adjuvants can also be used to boost the mucosal immune response. Two such adjuvants are CT and *E. coli* enterotoxin (LT) wherein expressed antigens are fused to the LTB and CTB mutants that maintain their strong mucosal adjuvant properties but have dramatically reduced toxicity.

EXAMPLE 3 VIRULENCE, LD_{50}

As described in Examples 1 and 2, *Helicobacter*-based vectors such as pHP3 and pHP1 are capable of providing protection against infection in a mammal, such as a mouse or human. In the present example, a murine model is used to demonstrate the utility of using the *Helicobacter*-based systems to provide delivery of a pharmacologically active molecule of interest to a mammal, including a human. The murine model is employed to demonstrate the activity of a transgenic strain of *H. pylori* to elicit a serological response to an expressed surface antigen *in vivo*.

Mice are infected with wild-type *H. pylori*, while other mice are inoculated by gavage with temperature-sensitive *H. pylori* as described in Example 2. Sera from both control and test animals are assayed for antibody and gastric histology are performed on sacrificed animals in accordance with the schedule shown in Table 1. A mouse urea breath test can also be used.

As shown in Table 2, a 50% decrease in virulence (from 75% to 40%) was observed. Specific antibody titre increased 4 fold above baseline, indicating a serological response. Serum samples were taken at baseline, 12, 24, 48 weeks. At these times 10, 10 and 20 animals were sacrificed and gastric histology performed.

TABLE 1

IN VIVO STUDY

Week	Mice* Remaining	Mice Used	Serology	Histology	Adjistment
0	50	0	40	0	0
12	50	10	40	10	109.2
24	40	10	30	10	218.4
48	30	30	20	20	1310.4
49	0				
Totals		50	130	40	

*C57BL/6J females, 40 test and 10 control

TABLE 2

POWER CALCULATION VIRULENCE STUDY

At α=0.05 and Power=80% and various Infection Rates of *H. pylori*
Normal=75% vs TSHP=50% n=58 mice in each group Normal=75% vs TSHP=40% n=30 mice in each group Sample size of 20 will only give you 37% Power: to detect a difference (75% vs 50%) and 62% Power: to detect a difference (75% vs 40%). In order to use a sample size of 20 you would need the infection rate in TSHP to be at least 32% or less (with 80% Power and α=0.05)

EXAMPLE 4 COMPARISON OF VIRULENCE AND ANTIGENICITY OF SEVERAL TEMPERATURE SENSITIVE *H. pylori* STRAINS

In order detect a major change in virulence related to expression/modification of an outer membrane protein, mice are inoculated with temperature-sensitive *H. pylori* as described in Example 3. An equal sized control group of mice were infected with a wild type *H. pylori* strain. Non-invasive means were used to determine presence or absence of *H. pylori*. Mice were bled at 3 and 6 months for antibody estimation. At sacrifice, histology was performed to assay gastritis and confirm colonization. Table 3 shows the serology and histology of the mice used.

TABLE 3

VIRULENCE AND SEROLOGY COMPARISON STUDY

Week	Mice Remaining	Mice Used	Serology	Histology	Adjistment
0	180		180		
24	180		180		
48	180	180	180	180	7862.4
49	0				
Totals		180	540	180	

EXAMPLE 5 LD_{50} STUDY TO EVALUATE TEMPERATURE SENSITIVE *H. pylori* VACCINE EFFICACY FOR A *PNEUMOCOCCAL* ANTIGEN

In order to demonstrate the *Helicobacter*-based vaccine protection effect from a standard pathogen (pneumococcus), mice were inoculated with temperature sensitive *H. pylori* by gavage. An equal sized control group was infected with the wild type *H. pylori* strain. Non-invasive means are used to determine presence or absence of *H. pylori* as described in Example 4. At 6 months post infection, all mice were given intraperitoneal challenge with 10 times the LD_{50} of live virulent pneumococci type 4 (~20 CFU/mouse), as per the method of Aaberge *et al.* (1995, *Microb. Pathog.* 18:141-152).

As shown in Table 4, allowing for 75% lethality in the controls, the study has a power of 0.8 to detect a 50% decrease in mortality (75% vs 50%).

TABLE 4

LD_{50} TO PNEUMOCOCCUS FOR IMMUNIZED MICE

Week	Mice Remaining	Mice Used	Serology	Histology	Adjistment
0	60	0	0		
24	60		0		
25	60	60	0	0	1365
26	0				
Totals		60	0	0	

EXAMPLE 6 DETERMINATION OF *H. pylori* STATUS OF MICE: BREATH TEST METHOD

In the present Example, the urea breath test used in humans was adapted use in mice.

Ten mice were fed a diet devoid of urease (uncooked soy). Mice were then administered 3.7 kBq $^{[14]}C$ urea in 200µl flavoured citrate by gavage and placed in air-filled 2L plastic ziplock bags for 20 minutes. Mice were then removed without exchanging the air within the bag. Hyamine, 0.1 mmol in ethanol, was then introduced and scintillant was added to the hyamine solution and counted for 10 min or up to a count of 1,000 dpm.

EXAMPLE 7 HUMAN STUDIES

To confirm virulence and antibody response in humans, a strain of *H. pylori* like the "Baylor Strain" will be employed and the following criteria will be adopted:

1. The infected individuals have no symptoms, no more than mild histologic damage, and no evidence of infection with hepatitis viruses or HIV.

2. The isolate is a single strain, *cagA* negative, and sensitive to metronidazole, clarithromycin, tetracycline, and amoxicillin.

3. Volunteers to receive a challenge are healthy with normal gastric histology, no history of peptic ulcer, no young children at home, no regular contact with young children, and no allergies to the antibiotics that might be required to treat the infection.

Challenge will consist of 40mg famotidine at bedtime followed by administration of *H. pylori* in beef broth

orally in the morning. Subjects are contacted daily for 14 days. A 13^C-UBT is performed after 7 and 14 days and endoscopy with quantitative culture and histology after 2 weeks and 3 months. Antibiotics are used to eradicate the infection.

EXAMPLE 8 DEVELOPMENT OF EXTERNAL CHEMICAL MARKER FOR THE DETECTION OF WILD TYPE AND/OR TSHP *IN VIVO*

An example of a chemical marker that may be used for the detection of wild type or TSHP *in vivo* is sulfasalazine (SSN), the structure of which is shown in Figure 4. Studies in germ free mice and conventional rats have shown that intestinal bacteria are solely responsible for the diazo-bond reduction, resulting in the reductive catabolism of SSN and the release of sulfapyridine and 5-aminosalicylate. The enzyme(s) which catalyses this reaction is referred to as diazoreductase(s) (synonym azoreductase(s)). Conventional rats given SSN excrete 5-aminosalicylate and sulfapyridine (and their respective conjugates) in urine and faeces, whereas germ-free rats show no evidence of SSN degradation.

Several bacterial species have been shown to have diazoreductases (AZR's). Preliminary bioinformatic studies have indicated that *H. pylori* may not contain the AZR gene. The presence of similar analogous sequences has also produced a negative result. Under these circumstances a transgenic strain of *H. pylori* (TSHP) which has a viable and functional azoreductase (azr + TSHP) can be used to assess the use of these markers.

Plasmid pTMI03-02 is digested by *Eco*R I and *Hind*III, and ligated with the Azoreductase (AZR) gene from *Bacillus subtilis* treated with *Eco*R I and *Hind*III, to generate a vector containing both *HopE* 168aa and AZR named pTMI03-azr.

This plasmid is transformed into *E. coli* to assess whether expression of *Hop*E and the *B. subtilis* AZR occurs. pTMI02 when similarly treated with full-length hepatitis C core antigen (HCCA) demonstrated transport of *Hop*E::HCCA to *E. coli* outer membrane employing western blots and anti-*Hop*E Abs.

Mice (n =30) are infected with the azr + TSHP by gavage and once AZR expression *in vivo* to produce 5-aminosalicylate and sulfapyridine (and their respective conjugates) in urine and faeces is established human trials can begin.

EXAMPLE 9 USE OF DIAGNEX BLUE AS A MARKER

The diagnostic agent "Diagnex Blue" consists of an ion exchange resin (Amberlite XE-96) conjugated with a dye (Azure-A). This test relies on the fact that the resin-dye combination disassociates at pH less than 2.5 after which the dye is absorbed and appears in the urine. Persons without dye in the urine are achlorhydric. This principle is shown in Figure 5.

The same principle can be used to test for *H. pylori*. For example, a dye-resin combination which disassociates at pH > 7.0 could detect urease if the resin was given with urea. This would produce a pH > 7.0 in the mucus layer where *H. pylori* resides thus releasing the dye.

Mice (n =30) are inoculated with a wild type *H. pylori* strain while germ-free mice (n =30) are used as controls (Pilot study). After an optimal period allowing for the *H. pylori* to establish an active infection, the test group and the controls are introduced with a predetermined quantity of the resin-dye complex by gavage. This will be followed by a urea solution. (Range 0.01M to 0.5M). The mice are kept in metabolic cages and the excretion of the azure dye are monitored and quantified. Different ratios of the

resin and urea concentrations are tested to verify the optimal combinations to be used.

EXAMPLE 10 DELIVERY FORMULATIONS

For administration by aerosol, the present invention can be delivered in the form of aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant. In the case of a pressurized aerosol, the dosage unit can be determined by providing a valve to deliver a metered amount. The formulation would be prepared as a powder for administration by inhalation. Administration by inhalation can also be carried out by atomizing solutions or suspensions which contain the compositions according to the invention.

The compositions according to the invention may also be formulated in a liquid for oral digestion for administration to a subject as an intravenous preparation.

All of the various preparations of the invention may be prepared by procedures familiar to those skilled in the art, if appropriate using further suitable pharmaceutical auxiliaries. Compositions according to the invention advantageously contain the species of *Helicobacter*, alone or in combination with other desired ingredients.

Any of the above individual or combination of *Helicobacter* formulations may be included in a pharmaceutical composition comprising the pharmaceutically acceptable composition according to the present invention. The pharmaceutical compositions as described herein may be in solid (e.g. powder, particles, granules, sachets, tablets, capsules etc.), semi-solid (gels, pastes etc.) or liquid (solutions, dispersions, suspensions, emulsions, mixtures etc) form and adapted for administration via e.g. the gastrointestinal tract and gastric mucosa. The

pharmaceutical compositions may thus be in powder or particulate form adapted to be dispersed in an aqueous medium before use.

A pharmaceutical composition in liquid form may be in the form of a dispersion comprising the *Helicobacter* composition and an electrolyte solution such as, e.g. a composition that is adapted to physiological conditions e.g. a physiologically acceptable solution.

A pharmaceutical composition according to the invention may further comprise another therapeutically, prophylactically and/or diagnostically active substance.

In another aspect, the invention relates to a pharmaceutical kit comprising a first and a second container, the first container comprising a recombinant *Helicobacter* composition comprising the plasmid and/or plasmid vector according to the invention and the second container comprising a dispersion medium for the *Helicobacter* composition, accompanied by instructions for administering and/or dosing the *Helicobacter* composition in the dispersion medium before use.

The *Helicobacter* composition according to the present invention contained in the kit may be in powder or particulate form.

A pharmaceutical kit according to the present invention may include instructions with recommendations for the time period during which the *Helicobacter* composition should be administered after dispersion in the dispersion medium.

EXAMPLE 11 IMMUNE MODULATION WITH *Helicobacter* - VACCINE PREPARATION

The TH1 response (T-helper cell type 1) is a cell mediated response. Over activity of this is a presumed cause of diseases such as rheumatoid arthritis (RA) and lupus. In contrast, TH-2 is an antibody type serological response characteristic of vaccines. The present example provides a technique to obtain a TH-2 type response in an animal when treated with a *Helicobacter*-based vaccine treatment preparation according to the present invention.

Use of the *Helicobacter* vectors and vector plasmid systems as described herein may be used to invoke antibody response in an animal. By way of example, a system employing a gene expression cassette in a construct that provided for the transformation of the bacterium, *Clostridium*, and the subsequent secretion of a protein (S-layer protein) from the surface of the transformed *Clostridium*, this resulting in initiation of mucosal vaccination, is described in WO0194599, which disclosure is hereby incorporated herein in its entirety. These constructs may also include a secretory leader sequence selected from ORF1, ORF3, ORF5-7., ORF7 or ORF11.

In accordance with some embodiments of the vaccine, the *Helicobacter*-based vectors and vector plasmids may comprise a sequence encoding a bacterial surface layer protein. A surface layer protein is defined herein as any molecule of proteinaceous nature, including e.g., protein, glycol-protein or lipoprotein occurring in the outer membrane of a bacterium and capable of being exposed on the surface of the bacterium. S-layer proteins may be continuously and spontaneously produced in larger amounts than any other class of protein in the cell.

A process for preparation of a recombinant cell preparation comprising a gram negative host cell, *Clostridium*, having the S-layer protein, is also provided in WO-97/28263. The process may be modified and followed in accord with the procedures described herein to incorporate an S-protein as part of the *Helicobacter* constructs of the present invention.

Accordingly, in some of the vector and vector plasmid constructs, a fusion protein is provided that comprises a Helicobacter sequence and a non-*Helicobacter* pharmacologically active molecule of interest. In order to enhance the immunogenicity of a vaccine employing the *Helicobacter* constructs of the present invention, the *Helicobacter* sequence of the fusion protein may comprise a sequence encoding an S-layer protein. Bacillus constructs that include the S-layer protein as part of a fusion protein have been reported to express the S-layer protein at the *Bacillus* surface. (See WO-95/19371, describing *Bacillus sphaericus*), thus enhancing the immunogenicity of the preparation.

Mucosal immunization is already provided against some diseases, including an oral polio vaccine and an oral (drinkable) vaccine against cholera and diarrhea due to *E. coli* (an inactivated vaccine). In some embodiments, it is contemplated that the vaccines of the invention may thus comprise an inactivated vaccine.

The present invention contemplates a live vaccine, as such will provide a single-dose, long lasting vaccination, because the carrier organism, *Helicobacter*, will continue to produce the antigen, i.e., non-*Helicobacter* pharmacologically active molecule of interest, and boost immunity *in vivo*. In addition, the vaccines will be administered in combination with an adjuvant. These adjuvants' comprise molecules such as aluminium hydroxide

or lipid vesicles that increase the exposure time for the vaccine by slowing its removal forte site of administration. Adjuvants' also act by evoking production of immunomodulatory peptides called cytokines and chemokines (Brewer *et al.* 1997, *J. Cytokines Cell Mol. Ther.*, 4:223-246). Thus, the present vaccines may comprise cytokine adjuvant to enhance immune response.

The transformed *Helicobacter* or *E. coli* bacterium, when administered orally to a mammal such as a human or animal, will provide for the gastro intestinal colonization, production and presentation of the desired polypeptide through the gastric wall, which is the natural site of colonization. The gastro intestinal tract is surrounded by an immense immune apparatus specialized in mounting immune response of various types. Gastro intestinal colonization by recombinant *Helicobacter* vaccine or peptide producer strain thus enables a much longer immune stimulus than traditional vaccination. Additionally, antigen can be presented preferentially to the gut wall or the lumen.

EXAMPLE 12 *Helicobacter* AND USES THEREOF AS AN APPETITE SUPPRESSANT

The present example is provided to demonstrate the utility of the present invention as a method for employing Helicobacter in preparations and treatment regimens that provide for appetite suppression. In particular, delivery to the gut mucosa of a construct that comprises attenuated *Helicobacter* together with a non-*Helicobacter* pharmacologically active molecule of interest that regulates the level of ghrelin (a hormone) or an agonist of ghrelin, is expected to provide an effective means for providing suppression of the gut-brain axis that regulates appetite and sanity.

Studies have suggested that ghrelin is an appetite stimulant, i.e., ghrelin increases food intake in mice

(Asakawa *et al.* 2003, *Gut*, 52(7):947-52). Ghrelin has also been reported to reduce fat utilization in adipose tissue in rodents (Tschop *et al.*, 2000, *Nature*, 407: 908-13), as well as to be involved in rat adipogenesis (Choi *et al.* (2003), *Endocrinology*, 144 (3):751-9). Ghrelin has also been reported to be a hunger signal, prompting the subject to eat when nutrition availability is low.

Ghrelin, an endogenous ligand for the growth hormone secretagogue receptor (GHS-R), stimulates growth hormone (GH) release from cultured pituitary cells in a dose-dependent manner, and is produced and secreted from the A-like cells found mainly in the oxyntic glands of the gastric fundus. Ghrelin is now known to play a role in not only GH release, but also in controlling the appetite and body weight.

Both parenterally and intracerebro-ventricularly administered ghrelin have been shown to stimulate food intake and increase the body weight of mice and rats with free access to food, even those animals with GH deficiency. The control of appetite and body weight may be independent of GH release.

Ghrelin, a 28-amino-acid peptide, is activated when its third serine residue is acylated by n-octanoic acid, and GHS-R is responsive to the first four or five residues including the octanylated serine residue of the whole ghrelin peptide. GHS-R has been shown to be present in the pituitary, hypothalamus, adrenal glands, thyroid, pancreas, myocardium, spleen and testes. Ghrelin stimulates the expression of both NPY and AGRP mRNA in the hypothalamus. The central orexigenic effect of ghrelin is mediated by the NPY/AGRP-expressing neurons in the hypothalamus. Ghrelin has also been reported to suppress vagal afferent activity. The peripheral orexigenic effect of ghrelin may be mediated, at least in part, by its suppressive effect on

the vagal afferent activity. IL-1β is a pro-inflammatory cytokine that mediates the cachectic process by stimulating the expression and release of leptin, and/or by mimicking the effect on the hypothalamus of excessive negative-feedback signalling from leptin.

It is proposed that antagonists to ghrelin if provided to the animal at the gut mucosa will reduce food intake by an animal and reduce body weight gain.

EXAMPLE 13 CELL WASTING ATTENDANT CANCER AND AIDS

The present example demonstrates the utility of the present invention for use as a preparation that will prevent or inhibit cell wasting, particularly cell wasting associated with the diseased states of AIDS and cancer.

Cachexia is a condition characterized by wasting, emaciation, feebleness and inanition. It was recently reported that the levels of both ghrelin peptide and ghrelin mRNA in the stomach were up-regulated in a mouse model of cancer cachexia. In cachectic mice with increased plasma levels of IL-1β, the plasma concentrations of ghrelin also increased with the progression of cachexia. This result suggests that a close relationship might exist between the ghrelin dynamics and the cachectic process mediated by IL-1. IL-1β is an anorexigenic substance, just like CCK, leptin, gastrin-related protein and bombesin, and antagonizes the actions of ghrelin.

Asakawa *et al.* reported that parenterally administered IL-1β decreased NPY mRNA expression in the hypothalamus and pre-proghrelin mRNA expression in the stomach, and that intraperitoneally administered ghrelin inhibited the severity of IL-1β-induced anorexia. *H. pylori* infection is known to be a major pathogenetic factor in the development of gastritis, peptic ulcer disease and gastric malignancy.

Attachment of *H. pylori* to the gastric mucosa induces inflammation, which is associated with the release of various cytokines, including IL-1β.

It has been observed clinically that *H. pylori* eradication is often followed by improvement of some nutritional parameters, such as the body weight and the serum levels of total cholesterol, total protein and albumin. *H. pylori* infection has been reported to be capable of modifying the plasma and gastric ghrelin dynamics in Mongolian gerbils. In humans, however, *H. pylori* infection has been reported not to be associated with any changes of the plasma ghrelin levels, although eradication of *H pylori* has been shown by some to be associated with increases of the plasma ghrelin levels.

It is proposed that *H. pylori* may be used as a carrier to provide amylin to a patient in need thereof, by, for example, acting to provide secretion of ghrelin to the gastric mucosa. In some embodiments, the *Helicobacter* vector will be constructed that include a sequence encoding amylin, amylin analogs, and/or derivatives thereof, and amylin agonists (including calcitonins, calcitonin gene-related peptides, and analogs therefore), so as to decrease ghrelin levels.

Amylin antagonists can increase ghrelin levels. Modulation of the effective levels of amylin, with amylin, amylin agonists, or other like compounds that decrease the effective level of amylin, may inhibit, or stimulate in the case of antagonists and antibodies, ghrelin secretion. Hence, some embodiments of the method are directed to modulating endogenous levels of ghrelin by increasing the effective level of amylin or amylin agonists in the body, by direct or indirect means, or by decreasing the effective level of amylin using amylin antagonists or inhibiting amylin production.

EXAMPLE 14 TREATMENT OF GAUCHERS DISEASE

The present example demonstrates the utility of the invention for use as a treatment for a disease resulting from an enzyme deficiency, such as Gaucher's disease. Gaucher's disease is the most common lysosomal storage disorder in humans, and results from a deficiency of the enzyme, glucocerebrosidae (GC). (Nolta *et al.*, (1992), *J. Clin. Invest.* 90 (2):342-348).

Enzyme replacement therapy is provided with a *Helicobacter* vaccine construct that comprises a sequence encoding chemical chaperones. (Sawker *et al.*, (2002), *PNAS USA* 99(24): 15428-15433) or glucocerebrosidae. An enhanced level of functional β-glycosidase (β-Glu, glucocerebrosidase) may thus be obtained. In particular, a sequence encoding the chemical chaperone deoxynojirimycin (NN-DNJ) may be used in the *H. pylori* construct and administered to the patient orally or intragastrically.

As part of yet another embodiment, a *Helicobacter*-based construct is provided comprising a vector having a non-*Helicobacter* pharmacologically active molecule of interest, in this case, encoding glucocerebrosidase (GC). Retroviral mediated transfer of glucocerebrosidase cultured Gaucher bone marrow is described as one approach for treating Gauchers disease in Nolta *et al.* (1992). However, this approach is extremely invasive. Alternative enzyme replacement therapy employing the *Helicobacter*-based constructs of the present invention that include a sequence encoding for the deficient enzyme, glucocerebrosidase, provides a much more attractive and less expensive alternative to such a therapy.

EXAMPLE 16

The present example is presented to demonstrate the utility of the present invention to provide a useful preparation that is suitable for treating and or inhibiting a bacterial induced malignancy, such as lymphoma, particularly MALT lymphoma, using a vaccination preparation comprising the *Helicobacter* vector and/or plasmid vectors as described herein.

Sutton *et al.* (2004) (*Vaccine*, 22 (20): 2541-6) report protection against a bacteria-induced malignancy, specifically primary gastric MALT lymphoma, as a result of vaccination/immunization of an animal against *Helicobacter felis*. Therefore, the *H. pylori* constructs of the present disclosure that include a vector and/or plasmid vector suitable for providing an immunizing preparation against other than *H. felis* may also be used to provide vaccination protection against a bacterial-induced malignancy, and in particular, against primary gastric MALT lymphoma.

All documents, patents, journal articles and other materials cited in the present application are hereby incorporated by reference.

Although the present invention has been fully described in conjunction with several embodiments thereof with reference to the accompanying drawings, it is to be understood that various changes and modifications may be apparent to those skilled in the art. Such changes and modifications are to be understood as included within the scope of the present invention as defined by the appended claims, unless they depart therefrom.

BIBLIOGRAPHY

The following references are specifically incorporated herein by reference.

1. U.S. 6,570,004- Blaser et al (2003).
2. U.S. 6,680,179 Collins et al.
3. U.S. 6,383,496 - Curtiss et al (2002).
4. U.S. 6,150,170 - Powell et al. (2000).
5. U.S. 6,410,012 - Seizmore et al. (2002).
6. U.S. 6,550.419 - Hone et al. (2002).
7. U.S. 6,531,313 - Goudsmit et al. (2003).
8. U.S. 6,682,729 - Powell et al (2004).
9. U.S. Patent Publication 2005/0075298A1 - Chen et al. (2005).
10. U.S. Patent Publication 2002/0176848A1 - Seizemore et al (2002).
11. U.S. Patent Publication 2005/0096288 A1 - Guevara et al. (2005).
12. U.S. Patent Publication 2004/0236072 A1 - Olmsted et al. (2004).
13. U.S. Patent Publication 2004/0203039 A1 - Hensel et al. (2004).
14. U.S. Patent Publication 2004/0005325 A1 - Kusters et al.(2004).
15. U.S. Patent Publication 2002/0032152 A1 - Torossian (2002).
16. U.S. Patent Publication 2003/0170264 A1 - Turner et al. (2003).
17. U.S. Patent Publication 2003 0204068- Blasec et al (2003).
18. U.S. Patent Publication 2002/0161192 A1- Meyer et al (2002).
19. WO 96/33274 - Covacci et al. (1996).
20. WO 99/21959 - Ellis et al. (1999).
21. WO 01/94599 - Burman et al. (2001).
22. WO 2005 021026 - Baron, et al. (2005).

23. Graham et al (2002), Gastroenterology, 123:1637-1648.
24. Liu et al (2005), World Journal Gastroenterology, 11(14): 2154-2156.
25. Conway, BR (2005), Curr. Pharm. Res., 11(6) 775-90.
26. Sawker et al (2002), PNAS USA, 99(24): 15428-15433.
27. Sutton, P et al (2004), Vaccine, 22(20): 2541-6.
28. Kang et al (2005), World Journal Gastroenterology, 11(3): 454-456.
29. Moschos et al (2004), Immunology and Cell Biology, 82(6): 628-637.
30. Reddy et al (2004), International Journal Antimicrob. Agents, 24(6): 536-47.
31. Bai et al. (2003), Sheng Wugong Cheng Xu Bao, 19(4):433-8.
32. Nolta et al. (1992), Journal of Clin. Invest, 90(2): 342-348.
33. Shi et al. (2005), Helicobacter, 10(1): 71-9.
34. Deml et al. (2005), Infection Immunity, 73(8): 4732-42.
35. Cosima et al. (2005), Trends in Immunology, 26(4): 199-207.
36. Velin et al. (2005), Gastroenterology, 129(1): 142-155.
37. Kong et al. (2000), Nucleic Acids Research, 28(17) 3216-3223.
38. Mao et al. (2003), World Journal of Gastroenterology, 9(7): 1529-1536.
39. Chu et al. (2005), World Journal of Gastroenterology, 11(23): 3518-22.
40. Kathy Parton. (2000), Institute of Veterinary, Animal and Biomedical Sciences, "*Helicobacter mustelae* as vector for biological control in stoats" Wildlife Health and Conservation Research Program; Landcare Research (Funding Body).
41. Forrester N.T., Parton, K. (2000), New Zealand Veterinary Journal, 48: 65-69. Title, "Isolation of *Helicobacter mustelae* from ferrets in New Zealand".

42. Spranger *et al.* (2005), Br. Nutr., 93 (6):765-71.
43. Garbom et al. (2004), Infect Immun., 72(3): 1333-1340.
44. Tschop et al. (2000) Nature, 407:908-13.
45. Choi et al (2003), Edocrinology, 144 (3).
46. Remington's Pharmaceutical Sciences, 20th edition, Mack Publishing Company.
47. Jones et al (2005) Nat. Med. 11 (7): 786-90.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A composition comprising a *Helicobacter* molecular construct having a *Helicobacter* sequence and a non-*Helicobacter* sequence encoding a non-*Helicobacter* pharmacologically active molecule of interest.

2. A composition according to claim 1, wherein the *Helicobacter* is *Helicobacter pylori*.

3. A composition according to claim 1 or claim 2, wherein the pharmacologically active molecule of interest is heterologous to the *Helicobacter pylori* species.

4. A composition according to any one of claims 1 to 3, further comprising a promoter sequence.

5. A composition according to any one of claims 1 to 4, wherein the pharmacologically active molecule of interest is ghrelin, amylin or an analog thereof.

6. A composition according to any one of claims 2 to 5, wherein the *Helicobacter pylori* is attenuated *Helicobacter pylori*.

7. A composition according to any one of claims 2 to 6, wherein the pharmacologically active molecule of interest comprises a protein, peptide or nucleic acid molecule.

8. A vaccine comprising a composition according to claim 4 and a pharmacologically acceptable carrier solution.

9. A vector plasmid comprising a composition according to claim 4.

10. A composition comprising a vaccine, wherein said vaccine comprises a *Helicobacter* vector plasmid comprising:

(a) a *Helicobacter* nucleotide sequence comprising a promoter sequence capable of controlling expression of a sequence encoding a non-*Helicobacter* pharmacologically active molecule of interest; and

(b) a sequence encoding a non-*Helicobacter* pharmacologically active molecule of interest.

11. A composition according to claim 10, further comprising an adjuvant.

12. A composition according to claim 10 or claim 11, further described as suitable for delivery to the mucosa.

13. A composition according to any one of claims 10 to 12, wherein the non-*Helicobacter* pharmacologically active molecule of interest is a protein, peptide or nucleic acid molecule.

14. A composition according to any one of claims 10 to 12, wherein the non-*Helicobacter* pharmacologically active molecule of interest is ghrelin, amylin or an antagonist or analog thereof.

15. A method of preparing an immunogenic composition comprising a non-*Helicobacter* immunogen comprising:

(a) providing a culture comprising *Helicobacter pylori* cells transformable with a plasmid vector;

(b) introducing into said culture a plasmid vector comprising a promoter sequence and a sequence encoding a non-*Helicobacter* immunogen under suitable conditions to provide transformed *Helicobacter pylori* cells, and

(c) selecting transformed cells that express said non-*Helicobacter* immunogen to provide an immunogenic composition,

wherein said promoter sequence is capable of controlling the expression of the sequence encoding the non-*Helicobacter* immunogen.

16. A method according to claim 15, wherein the promoter sequence comprises an inducible promoter.

17. A method according to claim 15 or claim 16, further comprising a non-antibiotic resistance gene marker.

18. A method according to claim 15, wherein the promoter is constitutively expressed in *Helicobacter pylori*.

19. A method according to claim 15, the promoter is an arabinose inducible promoter.

20. A method according to any one of claims 15 to 19, wherein the *Helicobacter pylori* is strain 26695.

21. A method according to any one of claims 15 to 20, wherein the plasmid vector is further defined as comprising an operon construct.

22. A method of treating an animal comprising the steps of:

(a) providing a composition according to any one of claims 10 to 14; and

(b) administering to the animal an effective amount of said composition.

23. A method according to claim 22, wherein the animal is a human.

24. A method according to claim 22, wherein the treated animal has an enhanced immune response.

25. A method according to any one of claims 22 to 24, wherein the composition is administrated at a mucosal surface of the animal.

26. A method according to any one of claims 22 to 25, wherein one or more effective amounts of the composition are administered to the animal.

27. A composition comprising a recombinant cell comprising a sequence encoding a *non-Helicobacter pylori* pharmacologically active molecule of interest comprising:

(a) at least one non-*Helicobacter* sequence encoding a pharmacologically active molecule of interest; and

(b) a *Helicobacter* sequence having a promoter sequence capable of controlling expression of the non-*Helicobacter* sequence,

wherein the recombinant cell is capable of expressing said nucleic acid or causing the expression of the nucleic acid molecule in a target cell.

28. A composition according to claim 27, wherein the nucleotide sequence encoding the recombinant cell further comprises a secretory signal polypeptide.

29. A composition according to claim 27, wherein said recombinant cell is a recombinant *Helicobacter pylori*.

30. A composition according to claim 29, wherein the recombinant *Helicobacter pylori* is pTM103-8.

31. A composition comprising a recombinant *Helicobacter pylori* comprising:

(a) a sequence encoding a fusion protein comprising a non-*Helicobacter* pharmacologically active molecule of interest and a *Helicobacter* sequence; and

(b) an inducible *Helicobacter pylori* promoter sequence.

32. A plasmid vector comprising a composition according to claim 31.

33. A pharmaceutical composition comprising as an active ingredient the plasmid vector of claim 32, together with a pharmaceutically acceptable dilute, carrier, adjuvant or combination thereof.

34. A method for preparing a recombinant *Helicobacter pylori* comprising:

(a) providing a culture of *Helicobacter pylori*; and

(b) providing a plasmid vector according to claim 32 to the culture to provide recombinant *Helicobacter pylori*,

wherein said recombinant *Helicobacter pylori* are capable of expressing or causing expression of said *non-Helicobacter* pharmaceutically active molecule in a target cell.

35. A composition according to claim 31, wherein said recombinant *Helicobacter* further comprises a second nucleic acid molecule encoding an immunomodulatory polypeptide, wherein said recombinant *Helicobacter* is capable of expressing said second nucleic acid molecule in a target cell.