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SUPERINTENDENTE DE INDUSTRIA Y COMERCIO

E. S. D.

REF: **Expediente 13-019-529**

TÍTULO SOLICITADO: **"COMPOSICIONES INMUNOGÉNICAS ANTI-INFLAMATORIAS"**

PUBLICACIÓN: **Gaceta 668 de 31 de mayo de 2013, Nº de publicación 627.**

PRIORIDAD: **RU 2010129824 20/05/2010**

SOLICITANTE: **QU BIOLOGICS INC.**

APODERADO: **LUZ CLEMENCIA SUAREZ DE PAEZ**

TRÁMITE: **SUSTENTACIÓN DE OPOSICIÓN**

JOSÉ LUIS REYES VILLAMIZAR, mayor de edad, identificado como aparece al pie de mi firma, abogado en ejercicio, titular de la tarjeta profesional número 44655 del Consejo Superior de la Judicatura, obrando en calidad de apoderado de la sociedad **TECNOQUIMICAS S.A.,** dentro del trámite de la referencia, en ejercicio de lo dispuesto por el artículo 42 inciso 2º de la Decisión 486 de 2000, por medio del presente escrito, procedo a sustentar y complementar la oposición presentada el 25 de junio de 2013, en los siguientes términos:

I. RATIFICACIÓN

La solicitud 12-217-148, referida a un método para formular una composición antiinflamatoria para tratar afecciones inflamatorias en un órgano o tejido específico, además de presentar materia no patentable de acuerdo con la legislación actual, carece de Claridad, Novedad y Nivel Inventivo, especialmente a la luz de las revelaciones de los documentos Gunn et al. de 1999, y WO 2008/049231 de 02/05/2008.

En primer lugar, se objetó la claridad, concisión y suficiencia de la descripción y reivindicaciones de la solicitud en trámite, dado que las características técnicas estructurales no definen claramente el objeto a proteger, es decir, no se define ninguno de los pasos del método de forma técnica, por lo que no existe claridad en el capítulo.

Se observó, igualmente, que existen diferencias altamente variables de todos los organismos que pueden incluirse en la reivindicación 1 por lo que no se asocia un efecto común en estructura y función.

Aparte, las reivindicaciones 4 – 5 y 8 – 9 definen la materia mediante resultados a alcanzar incluyendo materia dirigida a formulación para inyección subcutánea o intradérmica. Se observó además que por ejemplo en la reivindicación 6 existe una alta cantidad de sustituciones en la materia reclamada en diferentes propiedades de la misma.

Además de lo anterior, las reivindicaciones de usos tales como 16 - 19, 17, 18, 21, 22, mientras que reivindicaciones como 37 - 48, 50 – 56, 73 – 86, reclaman un método de tratamiento, por lo que dichas reclamaciones no cumplen con los criterios de patentabilidad, por tal razón deberá negarse la solicitud y producirse en consecuencia su archivo.

Por su parte, el documento de Gunn et al., se destaca por contener *la materia relevante* de las reivindicaciones debido a que este documento describe métodos para formular una composición inmunogénica para el tratamiento del cáncer situado en un órgano o tejido específico de un mamífero tal como un paciente humano.

Este documento objeta la novedad de la solicitud al incluir al menos un patógeno microbiano que es patogénico naturalmente en el órgano o tejido del mamífero dentro de que se sitúa el cáncer. Así mismo, una composición antigénica puede ser producida y puede incluir los determinantes antigénicas que juntos son específicos o son característicos del patógeno microbial.

Adicionalmente se identificó el uso de patógenos empleados por la solicitud tal como lo menciona el párrafo [0246] de la anterioridad y en especial de

Mycobacterium bovis, M vaccae, Corynebacterium parvum, Streptococcus pyogenes, Nocardia rubra, Lactobacillus case, Pseudomonas aeruginosa, entre otros.

En otras palabras, el documento citado como anterioridad, incluye todas las características técnicas del método definido por la solicitud en la reivindicación 1 y cualquier modalidad particular es evidente para una persona versada en la materia, quien habría llegado a los métodos reclamados al caracterizar los antígenos de cada microorganismo y su patología principal.

En consecuencia, los métodos reivindicados no son novedosos y no cumplen con el requisito de nivel inventivo, pues las características específicas incluyen aquellas que un técnico medio versado en la materia emplea rutinariamente; adicionalmente, la materia se encontraba consignada en la información divulgada por la anterioridad

II. COMPLEMENTO DE INFORMACIÓN

Es importante destacar que a la fecha no existen modificaciones al capitulo reivindicatorio de la solicitud en estudio; sin embargo, en el presente documento se reitera que la solicitud en curso no cumple con los requisitos de patentabilidad.

De forma más clara observemos que las reivindicaciones 1, 2 y 3 de la solicitud en trámite pretenden reclamar un método para formular una composición antiinflamatoria, tal que:

> *1-. Un método para formular una composición antiinflamatoria para tratar una afección caracterizada por inflamación en un órgano o tejido específico, que comprende:*
> *a. seleccionar al menos un patógeno que sea patógeno en el órgano o tejido específico;*
> *b. producir una composición antigénica que comprende determinantes antigénicos que en conjunto son específicos para el patógeno, y*
> *c. formular la composición antigénica para su administración como una composición antiinflamatoria que pueda producir una respuesta*

antiinflamatoria en el órgano o tejido específico, donde la afección caracterizada por inflamación no es un cáncer.

2-. El método de la reivindicación 1, que comprende adicionalmente un paso de diagnóstico de identificar el órgano o tejido específico dentro del cual la inflamación es sintomática antes de producir la composición antigénica.

3-. El método de la reivindicación 1, donde un tumor se encuentra ubicado en el órgano o tejido específico.

Con respecto a dicha materia, se observa que el documento WO 2005/120560 publicado por el mismo autor (en adelante Gunn, 2005) describe un método para tratar cáncer en un órgano, tejido o célula específico en un sujeto mediante la administración de una composición que comprende un antígeno de uno o más especies bacterianas patogénicas, tal como puede observarse en el sumario del documento:

SUMMARY OF THE INVENTION

The invention provides in part methods of treating cancers of a specific organ, tissue or cell in a subject by administering an antigen of one or more pathogenic bacterial species that are pathogenic in the specific organ, tissue or cell.

In one aspect, the invention provides a method of treating a cancer of a specific organ, tissue or cell in a subject by administering to the subject an effective amount of an antigen of one or more pathogenic bacterial species, where the pathogenic bacterial species is pathogenic in the specific organ, tissue or cell.

Dicha composición puede ser provista sola o en combinación con otros compuestos tanto en presencia de liposomas, adyuvantes, o cualquier transportador farmacéuticamente aceptable, en una forma adecuada para su administración.

Según la reivindicación 4 de la solicitud en estudio:

4. El método de la reivindicación 1, donde la composición antigénica se formula para inyección subcutánea o intradérmica.

El documento de Gunn, 2005, hace referencia a que los vehículos mencionados son adecuados para administración subcutánea, intravenosa, parenteral, intraperitoneal, intramuscular, sublingual, etc.

> The bacterial compositions of the invention can be provided alone or in combination with other compounds (for example, nucleic acid molecules, small molecules, peptides, or peptide analogues), in the presence of a liposome, an adjuvant, or any pharmaceutically acceptable carrier, in a form suitable for administration to mammals, for example, humans. As used herein "pharmaceutically acceptable carrier" or "excipient" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. The carrier can be suitable for any appropriate form of administration, including subcutaneous, intravenous, parenteral, intraperitoneal, intramuscular, sublingual, inhalational, or oral administration. Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for
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Así mismo, el método implica el uso de especies patogénicas tales como *Streptococcus pneumoniae, Moraxella catarrhalis, Mycoplasma pneumoniae, Klebsiella pneumoniae, Haemophilus influenzae, Staphylococcus aureus, Chlamydia pneumoniae, o Legionella pnaumophila* para cáncer de pulmón; aunque se incluyen los demás microorganismos para ser usados como antígenos en diferentes composiciones para el tratamiento de enfermedades localizadas:

> 2. The method of claim 1, wherein said cancer of a specific organ, tissue or cell is lung cancer and said pathogenic bacterial species are *Streptococcus pneumoniae, Moraxella catarrhalis, Mycoplasma pneumoniae, Klebsiella pneumoniae, Haemophilus influenzae, Staphylococcus aureus, Chlamydia pneumoniae,* or *Legionella pneumophila.*

Por lo tanto, los microorganismos determinados para cada tratamiento en el método reivindicado así como su uso en dicho tratamiento, no solo ya eran conocidos en el estado de la técnica para la fecha de la presentación de la solicitud tal como se observa de la lectura de las reivindicaciones, sino que había sido reclamado igualmente, bajo el mismo esquema.

En conclusión, un método para formular una composición antiinflamatoria para el tratamiento de enfermedades en un órgano específico o tejido, no puede ser patentable ya que no se diferencia del método del documento de Gunn 2005, además porque en dicho documento se incluye el crecimiento celular (que caracteriza la inflamación) y que técnicamente puede convertirse de una enfermedad benigna a una maligna.

Un tercer documento que presenta la materia divulgada por la solicitud es el publicado por Marshall, US 2007/0134264 el cual describe un método para tratamiento de una enfermedad que comprende la administración de recombinantes de *Helicobacter pillori* adecuados para uso en medicamentos. Las células transformadas con *Helicobacter* basadas en vectores plasmídicos pueden comprende células de *E. coli o H. pylori.* En algunos casos, la vacuna puede estar conformada por vacunas vivas atenuadas. Las enfermedades incluyen colitis ulcerativa, enfermedad de Crohn, enfermedades ulcerativas, síndrome de colon irritable y colitis

(57) **ABSTRACT**

Helicobacter based preparations comprising a pharmacologically active molecule of interest are disclosed, as well as methods of preparing and using said preparations. In particular, *Helicobacter pylori* vectors, vector plasmids and recombinant cells that include a sequence encoding a pharmacologically active molecule of interest useful in therapeutic treatments and/or vaccination against disease are provided. Delivery of the pharamacologically active molecules is provided at the mucosal surface, such as the gastric mucosa or nasal membranes, to provide effective and continuous delivery of a pharmacologically active agent. In some embodiments, the *Helicobacter* provides exposure of a desired molecule of interest though the surface of the *Helicobacter,* providing exposure of the antigen to the host at the gastric mucosa. Live *Helicobacter pylori* vaccines are also provided. Vectors and shuttle vector constructs of the *Helicobacter* are also disclosed.

Así, este documento incluye todas las características técnicas al reemplazar la materia particular en la formula general de las reivindicaciones de la solicitud así:

Se reclama un método para tratar una enfermedad que comprende la administración de una composición de vacuna que comprende *H. pylori* recombinante adecuado para el uso en un medicamento donde dicho constructo comprende:

a. una primera secuencia de ácidos nucleicos que codifica un primer antígeno de superficie de *H. pylori.*
b. Una segunda secuencia de ácidos nucleicos que codifica un segundo antígeno de superficie de *H. pylori* y
c. Una secuencia de ácidos nucleicos que codifica una molécula de interés (no *Helicobacter)* que tiene una primera y segunda terminal y una molécula de ácido nucleico que codifica a un antígeno de superficie de *H. pylori* es unido a dicha segunda terminal de las moléculas *no Helicobacter* de interés.

Es útil observar que dichas moléculas de interés que no son *Helicobacter*, pueden comprender diferentes proteínas o péptidos tales como insulina, prolactina, citoquinas, interleucinas, factores de crecimiento, interferones, EPO, miembros del factor de necrosis tumoral (TNF) tal como se observa en el párrafo 0067 del documento de Marshall:

> [0067] Specific examples of such biologically active agents include insulin, growth hormone, prolactin, calcitonin, luteinizing 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, CM-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, e.g. 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 EGF domain in the extracellular region, e.g., the epidermal growth factor family of cytokines, the chemokines characterized 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.

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y adicionalmente, se incluyen como moléculas de interés de la composición los antagonistas de las proteínas mencionadas, por lo que los anticuerpos se encuentran comprendidos en la materia anterior y no pueden ser objeto de patente tal como se pretende en la solicitud en trámite:

> [0068] Alternatively, the biologically active agent can be a receptor or antagonist for biologically active agent as defined above.

Por lo tanto, la materia pretendida por la reivindicación 35 de la solicitud que incluye medicamentos antagonistas de receptores y proteínas del factor de necrosis tumoral (TNF) tales como infliximab o adalimumab, no puede ser reclamada nuevamente y menos aún en una composición como la de la solicitud en trámite, la cual no cumple con los criterios de patentabilidad, en especial novedad o nivel inventivo.

Es importante observar detenidamente que la amplitud de las reivindicaciones y la falta de concisión de las mismas incluye materia divulgada y disponible comercialmente tal como se observa en la reivindicación mencionada:

> 35. El método de la reivindicación 34, donde el medicamento es sulfasalazina, mesalamina, un corticosteroide, azatioprina, mercaptopurina, infliximab, adalimumab, certolizumab pegol, metotrexato, ciclosporina o natalizumab.

Por lo anterior, los métodos reivindicados no revelan ninguna característica técnica novedosa además de ser ampliamente imprecisos y poco claros, por lo que además no se a demostrado un efecto técnico sorprendente o inesperado en la materia incorporada en la solicitud; en conclusión, la formulación reclamada carece de novedad y de un efecto técnico sorprendente que soporte la novedad y el nivel inventivo de la misma.

III PRUEBAS

- El documento de Gunn et al., WO 2008/049231, *"Tissue targeterd antigenic activation of the immune response to treat cancers"* publicado el 2 de mayo de 2008.

- El documento de Gunn, WO 2005/120560, *"Bacterial compositions for the treatment of cancer"* publicado el 22 de diciembre de 2005. Portada, páginas 25 y 64. Portada, página 3, 14, 32.

- El documento de Marshall Barry US 2007/0134264, *"Helicobacter system and uses thereof"*, portada, páginas 1y 16

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(54) Title: BACTERIAL COMPOSITIONS FOR THE TREATMENT OF CANCER

(57) Abstract: The invention provides in part methods of treating cancers of a specific organ, tissue or cell in a subject by administering an antigen of one or more pathogenic bacterial species that are pathogenic in the specific organ, tissue or cell.

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BACTERIAL COMPOSITIONS FOR THE TREATMENT OF CANCER

FIELD OF THE INVENTION

The invention provides in general methods of treating cancers. More specifically, the invention provides methods of treating cancers using bacterial compositions.

BACKGROUND OF THE INVENTION

More than one in three people in the developed nations are diagnosed with cancer. More than one in four die from it. Therapies for cancer have primarily relied upon treatments such as surgery, chemotherapy, and radiation. These approaches however, while beneficial for some types and stages of cancer, have proved to be of limited efficacy in many common types and stages of cancers. For example, surgical treatment of a tumor requires complete removal of cancerous tissue to prevent reoccurrence. Similarly, radiation therapy requires complete destruction of cancerous cells. This is difficult since, in theory, a single malignant cell can proliferate sufficiently to cause reoccurrence of the cancer. Also, both surgical treatment and radiation therapy are directed to localised areas of cancer, and are relatively ineffective when the cancer metastasises. Often surgery or radiation or both are used in combination with systemic approaches such as chemotherapy. Chemotherapy however has the problem of non-selectivity with the concomitant problem of deleterious side effects, as well as the possibility of the cancer cells developing resistance to the drugs.

Alternative approaches for the treatment of cancers have included therapies that involve stimulation of the immune system such as cytokine therapy (e.g., recombinant interleukin 2 and gamma interferon for kidney cancers), dendritic cell therapy, autologous tumor vaccine therapy, genetically-altered vaccine therapy, lymphocyte therapy, and bacterial vaccine therapy. The above therapies have all proved to be of limited use: cytokine therapy have been effective only in a minority of cases, as has dendritic cell therapy, and the latter is also time consuming and expensive. The diverse vaccine therapies have exhibited highly variable efficacy, and in the case of bacterial vaccines, have proved ineffective in a variety of cancers.

SUMMARY OF THE INVENTION

The invention provides in part methods of treating cancers of a specific organ, tissue or cell in a subject by administering an antigen of one or more pathogenic bacterial species that are pathogenic in the specific organ, tissue or cell.

In one aspect, the invention provides a method of treating a cancer of a specific organ, tissue or cell in a subject by administering to the subject an effective amount of an antigen of one or more pathogenic bacterial species, where the pathogenic bacterial species is pathogenic in the specific organ, tissue or cell.

If the cancer is lung cancer, the pathogenic bacterial species may be *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Mycoplasma pneumoniae*, *Klebsiella pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, *Chlamydia pneumoniae*, or *Legionella pneumophila*.

If the cancer is colon cancer, the pathogenic bacterial species may be *Bacteroides fragilis*, *Bacteroides vulgatus*, *Bacteroides thetaiotaomicron*, *Clostridium perfringens*, *Clostridium difficile*, *Escherichia coli*, *Salmonella enteriditis*, *Yersinia enterocolitica*, or *Shigella flexneri*.

If the cancer is kidney cancer, the pathogenic bacterial species may be *Escherichia coli*, *Proteus mirabilis*, *Proteus vulgatus*, *Providentia* species, *Morganella* species, or *Enterococcus faecalis*.

If the cancer is prostate cancer, the pathogenic bacterial species may be *Escherichia coli*, *Corynebacterium* species, *Enterococcus faecalis*, or *Neisseria gonorrhoeae*.

If the cancer is skin cancer, the pathogenic bacterial species may be *Staphylococcus aureus*, *Streptococcus pyogenes*, *Corynebacterium diphtheriae*, *Corynebacterium ulcerans*, or *Pseudomonas aeruginosa*.

If the cancer is bone cancer, the pathogenic bacterial species may be *Staphylococcus aureus*.

If the cancer is mouth cancer, the pathogenic bacterial species may be *Prevotella melaninogenicus*, anaerobic streptococci, viridans streptococci (e.g., *Streptococcus salivarius*, *Streptococcus sanguis*, or *Streptococcus mutans*), or *Actinomyces* species.

If the cancer is testicle cancer, the pathogenic bacterial species may be *Escherichia coli*, *Salmonella enteriditis*, or *Staphyloccocus aureus*.

cause of colon and urinary tract (kidney and bladder) infection. E. coli serotypes which commonly cause colon infection include 06:H16, 08:H9, 015:H22, 025:H-, 028ac:H-, 0136:H-, 0157:H7, 051:H11, and 077:H18. On the other hand, the most common E. coli serotypes to cause urinary tract infection are O groups 1, 2, 4, 6, 7, 18, 25, 50 and 75 (Infectious Diseases: A Treatise of Infectious Diseases; Editors: P. Hoeprich, M.C. Jordan, A. Ronald (1994) J.B. Lippincott Company: Philidelphia p. 602).

Bacterial Compositions, Dosages, And Administration

The compositions of the invention include antigens of pathogenic bacterial species that are pathogenic in a specific cell, tissue, or organ. The compositions may include whole bacterial species, or may include extracts or preparations of the pathogenic bacterial species of the invention, such as cell wall or cell membrane extracts or whole cell extracts. The compositions may also include one or more isolated antigens from one or more of the pathogenic bacterial species of the invention; in some embodiments, such compositions may be useful in situations where it may be necessary to precisely administer a specific dose of a particular antigen, or may be useful if administering a whole bacterial species or components thereof (e.g., toxins) may be harmful. Pathogenic bacterial species may be available commercially (from, for example, ATCC (Manassas, VA, USA), or may be clinical isolates from subjects having a bacterial infection of a cell, tissue, or organ (e.g., pneumonia).

The bacterial compositions of the invention can be provided alone or in combination with other compounds (for example, nucleic acid molecules, small molecules, peptides, or peptide analogues), in the presence of a liposome, an adjuvant, or any pharmaceutically acceptable carrier, in a form suitable for administration to mammals, for example, humans. As used herein "pharmaceutically acceptable carrier" or "excipient" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. The carrier can be suitable for any appropriate form of administration, including subcutaneous, intravenous, parenteral, intraperitoneal, intramuscular, sublingual, inhalational, or oral administration. Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for

WHAT IS CLAIMED IS:

1. A method of treating a cancer of a specific organ, tissue or cell in a subject, said method comprising administering to said subject an effective amount of an antigen of one or more pathogenic bacterial species, wherein said pathogenic bacterial species is pathogenic in said specific organ, tissue or cell.

2. The method of claim 1, wherein said cancer of a specific organ, tissue or cell is lung cancer and said pathogenic bacterial species are *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Mycoplasma pneumoniae*, *Klebsiella pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, *Chlamydia pneumoniae*, or *Legionella pneumophila*.

3. The method of claim 2, wherein said pathogenic bacterial species are *Streptococcus pneumoniae*, *Moraxella catarrhalis*, or *Mycoplasma pneumoniae*.

4. The method of claim 1, wherein said cancer of a specific organ, tissue or cell is colon cancer and said pathogenic bacterial species are *Escherichia coli*, *Bacteroides fragilis*, *Bacteroides vulgatus*, *Bacteroides thetaiotaomicron*, *Clostridium perfringens*, *Clostridium difficile*, *Salmonella enteriditis*, *Yersinia enterocolitica*, or *Shigella flexneri*.

5. The method of claim 1, wherein said cancer of a specific organ, tissue or cell is kidney cancer and said pathogenic bacterial species are *Escherichia coli*, *Proteus mirabilis*, *Proteus vulgatus*, *Providentia* species, *Morganella* species, or *Enterococcus faecalis*.

6. The method of claim 1, wherein said cancer of a specific organ, tissue or cell is prostate cancer and said pathogenic bacterial species are *Escherichia coli*, *Corynebacterium* species, *Enterococcus faecalis*, or *Neisseria gonorrhoeae*.

7. The method of claim 1, wherein said cancer of a specific organ, tissue or cell is skin cancer and said pathogenic bacterial species are *Staphylococcus aureus*,

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(54) **HELICOBACTER SYSTEM AND USES THEREOF**

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(57) **ABSTRACT**

Helicobacter based preparations comprising a pharmacologically active molecule of interest are disclosed, as well as methods of preparing and using said preparations. In particular, *Helicobacter pylori* vectors, vector plasmids and recombinant cells that include a sequence encoding a pharmacologically active molecule of interest useful in therapeutic treatments and/or vaccination against disease are provided. Delivery of the pharamacologically active molecules is provided at the mucosal surface, such as the gastric mucosa or nasal membranes, to provide effective and continuous delivery of a pharmacologically active agent. In some embodiments, the *Helicobacter* provides exposure of a desired molecule of interest though the surface of the *Helicobacter*, providing exposure of the antigen to the host at the gastric mucosa. Live *Helicobacter pylori* vaccines are also provided. Vectors and shuttle vector constructs of the *Helicobacter* are also disclosed.

HELICOBACTER SYSTEM AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 60/602,859, filed Aug. 20, 2004, and to Australian Patent Application No. 2004/904564, filed Aug. 13, 2004, and to U.S. Utility application Ser. No. 11/202,249, filed Aug. 12, 2005, the text of all applications being specifically incorporated herein by reference.

BACKGROUND

[0002] 1. Field of the Invention

[0003] The present invention relates generally to the field of *Helicobacter*-based vector, plasmid vector and shuttle vector systems, as novel *Helicobacter* constructs that include a non-*Helicobacter* pharmacologically active molecule of interest are provided. The invention also relates to the field of drug delivery, vaccines and treatment methods, as compositions that provide for the administration and/or delivery of non-*Helicobacter* molecules at the mucosa in vivo are disclosed.

[0004] 2. Related Art

[0005] *Helicobacter pylori* are 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.

[0006] *H. pylori* have been described as a causative agent of chronic infection. In particular, *Helicobacter* has been established to play a critical role in peptic ulcer, gastric adenocarcinoma, and primary gastric lymphoma.

[0007] *H. pylori* have 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. However, this particular application has not found application in mucosal delivery systems in part owing to its involvement in a variety of diseases. A need continues to exist for a delivery system employing these important organisms having a reduced risk of pathology to the host.

[0008] The development of mucosal vaccines has also been hindered by the poor immunogenicity of antigens delivered by conventional approaches because of natural barrier functions of the host that prevent access to the mucosal compartment. Hence, a need continues to exist in the medical arts 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 a mucosal surface sufficient to elicit a general humoral and mucosal immune response.

SUMMARY

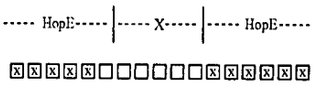
[0009] The present invention is directed to overcoming the above-mentioned challenges and others related to the use of *Helicobacter* and in the treatment of disease. The present invention is exemplified in a number of implementations and applications, some of which are summarized below.

[0010] 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* molecule of interest. 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* sequence encoding a molecule of interest.

Helicobacter Constructs:

[0011] In one aspect, the invention provides a composition comprising a *Helicobacter* construct, particularly a *Helicobacter pylori* nucleic acid construct. In some embodiments, the *Helicobacter* nucleotide sequence of the *Helicobacter* construct comprises a first *Helicobacter* sequence, Y1, a second *Helicobacter* sequence Y2, and a non-*Helicobacter* sequence X encoding a non-*Helicobacter* molecule of interest. A schematic of one embodiment of this construct appears in Formula 1:

Formula 1:



[0012] The non-*Helicobacter* nucleotide sequence of interest, "X", may comprise a nucleic acid sequence that encodes a pharmacologically active and/or biologically active molecule of interest. In some embodiments, the non-*Helicobacter* sequence X is heterologous to the *Helicobacter pylori* species. A pharmacologically active molecule of interest or a biologically active molecule of interest may comprise an enzyme, protein, peptide, or other molecule. This molecule of interest may be further described in some embodiments as capable of providing a beneficial and/or therapeutic effect to an animal delivered as an expressed product from a recombinant *Helicobacter* containing the construct that is introduced into an animal. In some embodiments, the molecule of interest is ghrelin, amylin or an analog thereof.

[0013] In some embodiments, the construct comprises a first *Helicobacter* sequence Y1 defined as a first portion of a native HopE gene sequence, a second *Helicobacter* sequence Y2 defined as a second portion of a native HopE gene sequence, and a non-*H. pylori* nucleotide sequence of interest, "X". By way of example, one such construct has a structure defined as: 580 bp HopE—"X" bp—502 bp HopE. In some embodiments, "X" is a nucleic acid sequence comprising 60 nucleotide bases to 300 nucleotide bases, or 69 nucleotide bases to 138 nucleotide bases.

[0014] In some embodiments, the *Helicobacter* construct comprises a construct as depicted in FIG. 7.

[0015] In other embodiments, *Helicobacter* construct is further defined as an attenuated *Helicobacter pylori* construct.

[0016] The *H. pylori* nucleic acid construct may further comprise, in some embodiments, a promoter sequence, a

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.

[0067] Specific examples of such biologically active agents include insulin, growth hormone, prolactin, calcitonin, luteinizing 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, CM-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, e.g. 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 EGF domain in the extracellular region, e.g., the epidermal growth factor family of cytokines, the chemokines characterized 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.

[0068] Alternatively, the biologically active agent can be a receptor or antagonist for biologically active agent as defined above.

[0069] In some embodiments, the *H. pylori*-based vector and/or vector plasmid construct is employed to create a transformed cell (such as an *E. coli* cell or *Helicobacter* cell) that permits the expression and secretion of a non-*Helicobacter* pharmacologically active molecule of interest at the mucosal membrane of a host to which the transformed cell preparation is administered. The isolated nucleic acid molecule contained within the transformed cell (or vector) may comprise one or more nucleic acid constructs in which nucleic acid encoding the pharmacologically active molecule of interest is under control of *H. pylori* regulatory sequences.

[0070] Suitable vectors and shuttle vector sequences 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, e.g. phage, or phagemid, as appropriate. For further details, for example, see Sambrook et al., supra. Many techniques and protocols are known for the 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, as 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 specifically herein by reference.

[0071] In some embodiments, the coding sequence(s) for the pharmacologically active molecules of interest is contained in an operon, i.e., a nucleic acid construct for multicistronic 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 (pharmacologically active molecule of interest) can be translated from a single mRNA. Use of an operon enables expression of the pharmacologically active molecule of interest to be coordinated.

[0072] A nucleic acid construct or vector comprising a coding sequence for a pharmacologically active molecule of interest is preferably under the control of a promoter for expression in *H. pylori*.

[0073] In one embodiment, the promoter employed in accordance with the present invention is expressed constitutively in *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 may be 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%.

[0074] In some embodiments, a method is provided comprising delivering a messenger nucleic acid sequence, such as an mRNA sequence, corresponding to a nucleic acid sequence encoding a molecule of interest to an animal. By way of example, such a messenger nucleic acid sequence (mRNA) corresponding to a therapeutic peptide, protein, hormone or pro-hormone may be prepared so as to provide a peptide, protein, or hormone to the animal upon expression of that messenger nucleic acid sequence in the animal. For example, such a hormone may be insulin, and such a pro-hormone may be pro-insulin. Thus, it is envisioned that the present invention has application as a gene therapy method for the treatment of human disease, such as for the treatment of diabetes.

[0075] 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 an arabinose inducible promoter. Other promoters include FlaB sigma 54 promoter (Josenhans et at., 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).

[0076] In another embodiment the promoter is inducible. Inducible promoters that may be used with clinical grade vectors include, but are not limited to, an inducible promoter as described in U.S. Pat. No. 6,242,194 issued to Kullen et at., a lactose inducible promoter such, as that used in *E. coli*

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