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DESCRIPTION

FIELD OF THE INVENTION

[0001] In various aspects, the invention relates to immunological therapies for treating Crohn's disease.

BACKGROUND OF THE INVENTION

[0002] 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 localized areas of cancer, and are relatively ineffective when the cancer metastasizes. 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.

[0003] The inherent shortcomings of chemotherapy have led to disparate efforts to recruit various aspects of the immune system to treat cancers. A subset of this work relates to immunization with microbial vaccines. Although this approach has a relatively long history, as discussed in more detail below, the field is a very confused mixture of sometimes intriguing successes mixed with many failures that together have failed to produce a cohesive therapeutic approach amenable to widespread clinical adoption.

[0004] Alternative approaches for the treatment of cancers have included therapies that involve augmentation of immune system function such as cytokine therapy (for 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 microbial vaccine therapies, the latter being thought to engage the host system in a non-specific manner. Microbial vaccines have been used to vaccinate subjects against pathogens that are associated with cancer, such as the human papillomavirus. Immunostimulatory microbial vaccines that are not targeted to cancer-causing organisms, *i.e.*, non-specific immunostimulatory vaccines, such as pyrogenic vaccines, have a long clinical history that includes reports of successes and failures in treating a variety of cancers. For example, Coley's vaccine (a combination of *Streptococcus pyogenes* and *Serratia marcescens*) has

been reported to be helpful for the treatment of sarcomas, and lymphomas (see, for *e.g.*, Nauts HC, Fowler GAA, Bogato FH. A review of the influence of bacterial infection and of bacterial products [Coley's toxins] on malignant tumors in man. Acta Med Scand 1953; 145 [Suppl. 276]:5-103). Clinical trials have reportedly demonstrated the benefit of Coley's vaccine treatment for lymphoma and melanoma (see, for *e.g.,* Kempin S, Cirrincone C, Myers J et al: Combined modality therapy of advanced nodular lymphomas: the role of nonspecific immunotherapy [MBV] as an important determinant of response and survival. Proc Am Soc Clin Oncol 1983;24:56; Kolmel KF, Vehmeyer K. Treatment of advanced malignant melanoma by a pyrogenic bacterial lysate: a pilot study. Onkologie 1991;14:411-17).

[0005] It has been suggested that the effectiveness of some non-specific bacterial cancer vaccines is attributable to particular bacterial components or products, such as bacterial DNA or endotoxin (LPS), or because they induce the expression of particular factors, such as tumor necrosis factor (TNF) or interleukin-12. A correspondingly broad range of physiological mechanisms have been ascribed to such treatments, ranging from generalized effects of fever to anti-angiogenic mechanisms. In accordance with these various principles, a wide variety of microbial vaccines have been tested as general immune stimulants for the treatment of cancer. While most have shown negative results, a few have shown some intruiguing positive results in certain contexts, as discussed below.

[0006] Intradermal BCG (*Mycobacterium bovis)* vaccine treatment has been reported to be effective for the treatment of stomach cancer (see, for *e.g.,* Ochiai T, Sato J, Hayashi R, et al: Postoperative adjuvant immunotherapy of gastric cancer with BCG-cell wall endoskeleton. Three- to six-year follow-up of a randomized clinical trial. Cancer Immunol Immunother 1983; 14:167-171) and colon cancer (Smith RE, Colangelo L, Wieand HS, Begovic M, Wolmark N. Randomized trial of adjuvant therapy in colon carcinoma: 10-Year results of NSABP protocol C-01. J. NCI 2004;96[15]:1128-32; Uyl-de Groot CA, Vermorken JB, Hanna MG, Verboon P, Groot MT, Bonsel GJ, Meijer CJ, Pinedo HM. Immunotherapy with autologous tumor cell-BCG vaccine in patients with colon cancer: a prospective study of medical and economic benefits Vaccine 2005; 23[17-18]:2379-87).

[0007] *Mycobacterium* w vaccine therapy, in combination with chemotherapy and radiation, was found to significantly improve quality of life and response to treatment in patients with lung cancer (see for *e.g.*, Sur P, Dastidar A. Role of Mycobacterium w as adjuvant treatment of lung cancer [non-small cell lung cancer]. J Indian Med Assoc 2003 Feb; 101 [2]:118-120). Similarly, *Mycobacterium vaccae* vaccine therapy was found to improve quality of life (see, for *e.g.*, O'Brien M, Anderson H, Kaukel E, et al. SRL172 [killed Mycobacterium vaccae] in addition to standard chemotherapy improves quality of life without affecting survival, in patients with advanced non-small-cell lung cancer: phase III results. Ann Oncol 2004 Jun;15[6];906-14) and symptom control (Harper-Wynne C, Sumpter K, Ryan C, et al. Addition of SRL 172 to standard chemotherapy in small cell lung cancer [SCLC] improves symptom control. Lung Cancer 2005 Feb;47[2]:289-90) in lung cancer patients.

[0008] *Corynebacterium parvum* vaccine was linked with a trend towards improved survival for

the treatment of melanoma (see, for *e.g.,* Balch CM, Smalley RV, Bartolucci AA, et al. A randomized prospective trial of adjuvant C. parvum immunotherapy in 260 patients with clinically localized melanoma [stage I]. Cancer 1982 Mar 15;49[6]:1079-84).

[0009] Intradermal *Streptococcus pyogenes* vaccine therapy was found to be effective for the treatment of stomach cancer (see, for *e.g.*, Hanaue H, Kim DY, Machimura T, et al. Hemolytic streptococcus preparation OK-432; beneficial adjuvant therapy in recurrent gastric carcinoma. Tokai J Exp Clin Med 1987 Nov;12[4]:209-14).

[0010] *Nocardia rubra* vaccine was found to be effective for the treatment of lung cancer (see, for *e.g.*, Yasumoto K, Yamamura Y. Randomized clinical trial of non-specific immunotherapy with cell-wall skeleton of Nocardia rubra. Biomed Pharmacother 1984;38[1]:48-54; Ogura T. Immunotherapy of respectable lung cancer using Nocardia rubra cell wall skeleton. Gan To Kagaku Ryoho 1983 Feb;10[2 Pt 2]:366-72) and linked to a trend to improved survival for the treatment acute myelogenous leukemia (Ohno R, Nakamura H, Kodera Y, et al. Randomized controlled study of chemoimmunotherapy of acute myelogenous leukemia [AML] in adults with Nocardia rubra cell-wall skeleton and irradiated allogeneic AML cells. Cancer 1986 Apr 15;57[8]: 1483-8).

[0011] *Lactobacillus casei* vaccine treatment combined with radiation was found to more effective for the treatment of cervical cancer than radiation alone. (see, for *e.g.*, Okawa T, Kita M, Arai T, et al. Phase II randomized clinical trial of LC9018 concurrently used with radiation in the treatment of carcinoma of the uterine cervix. Its effect on tumor reduction and histology. Cancer 1989 Nov 1;64[9]:1769-76)

[0012] *Pseudomonas aeruginosa* vaccine treatment was found to increase the effectiveness of chemotherapy in the treatment of lymphoma and lung cancer (see, for *e.g.,* Li Z, Hao D, Zhang H, Ren L, et al. A clinical study on PA_MSHA vaccine used for adjuvant therapy of lymphoma and lung cancer. Hua Xi Yi Ke Da Xue Xue Bao 2000 Sep;31[3]:334-7).

[0013] Childhood vaccination with the smallpox vaccine (*i.e.,* Vaccinia virus vaccine) was found to be associated with a decreased risk of melanoma later in life (see, for *e.g.*, Pfahlberg A, Kolmel KF, Grange JM. et al. Inverse association between melanoma and previous vaccinations against tuberculosis and smallpox: results of the FEBIM study. J Invest Dermatol 2002[119]:570-575) as well as decreased mortality in those patients who did develop melanoma (see, for *e.g.*, Kolmel KF, Grange JM, Krone B, et al. Prior immunization of patients with malignant melanoma with vaccinia or BCG is associated with better survival. European Organization for Research and Treatment of Cancer cohort study on 542 patients. Eur J Cancer 41[2005]:118-125).

[0014] Treatment with rabies virus vaccine was found to result in temporary remission in 8 of 30 patients with melanoma (see, for *e.g.*, Higgins G, Pack G. Virus therapy in the treatment of tumors. Bull Hosp Joint Dis 1951;12:379-382; Pack G. Note on the experimental use of rabies vaccine for melanomatosis. Arch Dermatol 1950;62:694-695).

[0015] In spite of substantial efforts to engage the immune system to combat cancers using non-specific immunostimulatory microbial vaccines, the vast majority of these efforts have failed and there is little clinical or research evidence of widespread success in improving the survival of cancer patient populations. While it has been recognized that immunostimulatory microbial vaccine approaches have promise, it has also been recognized that significant challenges characterize the field (see, for *e.g.,* Ralf Kleef, Mary Ann Richardson, Nancy Russell, Cristina Ramirez. "Endotoxin and Exotoxin Induced Tumor Regression with Special Reference to Coley Toxins: A Survey of the Literature and Possible Immunological Mechanisms." Report to the National Cancer Institute Office of Alternative and Complementary Medicine August 1997; DL Mager. "Bacteria and Cancer: Cause, Coincidence or Cure? A Review." Journal of Translational Medicine 28 March 2006 4[14]:doi:10.1186/1479-5876-4-14).

[0016] Inflammatory bowel disease (IBD) is a name frequently given to a group of inflammatory conditions of the colon and small intestine, generally characterized by similar symptoms and indeterminate etiology. Major sub-types of IBD are recognized clinically as Crohn's disease and ulcerative colitis. In addition to Crohn's disease and ulcerative colitis, IBD may also include conditions recognized as any one of the following: collagenous colitis, lymphocytic colitis, ischaemic colitis, diversion colitis, Behçet's syndrome or indeterminate colitis. The difference between these conditions relate primarily to the location and nature of the inflammatory changes in the gastrointestinal tract (GIT). Crohn's disease, for example, is generally recognized as potentially affecting any part of the gastrointestinal tract, from mouth to anus, with a majority of the cases marked by relapsing and remitting granulomatous inflammation of the alimentary tract in the terminal ileum and colon. Ulcerative colitis, in contrast, is generally considered to be restricted to the colon and the rectum. The various regions of the gastrointestinal tract in which these inflammatory conditions may exhibit symptoms include: the bowel or intestine, including: the small intestine (which has three parts: the duodenum, the jejunum, and the ileum); the large intestine (which has three parts: the cecum, the colon, which includes the ascending colon, transverse colon, descending colon and sigmoid flexure; and the rectum); and, the anus.

[0017] The understanding of inflammatory bowel diseases is evolving, but is as yet incomplete in many respects (see, for e.g., Baumgart DC, Carding SR (2007) "Inflammatory bowel disease: cause and immunobiology" The Lancet 369 (9573): 1627-40; Baumgart DC, Sandborn WJ (2007) "Inflammatory bowel disease: clinical aspects and established and evolving therapies" The Lancet 369 (9573): 1641-57; Xavier RJ, Podolsky DK (2007) "Unravelling the pathogenesis of inflammatory bowel disease" Nature 448 (7152): 427-34; J. H. Cho (2008) "The genetics and immunopathogenesis of inflammatory bowel disease" Nature Reviews Immunology 8, 458-466).

[0018] Anti-inflammatory drugs and immune system suppressants may be used in the treatment of IBD, such as sulfasalazine (Azulfidine™), mesalamine (Asacol™, Rowasa™), corticosteroids (e.g. prednisone), azathioprine (Imuran™), mercaptopurine (Purinethol™), infliximab (Remicade™), adalimumab (Humira™), certolizumab pegol (Cimzia™),

methotrexate (Rheumatrex™), cyclosporine (Gengraf™, Neoral™, Sandimmune™) or natalizumab (Tysabri™).

[0019] Alternative treatments for IBD have been suggested, including the use of various biological agents, or treatments that purportedly adjust natural intestinal flora, sometimes called probiotic treatments (US 2007/0258953; US 2008/0003207; WO 2007/076534; WO 2007/136719; WO 2010/099824). It has for example been reported that IBD may be treated with a deliberate infestation of parasitic worms, for example by consumption of the live ova of the Trichuris suis helminth (Summers et al. (2003) "Trichuris suis seems to be safe and possibly effective in the treatment of inflammatory bowel disease". Am. J. Gastroenterol. 98 (9): 2034-41; Büning et al., (2008) "Helminths as governors of inflammatory bowel disease" Gut 57:1182-1183; Weinstock and Elliott (2009) "Helminths and the IBD hygiene hypothesis" Inflamm Bowel Dis. 2009 Jan;15(1):128-33). Torres et al. (1995) "Evaluation of formalin-inactivated Clostridium difficile vaccines administered by parenteral and mucosal routes of immunization in hamsters". Infection and Immunity. 63 (12): 4619-27 reports on the evaluation of valuated *C. difficile* antigens as vaccines to protect against systemic and intestinal disease in a hamster model of clindamycin colitis, and found that hamsters immunized by the intranasal, intraperitoneal, and subcutaneous routes were 100% protected against death and partially protected (40, 40, and 20%, respectively) against diarrhea, whereas hamsters immunized intranasally and revaccinated intraperitoneally were 100% protected against both death and diarrhea. The authors concluded that protection against death and diarrhea correlated with antibody responses to all antigens tested, and that optimal protection against *C. difficile* disease can be achieved with combined parenteral and mucosal immunization.

SUMMARY OF THE INVENTION

[0020] In one aspect, the present invention provides an antigenic composition for use in treating a human patient for Crohn's disease, wherein the antigenic composition comprises whole killed or attenuated pathogenic *Escherichia coli* cells, and wherein the antigenic composition is administered intradermally or subcutaneously at an administration site in successive doses given at a dosage interval of between one hour and one month, over a dosage duration of at least 1 week, 2 weeks, 2 months or 6 months.

[0021] Optionally, the the antigenic composition may be formulated for injection to produce a localized skin immune response at a site of administration.

[0022] The antigenic composition for use in accordance with the present invention is formulated for repeated subcutaneous or intradermal administration as described above. A method for preparing the antigenic composition may involve killing the E. *coli* pathogen to formulate the antigenic composition as a whole killed pathogen composition.

[0023] The present application also describes a method of comparing immune responses. The method involves administering to an animal having an organ or tissue a medicament

having an antigenic composition having antigenic determinants selected or formulated so that together the antigenic determinants are specific for at least one microbial pathogen that is pathogenic in the organ or tissue, extracting a quantifiable immune sample from the organ or tissue, measuring a characteristic of the immune response in the organ or tissue in the quantifiable immune sample following the administration of the medicament, and, comparing the characteristic of the immune response in the quantifiable immune sample to a corresponding characteristic of the immune response in a reference immune sample obtained from a corresponding organ or tissue. Optionally, the reference immune sample may be obtained from the corresponding organ or tissue in the animal prior to the step of administering the medicament. Optionally, the reference immune sample may be obtained from the corresponding organ or tissue in a second animal. Optionally, the animal may have a cancer situated in the organ or tissue.

[0024] Comparing the characteristic of the immune response may involve comparing, in the quantifiable and reference immune samples, an indication of the numbers of any one or more of the following cells: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or NK cells. Optionally, the macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages. Further, comparing the characteristic of the immune response may involve comparing a shift in an activation state of macrophages. Optionally, the macrophages may shift from being M2-like macrophages to being M1-like macrophages. Further and optionally, the macrophages may shift from being M1-like macrophages to being M2-like macrophages.

[0025] Optionally, comparing the characteristic of the immune response may involve identifying, in the quantifiable and reference immune samples, cellular markers on any one or more of the following cells: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or NK cells. The macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages.

[0026] Optionally, comparing the characteristic of the immune response may involve identifying, in the quantifiable and reference immune samples, cytokines produced by any one or more of the following cells: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or NK cells. As detailed herein, the macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages. Optionally, the cytokines are produced as a result of a shift in an activation state of the macrophages. Optionally, the macrophages shift from being M2-like macrophages to being M1-like macrophages. Further and optionally, the macrophages shift from being M1-like macrophages to being M2-like macrophages.

[0027] Optionally, comparing the characteristic of the immune response may involve identifying, in the quantifiable and reference immune samples, differential gene expression produced by any one or more of the following cells: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or

NK cells. The macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages. Optionally, the differential gene expression is produced as a result of a shift in an activation state of the macrophages. Optionally, macrophages may shift from being M2-like macrophages to being M1-like macrophages. Further and optionally, the macrophages shift from being M1-like macrophages to being M2-like macrophages.

[0028] In accordance with the present invention, the antigenic composition is administered administered intradermally or subcutaneously at an administration site in successive doses given at a dosage interval of between one hour and one month, over a dosage duration of at least 1 week, 2 weeks, 2 months or 6 months. Optionally, the antigenic composition may be administered in a dose so that each dose is effective to cause a visible localized inflammatory immune response at the administration site. Optionally, the medicament may be administered so that visible localized inflammation at the administration site occurs within 1 to 48 hours.

[0029] The method detailed herein may further involve measuring the characteristic of the immune response in a pre-treatment reference sample, wherein the pre-treatment reference sample was obtained from the specific organ or tissue before, at the same time as or after commencement of the treatment regime, but prior to obtaining the post-treatment immune sample, and comparing the characteristic of the immune response in the pre-treatment and post-treatment samples, wherein an increase in the magnitude of the immune response in the post-treatment immune sample compared to the pre-treatment reference sample is indicative of the efficacy of the treatment regime. Optionally, measuring the characteristic of the immune response may involve determining an indication of the number of inflammatory monocytes in a sample of the organ or tissue. Optionally, measuring the characteristic of the immune response may involve determining an indication of the number of macrophages in a sample of the organ or tissue. The macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages.

[0030] Optionally, measuring the characteristic of the immune response may involve determining an indication of the number of CD11b+ Gr-1+ cells in a sample of the organ or tissue or determining an indication of the number of dendritic cells in a sample of the organ or tissue. Further and optionally, measuring the characteristic of the immune response may involve determining an indication of the number of CD11c+ MHC class II+ cells in a sample of the organ or tissue or determining an indication of the number of CD4+ T cells in a sample of the organ or tissue or determining an indication of the number of CD8+ T cells in a sample of the organ or tissue.

[0031] Optionally, measuring the magnitude of the immune response may involve determining an indication of the number of NK cells in a sample of the organ or tissue. Further and optionally, comparing the characteristic of the immune response may involve identifying, in the reference and immune samples, cellular markers on any one or more of the following cells: inflammatory monocytes, macrophages, CD11b+ Gr-1 + cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or NK cells. Optionally, the macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages.

[0032] Further and optionally, comparing the characteristic of the immune response may involve identifying, in the reference and immune samples, cytokines produced by any one or more of the following cells: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or NK cells. The macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages. Optionally, the cytokines may be produced as a result of a shift in an activation state of the macrophages. The macrophages may shift from being M2-like macrophages to being M1-like macrophages. Further and optionally, the macrophages may shift from being M1-like macrophages to being M2-like macrophages.

[0033] Optionally, comparing the characteristic of the immune response may involve identifying, in the reference and immune samples, differential gene expression produced by any one or more of the following cells: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or NK cells. The macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages. The differential gene expression may be produced as a result of a shift in an activation state of the macrophages. The macrophages may shift from being M2-like macrophages to being M1-like macrophages. Optionally, the macrophages may shift from being M1-like macrophages to being M2-like macrophages.

[0034] The antigenic composition for use in accordance with the present invention is formulated for subcutaneous injection or intradermal injection. In embodiments, the dosing or formulation of the antigenic composition may be adjusted in order to produce a localized immune reaction visible in the skin at the site of administration, for example an area of inflammation from 2mm to 100mm in diameter appearing, for example, 2 - 48 hours after administration and lasting, for example, 2 - 72 hours or longer.

[0035] The antigenic composition may be used in accordance with the present invention by administration, by subcutaneous or intradermal injection, at an administration site, in successive doses given at a dosage interval, for example of between one hour and one month, over a dosage duration, for example of at least 1 week, 2 weeks, 2 months, 6 months, 1, 2, 3, 4, or 5 years or longer. Each injection dose may for example be metered so that it is effective to cause visible localized inflammation at the administration site, appearing, for example, 1 - 48 hours after injection.

[0036] In one embodiment, the whole killed or attenuated pathogenic Escherichia coli cells used in the antigenic composition of the present invention may have been capable of causing infection naturally, (i.e., without human intervention) in the gastrointestinal tract in a healthy subject, or may have caused an infection in the gastrointestinal tract in a healthy subject.

[0037] Optionally, the whole *Escherichia coli* cells used in the antigenic composition of the present invention may be killed, and thus rendered non-infectious.

[0038] Accordingly, the present invention provides an immunogenic composition, as defined above, for use in treating Crohn's disease. The treatment of the patient may involve a diagnostic step of identifying the region of the GIT within which the IBD is symptomatic. The antigenic composition may comprise whole killed or attenuated Escherichia coli cells that are pathogenic in the effected region of the GIT. The formulation may be prepared for administration as an immunogenic composition capable of eliciting an immune reaction to treat the Crohn's disease, in accordance with the use defined by Claim 1. The composition is formulated for subcutaneous injection or intradermal injection, for example to produce a localized skin immune response, such as an inflammatory response, at a site of administration.

DETAILED DESCRIPTION OF THE INVENTION

[0039] The present inventors have made the surprising discovery that administration, for example at a site distant from the cancer, of microbial pathogens, such as killed microbial pathogens, that are pathogenic in a particular tissue or organ is effective in treating cancer situated in that specific tissue or organ. Accordingly, as described herein, an antigenic compositions derived from these microbial pathogens, including whole killed bacterial or viral species, or components thereof, may be used for the treatment of cancer.

[0040] Based on observations from treating patients, it was found that administering compositions of killed bacteria which included many of the bacterial species that commonly cause lung infection was surprisingly and unexpectedly effective in improving the clinical course of cancer of the lung. Similarly, it was found that administering compositions including killed *Staphylococcus aureus,* one of the most common causes of bone, breast, skin, perineal and lymph node infection and septicemia was surprising and unexpectedly effective in improving the clinical course of cancer of the bone, breast, skin, perineum, and lymphoma (cancer of the lymph glands) and multiple myeloma (a type of hematological cancer). Similarly, it was surprisingly and unexpectedly found that administering a composition including *Escherichia coli,* which is a common cause of colon, kidney, peritoneal, liver, abdominal, pancreatic and ovarian infection, was effective in improving the clinical course of cancer in the colon, kidney, peritoneum, liver, abdominal lymph nodes, pancreas and ovary.

[0041] These results indicate that a composition including antigens of pathogenic microbial species that cause infection in a particular tissue or organ will be an effective formulation for treating a cancer in that tissue or organ. For example, cancer in the lung is effectively treated with a microbial composition including one or more pathogenic species that commonly cause lung infection, while cancer in the colon is effectively treated with a composition including pathogenic microbial species that commonly cause colon infections.

[0042] However, more particularly, the present invention provides an antigenic composition for use in treating a human patient for Crohn's disease, wherein the antigenic composition comprises whole killed or attenuated pathogenic *Escherichia coli* cells, and wherein the antigenic composition is administered intradermally or subcutaneously at an administration site

in successive doses given at a dosage interval of between one hour and one month, over a dosage duration of at least 1 week, 2 weeks, 2 months or 6 months.

[0043] Antigenic compositions of the invention may be produced that include antigenic determinants that together are specific for or characteristic of a microbial pathogen. In this context, by "specific", it is meant that the antigenic determinants are sufficiently characteristic of the pathogen that they could be used to raise an immune response, such as an adaptive immune response, against the pathogen in the patient, if the antigenic determinants were to be administered in an appropriate manner to have that effect. It will be recognized that the antigenic determinants need not be so specific that they are characteristic of only one particular strain or species of pathogen, since even a specific immune response against a particular pathogen may be cross reactive with other closely related organisms that are also naturally pathogenic in the tissue or organ in which the cancer is situated and that the antigenic composition is formulated or selected to target.

[0044] Various alternative embodiments and examples of the invention are described herein. These embodiments and examples are illustrative and should not be construed as limiting the scope of the invention.

[0045] A "cell" is the basic structural and functional unit of a living organism. In higher organisms, *e.g.,* animals, cells having similar structure and function generally aggregate into "tissues" that perform particular functions. Thus, a tissue includes a collection of similar cells and surrounding intercellular substances, *e.g.,* epithelial tissue, connective tissue, muscle, nerve. An "organ" is a fully differentiated structural and functional unit in a higher organism that may be composed of different types of tissues and is specialized for some particular function, *e.g.*, kidney, heart, brain, liver, *etc.* Accordingly, by "specific organ, tissue, or cell" is meant herein to include any particular organ, and to include the cells and tissues found in that organ.

[0046] "Pathogenic" agents are agents, such as microbes, such as bacteria or viruses, which are known to cause infection in a host in nature, and in this sense, "pathogenic" is used in the context of the present invention to mean "naturally pathogenic". Although a wide variety of microbes may be capable of causing infection under artificial conditions, such as artificial innoculations of a microbe into a tissue, the range of microbes that naturally cause infection is necessarily limited, and well established by medical practice.

[0047] An "infection" is the state or condition in which the body or a part of it is invaded by a pathogenic agent (e.g., a microbe, such as a bacterium) which, under favorable conditions, multiplies and produces effects that are injurious (Taber's Cyclopedic Medical Dictionary, 14th Ed., C.L. Thomas, Ed., F.A. Davis Company, PA, USA). An infection may not always be apparent clinically and may result in only localized cellular injury. Infections may remain subclinical, and temporary if the body's defensive mechanisms are effective. Infections may spread locally to become clinically apparent as an acute, a subacute, or a chronic clinical infection or disease state. A local infection may also become systemic when the pathogenic agent gains access to the lymphatic or vascular system (On-Line Medical Dictionary,

http://cancerweb.ncl.ac.uk/omd/). Infection is usually accompanied by inflammation, but inflammation may occur without infection.

[0048] "Inflammation" is the characteristic tissue reaction to injury (marked by swelling, redness, heat, and pain), and includes the successive changes that occur in living tissue when it is injured. Infection and inflammation are different conditions, although one may arise from the other (Taber's Cyclopedic Medical Dictionary, *supra*). Accordingly, inflammation may occur without infection and infection may occur without inflammation (although inflammation typically results from infection by pathogenic bacteria or viruses). Inflammation is characterized by the following symptoms: redness (rubor), heat (calor), swelling (tumor), pain (dolor). Localized visible inflammation on the skin may be apparent from a combination of these symptoms, particularly redness at a site of administration.

[0049] Although it is envisaged that various subjects may be treated, human patients with Crohn's disease are treated in accordance with the present invention. As used herein, a "subject" is an animal, for *e.g*, a mammal, to whom the specific pathogenic bacteria, bacterial antigens, viruses, viral antigens or compositions may be administered. In some embodiments, the terms "subject" and "patient" may be used interchangeably. A healthy subject may be a human who is not suffering from a cancer or suspected of having a cancer, or who is not suffering from a chronic disorder or condition. A "healthy subject" may also be a subject who is not immunocompromised. By immunocompromised is meant any condition in which the immune system functions in an abnormal or incomplete manner. Immunocompromisation may be due to disease, certain medications, or conditions present at birth. Immunocompromised subjects may be found more frequently among infants, the elderly, and individuals undergoing extensive drug or radiation therapy.

[0050] An "immune response" includes, but is not limited to, one or more of the following responses in a mammal: induction or activation of antibodies, neutrophils, monocytes, macrophages (including both M1-like macrophages and M2-like macrophages as described herein), B cells, T cells (including helper T cells, natural killer cells, cytotoxic T cells, □□T cells), such as induction or activation by the antigen(s) in a composition or vaccine, following administration of the composition or vaccine. An immune response to a composition or vaccine thus generally includes the development in the host animal of a cellular and/or antibody-mediated response to the composition or vaccine of interest. In some embodiments, the immune response is such that it will also result in slowing or stopping the progression of a cancer in the animal. An immune response includes both cellular immune responses and humoral immune responses as understood by those persons skilled in the art.

Bacteria and Bacterial Colonizations and Infections

[0051] Most animals are colonized to some degree by other organisms, such as bacteria, which generally exist in symbiotic or commensal relationships with the host animal. Thus, many species of normally harmless bacteria are found in healthy animals, and are usually localized

to the surface of specific organs and tissues. Often, these bacteria aid in the normal functioning of the body. For example, in humans, symbiotic *Escherichia coli* bacteria may be found in the intestine, where they promote immunity and reduce the risk of infection with more virulent pathogens.

[0052] Bacteria that are generally harmless, such as *Escherichia coli,* can cause infection in healthy subjects, with results ranging from mild to severe infection to death. Whether or not a bacterium is pathogenic *(i.e.,* causes infection) depends to some extent on factors such as the route of entry and access to specific host cells, tissues, or organs; the intrinsic virulence of the bacterium; the amount of the bacteria present at the site of potential infection; or the health of the host animal. Thus, bacteria that are normally harmless can become pathogenic given favorable conditions for infection, and even the most virulent bacterium requires specific circumstances to cause infection. Accordingly, microbial species that are members of the normal flora can be pathogens when they move beyond their normal ecological role in the endogenous flora. For example, endogenous species can cause infection outside of their ecological niche in regions of anatomical proximity, for example by contiguous spread. When this occurs, these normally harmless endogenous bacteria are considered pathogenic.

[0053] Specific bacterial species and viruses are known to cause infections in specific cells, tissues, or organs in otherwise healthy subjects. Examples of bacteria and viruses that commonly cause infections in specific organs and tissues of the body are listed below; it will be understood that these examples are not intended to be limiting and that a skilled person would be able to readily recognize and identify infectious or pathogenic bacteria that cause infections, or commonly cause infections, in various organs and tissues in healthy adults (and recognize the relative frequency of infection with each bacterial species) based on the knowledge in the field as represented, for example, by the following publications: Manual of Clinical Microbiology 8th Edition, Patrick Murray, Ed., 2003, ASM Press American Society for Microbiology, Washington DC, USA; Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases 5th Edition, G. L. Mandell, J.E. Bennett, R. Dolin, Eds., 2000, Churchill Livingstone, Philadelphia, PA, USA.

[0054] Infections of the skin are commonly caused by the following bacterial species: *Staphylococcus aureus,* Beta hemolytic streptococci group A, B, C or G, *Corynebacterium diptheriae, Corynebacterium ulcerans,* or *Pseudomonas aeruginosa*; or viral pathogens: rubeola, rubella, varicella-zoster, echoviruses, coxsackieviruses, adenovirus, vaccinia, herpes simplex, or parvo B19.

[0055] Infections of the soft tissue *(e.g.,* fat and muscle) are commonly caused by the following bacterial species: *Streptococcus pyogenes, Staphylococcus aureus, Clostridium perfringens,* or other Clostridium spp.; or viral pathogens: influenza, or coxsackieviruses.

[0056] Infections of the breast are commonly caused by the following bacterial species: *Staphylococcus aureus,* or *Streptococcus pyogenes.*

[0057] Infections of the lymph nodes of the head and neck are commonly caused by the following bacterial species: Staphylococcus *aureus, or Streptococcus pyogenes*; or viral pathogens: Epstein-Barr, cytomegalovirus, adenovirus, measles, rubella, herpes simplex, coxsackieviruses, or varicella-zoster.

[0058] Infections of the lymph nodes of the arm/axillae are commonly caused by the following bacterial species: Staphylococcus *aureus, or Streptococcus pyogenes*; or viral pathogens: measles, rubella, Epstein-Barr, cytomegalovirus, adenovirus, or varicella-zoster.

[0059] Infections of the lymph nodes of the mediastinum are commonly caused by the following bacterial species: viridans streptococci, Peptococcus spp., Peptostreptococcus spp., Bacteroides spp., Fusobacterium spp., or Mycobacterium *tuberculosis*; or viral pathogens: measles, rubella, Epstein-Barr, cytomegalovirus, varicella-zoster, or adenovirus.

[0060] Infections of the pulmonary hilar lymph nodes are commonly caused by the following bacterial species: *Streptococcus pneumoniae, Moraxella catarrhalis, Mycoplasma pneumoniae, Klebsiella pneumoniae, Haemophilus influenza, Chlamydophila pneumoniae, Bordetella pertussis or Mycobacterium tuberculosis;* or viral pathogens: influenza, adenovirus, rhinovirus, coronavirus, parainfluenza, respiratory syncytial virus, human metapneumovirus, or coxsackievirus.

[0061] Infections of the intra-abdominal lymph nodes are commonly caused by the following bacterial species: *Yersinia enterocolitica, Yersinia pseudotuberculosis,* Salmonella spp., *Streptococcus pyogenes, Escherichia coli, Staphylococcus aureus,* or *Mycobacterium tuberculosis;* or viral pathogens: measles, rubella, Epstein-Barr, cytomegalovirus, varicella-zoster, adenovirus, influenza, or coxsackieviruses.

[0062] Infections of the lymph nodes of the leg/inguinal region are commonly caused by the following bacterial species: *Staphylococcus aureus,* or *Streptococcus pyogenes*; or viral pathogens: measles, rubella, Epstein-Barr, cytomegalovirus, or herpes simplex.

[0063] Infections of the blood (*i.e.*, septicemia) are commonly caused by the following bacterial species: *Staphylococcus aureus, Streptococcus pyogenes,* coagulase-negative staphylococci, Enterococcus spp., *Escherichia coli,* Klebsiella spp., Enterobacter spp., Proteus spp., *Pseudomonas aeruginosa, Bacteroides fragilis, Streptococcus pneumoniae,* or group B streptococci; or viral pathogens: rubeola, rubella, varicella-zoster, echoviruses, coxsackieviruses, adenovirus, Epstein-Barr, herpes simplex, or cytomegalovirus.

[0064] Infections of the bone are commonly caused by the following bacterial species: *Staphylococcus aureus,* coagulase-negative staphylococci, *Streptococcus pyogenes, Streptococcus pneumoniae, Streptococcus agalactiae,* other streptococci spp., *Escherichia coli,* Pseudomonas spp., Enterobacter spp., Proteus spp., or Serratia spp.; or viral pathogens: parvovirus B19, rubella, or hepatitis B.

[0065] Infections of the meninges are commonly caused by the following bacterial species: *Haemophilus influenzae, Neisseria meningitidis, Streptococcus pneumoniae, Streptococcus agalactiae,* or *Listeria monocytogenes*; or viral pathogens: echoviruses, coxsackieviruses, other enteroviruses, or mumps.

[0066] Infections of the brain are commonly caused by the following bacterial species: Streptococcus spp. (including *S. anginosus, S. constellatus, S. intermedius*), *Staphylococcus aureus,* Bacteroides spp., Prevotella spp., Proteus spp., *Escherichia coli,* Klebsiella spp., Pseudomonas spp., Enterobacter spp., or *Borrelia burgdorferi*; or viral pathogens: coxsackieviruses, echoviruses, poliovirus, other enteroviruses, mumps, herpes simplex, varicella-zoster, flaviviruses, or bunyaviruses.

[0067] Infections of the spinal cord are commonly caused by the following bacterial species: *Haemophilus influenzae, Neisseria meningitidis, Streptococcus pneumoniae, Streptococcus agalactiae, Listeria monocytogenes,* or *Borrelia burgdorferi*; or viral pathogens: coxsackieviruses, echoviruses, poliovirus, other enteroviruses, mumps, herpes simplex, varicella-zoster, flaviviruses, or bunyaviruses.

[0068] Infections of the eye/orbit are commonly caused by the following bacterial species: *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae, Streptococcus milleri, Escherichia coli, Bacillus cereus, Chlamydia trachomatis, Haemophilus influenza,* Pseudomonas spp., Klebsiella spp., or *Treponema pallidum*; or viral pathogens: adenoviruses, herpes simplex, varicella-zoster, or cytomegalovirus.

[0069] Infections of the salivary glands are commonly caused by the following bacterial species: *Staphylococcus aureus,* viridans streptococci (e.g., *Streptococcus salivarius, Streptococcus sanguis, Streptococcus mutans*), Peptostreptococcus spp., or Bacteroides spp., or other oral anaerobes; or viral pathogens: mumps, influenza, enteroviruses, or rabies.

[0070] Infections of the mouth are commonly caused by the following bacterial species: *Prevotella melaninogenicus,* anaerobic streptococci, viridans streptococci, Actinomyces spp., Peptostreptococcus spp., or Bacteroides spp., or other oral anaerobes; or viral pathogens: herpes simplex, coxsackieviruses, or Epstein-Barr.

[0071] Infections of the tonsils are commonly caused by the following bacterial species: *Streptococcus pyogenes,* or Group C or G B-hemolytic streptococci; or viral pathogens: rhinoviruses, influenza, coronavirus, adenovirus, parainfluenza, respiratory syncytial virus, or herpes simplex.

[0072] Infections of the sinuses are commonly caused by the following bacterial species: *Streptococcus pneumoniae, Haemophilus influenza, Moraxella catarrhalis,* α-streptococci, anaerobic bacteria (*e.g.,* Prevotella spp.), or *Staphylococcus aureus*; or viral pathogens: rhinoviruses, influenza, adenovirus, or parainfluenza.

[0073] Infections of the nasopharynx are commonly caused by the following bacterial species: *Streptococcus pyogenes,* or Group C or G B-hemolytic streptococci; or viral pathogens: rhinoviruses, influenza, coronavirus, adenovirus, parainfluenza, respiratory syncytial virus, or herpes simplex.

[0074] Infections of the thyroid are commonly caused by the following bacterial species: *Staphylococcus aureus, Streptococcus pyogenes, or Streptococcus pneumoniae*; or viral pathogens: mumps, or influenza.

[0075] Infections of the larynx are commonly caused by the following bacterial species: *Mycoplasma pneumoniae, Chlamydophila pneumoniae,* or *Streptococcus pyogenes*; or viral pathogens: rhinovirus, influenza, parainfluenza, adenovirus, corona virus, or human metapneumovirus.

[0076] Infections of the trachea are commonly caused by the following bacterial species: *Mycoplasma pneumoniae*; or viral pathogens: parainfluenza, influenza, respiratory syncytial virus, or adenovirus.

[0077] Infections of the bronchi are commonly caused by the following bacterial species: *Mycoplasma pneumoniae, Chlamydophila pneumoniae, Bordetella pertussis, Streptococcus pneumoniae,* or *Haemophilus influenzae*; or viral pathogens: influenza, adenovirus, rhinovirus, coronavirus, parainfluenza, respiratory syncytial virus, human metapneumovirus, or coxsackievirus.

[0078] Infections of the lung are commonly caused by the following bacterial species: *Streptococcus pneumoniae, Moraxella catarrhalis, Mycoplasma pneumoniae, Klebsiella pneumoniae,* or *Haemophilus influenza*; or viral pathogens: influenza, adenovirus, respiratory syncytial virus, or parainfluenza.

[0079] Infections of the pleura are commonly caused by the following bacterial species: *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae, Haemophilus influenzae, Bacteroides fragilis,* Prevotella spp., *Fusobacterium nucleatum,* peptostreptococcus spp., or *Mycobacterium tuberculosis;* or viral pathogens: influenza, adenovirus, respiratory syncytial virus, or parainfluenza.

[0080] Infections of the mediastinum are commonly caused by the following bacterial species: viridans streptococci, Peptococcus spp., Peptostreptococcus spp., Bacteroides spp., Fusobacterium spp., or *Mycobacterium tuberculosis*; or viral pathogens: measles, rubella, Epstein-Barr, or cytomegalovirus.

[0081] Infections of the heart are commonly caused by the following bacterial species: Streptococcus spp. (including *S. mitior, S. bovis, S. sanguis, S. mutans, S. anginosus*), Enterococcus spp., Staphylococcus spp., *Corynebacterium diptheriae, Clostridium perfringens, Neisseria meningitidis,* or Salmonella spp.; or viral pathogens: enteroviruses, coxsackieviruses,

echoviruses, poliovirus, adenovirus, mumps, rubeola, or influenza.

[0082] Infections of the esophagus are commonly caused by the following bacterial species: Actinomyces spp., *Mycobacterium avium, Mycobacterium tuberculosis,* or Streptococcus spp.; or viral pathogens: cytomegalovirus, herpes simplex, or varicella-zoster.

[0083] Infections of the stomach are commonly caused by the following bacterial species: *Streptococcus pyogenes or Helicobacter pylori*; or viral pathogens: cytomegalovirus, herpes simplex, Epstein-Barr, rotaviruses, noroviruses, or adenoviruses.

[0084] Infections of the small bowel are commonly caused by the following bacterial species: *Escherichia coli, Clostridium difficile, Bacteroides fragilis, Bacteroides vulgatus, Bacteroides thetaiotaomicron, Clostridium perfringens, Salmonella enteriditis, Yersinia enterocolitica,* or *Shigella flexneri*; or viral pathogens: adenoviruses, astroviruses, caliciviruses, noroviruses, rotaviruses, or cytomegalovirus.

[0085] Infections of the colon/rectum are commonly caused by the following bacterial species: *Escherichia coli, Clostridium difficile, Bacteroides fragilis, Bacteroides vulgatus, Bacteroides thetaiotaomicron, Clostridium perfringens, Salmonella enteriditis, Yersinia enterocolitica,* or *Shigella flexneri*; or viral pathogens: adenoviruses, astroviruses, caliciviruses, noroviruses, rotaviruses, or cytomegalovirus.

[0086] Infections of the anus are commonly caused by the following bacterial species: *Streptococcus pyogenes,* Bacteroides spp., Fusobacterium spp., anaerobic streptococci, Clostridium spp., *Escherichia coli,* Enterobacter spp., *Pseudomonas aeruginosa,* or *Treponema pallidum*; or viral pathogens: herpes simplex.

[0087] Infections of the perineum are commonly caused by the following bacterial species: *Escherichia coli,* Klebsiella spp., Enterococcus spp., Bacteroides spp., Fusobacterium spp., Clostridium spp., *Pseudomonas aeruginosa,* anaerobic streptococci, Clostridium spp., or Enterobacter spp.; or viral pathogens: herpes simplex.

[0088] Infections of the liver are commonly caused by the following bacterial species: *Escherichia coli,* Klebsiella spp., Streptococcus (anginosus group), Enterococcus, spp. other viridans streptococci, or Bacteroides spp.; or viral pathogens: hepatitis A, Epstein-Barr, herpes simplex, mumps, rubella, rubeola, varicella-zoster, coxsackieviruses, or adenovirus.

[0089] Infections of the gallbladder are commonly caused by the following bacterial species: *Escherichia coli,* Klebsiella spp., Enterobacter spp., enterococci, Bacteroides spp., Fusobacterium spp., Clostridium spp., *Salmonella enteriditis, Yersinia enterocolitica,* or *Shigella flexneri.*

[0090] Infections of the biliary tract are commonly caused by the following bacterial species: *Escherichia coli,* Klebsiella spp., Enterobacter spp., enterococci, Bacteroides spp.,

Fusobacterium spp., Clostridium spp., *Salmonella enteriditis, Yersinia enterocolitica,* or *Shigella flexneri*; or viral pathogens: hepatitis A, Epstein-Barr, herpes simplex, mumps, rubella, rubeola, varicella-zoster, cocsackieviruses, or adenovirus.

[0091] Infections of the pancreas are commonly caused by the following bacterial species: *Escherichia coli,* Klebsiella spp., Enterococcus spp., Pseudomonas spp., Staphylococcal spp., Mycoplasma spp., *Salmonella typhi,* Leptospirosis spp., or Legionella spp.; or viral pathogens: mumps, coxsackievirus, hepatitis B, cytomegalovirus, herpes simplex 2, or varicella-zoster.

[0092] Infections of the spleen are commonly caused by the following bacterial species: Streptococcus spp., Staphylococcus spp., Salmonella spp., Pseudomonas spp., *Escherichia coli,* or Enterococcus spp.; or viral pathogens: Epstein-Barr, cytomegalovirus, adenovirus, measles, rubella, coxsackieviruses, or varicella-zoster.

[0093] Infections of the adrenal gland are commonly caused by the following bacterial species: Streptococcus spp., Staphylococcus spp., Salmonella spp., Pseudomonas spp., *Escherichia coli,* or Enterococcus spp.; or viral pathogens: varicella-zoster.

[0094] Infections of the kidney are commonly caused by the following bacterial species: *Escherichia coli, Proteus mirabilis, Proteus vulgatus,* Providentia spp., Morganella spp., *Enterococcus faecalis,* or *Pseudomonas aeruginosa*; or viral pathogens: BK virus, or mumps.

[0095] Infections of the ureter are commonly caused by the following bacterial species: *Escherichia coli, Proteus mirabilis, Proteus vulgatus,* Providentia spp., Morganella spp., or Enterococcus spp.

[0096] Infections of the bladder are commonly caused by the following bacterial species: *Escherichia coli, Proteus mirabilis, Proteus vulgatus,* Providentia spp., Morganella spp., *Enterococcus faecalis,* or *Corynebacterium jekeum*; or viral pathogens: adenovirus, or cytomegalovirus.

[0097] Infections of the peritoneum are commonly caused by the following bacterial species: *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumonia, Escherichia coli,* Klebsiella spp., Proteus spp., enterococci, *Bacteroides fragilis, Prevotella melaninogenica,* Peptococcus spp., Peptostreptococcus spp., Fusobacterium spp., or Clostridium spp.

[0098] Infections of the retroperitoneal area are commonly caused by the following bacterial species: *Escherichia coli,* or *Staphylococcus aureus.*

[0099] Infections of the prostate are commonly caused by the following bacterial species: *Escherichia coli,* Klebsiella spp., Enterobacter spp., *Proteus mirabilis,* enterococci spp., Pseudomonas spp., Corynebacterium spp., or *Neisseria gonorrhoeae*; or viral pathogens: herpes simplex.

[0100] Infections of the testicle are commonly caused by the following bacterial species: *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa,* Staphylococcus spp., Streptococcus spp., or *Salmonella enteriditis*; or viral pathogens: mumps, coxsackievirus, or lymphocytic choriomeningitis virus.

[0101] Infections of the penis are commonly caused by the following bacterial species: *Staphylococcus aureus, Streptococcus pyogenes, Neisseria gonorrhoeae,* or *Treponema pallidum*; or viral pathogens: herpes simplex.

[0102] Infections of the ovary/adnexae are commonly caused by the following bacterial species: *Neisseria gonorrhoeae, Chlamydia trachomatis, Gardenerella vaginalis,* Prevotella spp., Bacteroides spp., Peptococcus spp. Streptococcus spp., or *Escherichia coli.*

[0103] Infections of the uterus are commonly caused by the following bacterial species: *Neisseria gonorrhoeae, Chlamydia trachomatis, Gardenerella vaginalis,* Prevotella spp., Bacteroides spp., Peptococcus spp., Streptococcus spp., or *Escherichia coli.*

[0104] Infections of the cervix are commonly caused by the following bacterial species: *Neisseria gonorrhoeae, Chlamydia trachomatis,* or *Treponema pallidum*; or viral pathogens: herpes simplex.

[0105] Infections of the vagina are commonly caused by the following bacterial species: *Gardenerella vaginalis,* Prevotella spp., Bacteroides spp., peptococci spp., *Escherichia coli, Neisseria gonorrhoeae, Chlamydia Trachomatis,* or *Treponema pallidum*; or viral pathogens: herpes simplex.

[0106] Infections of the vulva are commonly caused by the following bacterial species: *Staphylococcus aureus, Streptococcus pyogenes,* or *Treponema pallidum*; or viral pathogens: herpes simplex.

Bacterial Strains/Viral Subtypes

[0107] It will be understood by a skilled person in the art that bacterial species are classified operationally as collections of similar strains (which generally refers to groups of presumed common ancestry with identifiable physiological but usually not morphological distinctions, and which may be identified using serological techniques against bacterial surface antigens). Thus, each bacterial species (e.g., Streptococcus pneumoniae) has numerous strains (or serotypes), which may differ in their ability to cause infection or differ in their ability to cause infection in a particular organ/site. For example, although there are at least 90 serotypes of *Streptococcus pneumoniae,* serotypes 1, 3, 4, 7, 8, and 12 are most frequently responsible for pneumococcal disease in humans.

[0108] As a second example, certain strains of *Escherichia coli,* referred to as extraintestinal

pathogenic *E. coli* (ExPEC), are more likely to cause urinary tract infection or other extraintestinal infections such as neonatal meningitis, whereas other strains, including enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), Shiga toxin-producing *E. coli* (STEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC) and diffuse adhering *E. coli* (DAEC) are more likely to cause gastrointestinal infection/diarrhea. Even among the sub-category of ExPEC strains, specific virulence factors (*e.g.*, production of type-1 fimbriae) enable certain strains to be more capable of causing infection of the bladder, while other virulence factors (*e.g.*, production of P fimbriae) enable other strains to be more capable of causing infection in the kidneys. For example, an ExPEC strain(s) that is more likely to cause infection in the bladder may be chosen for a formulation to target bladder cancer, whereas an ExPEC strain(s) that is more likely to cause infection in the kidney may be chosen for a formulation to target kidney cancer. Likewise, one or more of an ETEC, EPEC, EHEC, STEC, EAEC, EIEC or DAEC strains of *E. coli* (*i.e.,* strains that cause colon infection), may be chosen for a formulation to treat colon cancer.

[0109] Similarly, there may be numerous subtypes of specific viruses. For example, there are three types of influenza viruses, influenza A, influenza B and influenza C, which differ in epidemiology, host range and clinical characteristics. For example, influenza A is more likely to be associated with viral lung infection, whereas influenza B is more likely to be associated with myositis (*i.e.,* muscle infection). Furthermore, each of these three types of influenza virus have numerous subtypes, which also may differ in epidemiology, host range and clinical characteristics. One may, for example, choose an influenza A subtype most commonly associated with lung infection to target lung cancer, whereas one may choose an influenza B strain most commonly associated with myositis to treat cancer of the muscle/soft tissues.

[0110] It is understood that a clinical microbiologist skilled in the art would therefore be able to select, based on the present disclosure and the body of art relating to bacterial strains for each species of bacteria (and viral subtypes for each type of virus), the strains of a particular bacterial species (or subtype of a particular virus) to target a specific organ or tissue.

Bacterial Compositions Dosages, and Administration

[0111] The compositions of the invention include whole killed or attenuated *Escherichia coli* cells that are pathogenic in the gastrointestinal tract. 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 tissue or organ.

[0112] The antigenic 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 subcutaneous or intradermaladministration. 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 pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound *(i.e.,* the specific whole killed or attenuated *Escherichia coli* cells, or compositions thereof of the invention), use thereof in the pharmaceutical compositions of the invention is contemplated. Supplementary active compounds can also be incorporated into the compositions.

[0113] If desired, treatment with whole killed or attenuated *Escherichia coli* cellsaccording to the invention may be combined with any other therapy intended to stimulate the immune system, reduce inflammation or otherwise benefit the subject, such as nutrients, vitamins and supplements. For example, vitamin A, vitamin D, vitamin E, vitamin C, vitamin B complex, selenium, zinc, co-enzyme Q10, beta carotene, fish oil, curcumin, green tea, bromelain, resveratrol, ground flaxseed, garlic, lycopene, milk thistle, melatonin, other antioxidants, cimetidine, indomethacin, or COX-2 Inhibitors (*e.g.*, Celebrex™ [celecoxib] or Vioxx™ [rofecoxib]) may be also be administered to the subject.

[0114] Methods well known in the art for making formulations are found in, for example, "Remington's Pharmaceutical Sciences" (20th edition), ed. A. Gennaro, 2000, Mack Publishing Company, Easton, PA. Formulations for parenteral administration may, for example, contain excipients, sterile water, or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated napthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the compounds. Other potentially useful parenteral delivery systems for include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes.

[0115] An "effective amount" of a whole killed or attenuated *Escherichia coli* cells according to the invention includes a therapeutically effective amount or a prophylactically effective amount. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the compound to elicit a desired response in the individual. Dosage regimens may be adjusted to provide the optimum therapeutic response. A therapeutically effective amount may also be one in which any toxic or detrimental effects of the whole killed or attenuated *Escherichia coli* cellsare outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result.

[0116] For administration by subcutaneous or intradermal injection, an exemplary range for therapeutically or prophylactically effective amounts of whole killed or attenuated *Escherichia coli* cells may be about 1 million to 100,000 million organisms per ml, or may be 100 million to

7000 million organisms per ml, or may be 500 million to 6000 million organisms per ml, or may be 1000 million to 5000 million organisms per ml, or may be 2000 million to 4000 million organisms per ml, or any integer within these ranges. The total concentration of bacteria per ml may range from 1 million to 100,000 million organisms per ml, or may be 50 million to 7000 million organisms per ml, or may be 100 million to 6000 million organisms per ml, or may be 500 million to 5000 million organisms per ml, or may be 1000 million to 4000 million organisms per ml, or any integer within these ranges. The range for therapeutically or prophylactically effective amounts of antigens of a pathogenic bacterial species may be any integer from 0.1 nM-0.1 M, 0.1 nM-0.05M, 0.05 nM-15μM or 0.01 nM-10μM.

[0117] It is to be noted that dosage concentrations and ranges may vary with the severity of the condition to be alleviated, or may vary with the subject's immune response. In general, the goal is to achieve an adequate immune response. For administration by subcutaneous or intradermal infection, the extent of an immune response may be determined, for example, by size of delayed local immune skin reaction at the site of injection (*e.g.,* from 0.25 inch to 4 inch diameter). The dose required to achieve an appropriate immune response may vary depending on the individual (and their immune system) and the response desired. Standardized dosages may also be used. In the context of subcutaneous or intradermal administration, if the goal is to achieve a 2 inch local skin reaction, the total bacterial composition dose may, for example, range from 2 million bacteria *(e.g.,* 0.001 ml of a vaccine with a concentration of 2,000 million organisms per ml) to more than 20,000 million bacteria *(e.g.,* 1 ml of a vaccine with a concentration of 20,000 million organisms per ml). The concentrations of whole killed or attenuated *Escherichia coli* cellswithin a composition may also be considered. For example, if the concentration of the whole killed or attenuated *Escherichia coli* cells, cell size of that species or antigenic load thereof is much higher relative to the other pathogenic bacterial species in the vaccine, then the local immune skin reaction of an individual may be likely due to its response to this specific bacterial species. In some embodiments, the immune system of an individual may respond more strongly to one bacterial species within a composition than another, depending for example on past history of exposure to infection by a particular species, so the dosage or composition may be adjusted accordingly for that individual. However, in some embodiments detailed herein, an immune response will not be monitored by way of a skin reaction. For example, in some mouse models utilized herein, the effective treatment of such animals with antigenic compositions may not result in corresponding skin reactions. A person skilled in the art will understand that there are alternate ways in which an immune response can be monitored beside relying on the presence or absence of a skin reaction.

[0118] For any particular subject, the timing and dose of treatments may be adjusted over time (e.g., timing may be daily, every other day, weekly, monthly) according to the individual need and the professional judgement of the person administering or supervising the administration of the compositions. For example, in the context of subcutaneous or intradermal administration, the compositions may be administered every second day. An initial dose of approximately 0.05 ml may be administered subcutaneously, followed by increases from 0.01-0.02 ml every second day until an adequate skin reaction is achieved at the injection site (for

example, a 1 inch to 2 inch diameter delayed reaction of visible redness at the injection site). Once this adequate immune reaction is achieved, this dosing is continued as a maintenance dose. The maintenance dose may be adjusted from time to time to achieve the desired visible skin reaction (inflammation) at the injection site. Dosing may be for a dosage duration, for example of at least 2 weeks, 2 months, 6 months, 1, 2, 3, 4, or 5 years or longer.

[0119] In some embodiments, the invention may include antigenic compositions administered administered intradermally or subcutaneously to one or more epithelial tissues *(i.e.,* skin by intradermal or subcutaneous injection) simultaneously or sequentially. Accordingly, in some embodiments the antigenic compositions of the invention are administered so as to provoke an immune response in an epithelial tissue. In some embodiments, one or more epithelial routes of intradermal and/or subcutaneous administration may be combined with one or more additional routes of administration, such as intramuscular or intravenous administration.

[0120] In various aspects of the invention, the antigenic compositions that are administered to a patient may be characterized as having an antigenic signature, *i.e.,* a combination of antigens or epitopes that are sufficiently specific that the antigenic composition is capable of eliciting an immune response that is specific to a particular *Escherichia coli* pathogen, such as an adaptive immune response.

[0121] Dosage ranges set forth herein are exemplary only and do not limit the route of intradermally or subcutaneously administration and dosage ranges that may be selected by medical practitioners. The amount of whole killed or attenuated *Escherichia coli* cells in the composition may vary according to factors such as the disease state, age, sex, and weight of the individual. Dosage regimens may be adjusted to provide the optimum therapeutic response. For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It may be advantageous to formulate intradermal or subcutaneous compositions in dosage unit form for ease of administration and uniformity of dosage.

[0122] In the case of antigenic formulations (analogous to a vaccine), an immunogenically effective amount of whole killed or attenuated *Escherichia coli* cellscan be provided, alone or in combination with other compounds, with an immunological adjuvant. The whole killed or attenuated *Escherichia coli* cellsmay also be linked with a carrier molecule, such as bovine serum albumin or keyhole limpet hemocyanin to enhance immunogenicity. An antigenic composition ("vaccine") is a composition that includes materials that elicit a desired immune response. An antigenic composition may select, activate or expand, without limitation: memory B, T cells, neutrophils, monocytes or macrophages of the immune system to, for example, reduce or eliminate the growth or proliferation of cancerous cells or tissue. In some embodiments, whole killed or attenuated *Escherichia coli* cells, or compositions thereof of the invention are capable of eliciting the desired immune response in the absence of any other agent, and may therefore be considered to be an antigenic composition. In some embodiments, an antigenic composition includes a suitable carrier, such as an adjuvant, which

is an agent that acts in a non-specific manner to increase the immune response to the whole killed or attenuated *Escherichia coli* cells enabling the reduction of the quantity of whole killed or attenuated *Escherichia coli* cells in any given vaccine dose, or the reduction of the frequency of dosage required to generate the desired immune response. A antigenic composition of whole killed or attenuated *Escherichia coli* cells may include live or dead bacteria capable of inducing an immune response against antigenic determinants normally associated with the bacteria. In some embodiments, an antigenic composition may include live bacteria that are of less virulent strains (attenuated), and therefore cause a less severe infection.

[0123] An antigenic composition comprising whole killed *Escherichia coli* bacteria for administration by intradermal or subcutaneous injection may be made as follows. The *Escherichia coli* bacteria may be grown in suitable media, and washed with physiological salt solution. The bacteria may then be centrifuged, resuspended in saline solution, and killed with heat. The suspensions may be standardized by direct microscopic count, mixed in required amounts, and stored in appropriate containers, which may be tested for safety, shelf life, and sterility in an approved manner. In addition to the pathogenic *Escherichia coli* bacterial species, a killed bacterial vaccine suitable for administration to humans may include 0.4% phenol preservative and/or 0.9% sodium chloride. The *Escherichia coli* bacterial vaccine may also include trace amounts of brain heart infusion (beef), peptones, yeast extract, agar, sheep blood, dextrose, sodium phosphate and/or other media components.

[0124] In antigenic compositions comprising whole killed or attenuated *Escherichia coli* cells, the concentrations of specific bacterial species in compositions for subcutaneous or intradermal injection may be about 1 million to 100,000 million organisms per ml, or may be 100 million to 7000 million organisms per ml, or may be 500 million to 6000 million organisms per ml, or may be 1000 million to 5000 million organisms per ml, or may be 2000 million to 4000 million organisms per ml, or any integer within these ranges. The total concentration of bacteria per ml may range from 1 million to 100,000 million organisms per ml, or may be 50 million to 7000 million organisms per ml, or may be 100 million to 6000 million organisms per ml, or may be 500 million to 5000 million organisms per ml, or may be 1000 million to 4000 million organisms per ml, or any integer within these ranges.

[0125] In general, the antigenic composition comprising whole killed or attenuated *Escherichia coli* cellsof the invention should be used without causing substantial toxicity. Toxicity of the compounds of the invention can be determined using standard techniques, for example, by testing in cell cultures or experimental animals and determining the therapeutic index, *i.e.,* the ratio between the LD50 (the dose lethal to 50% of the population) and the LD100 (the dose lethal to 100% of the population).

[0126] In some aspects, the invention involves the use of an anti-inflammatory in conjunction with vaccinations. In these embodiments, a wide variety of anti-inflammatory treatments may be employed, including effective amounts of non-steroidal anti-inflammatory drugs (NSAIDs), including but not limited to: diclofenac potassium, diclofenac sodium, etodolac, indomethicin, ketorolac tromethamine, sulindac, tometin sodium, celecoxib, meloxicam, valdecoxib,

floctafenine, mefenamic acid, nabumetone, meloxicam, piroxicam, tenoxicam, fenoprofen calcium, flubiprofen, ibuprofen, ketoprofen, naproxen, naproxen sodium, oxaprozin, tiaprofenic acid, acetylsalicylic acid, diflunisal, choline magnesium trisalicylate, choline salicylate, triethanolamine salicylate, COX1 inhibitors, COX2 inhibitors (*e.g.,* Vioxx™, and Celebrex™). A variety of herbs and natural health products may also be used to provide anti-inflammatory treatment, including but not limited to: green tea, fish oil, vitamin D, antioxidant vitamins and minerals (*e.g.,* B carotene, vitamin A, vitamin C, vitamin D, vitamin E, co-enzyme Q10, selenium, *etc.*), resveratrol, turmeric, bromelain, boswellia, feverfew, quercetin, ginger, rosemary, oregano, cayenne, clove, nutmeg, willowbark. Alternative anti-inflammatory modalities may also include lifestyle modifications, such as: exercise, weight loss, smoking cessation, stress reduction, seeking social support, treatment of depression, stress management, abdominal breath work and dietary change (such as adopting a mediterranean diet, a low glycemic diet, eating non-charred foods, including foods having omega-3 fatty acids).

[0127] As detailed herein a method of comparing immune responses is described. The method involves administering to an animal having an organ or tissue a medicament having an antigenic composition, as defined herein. The antigenic composition may have antigenic determinants selected or formulated so that together the antigenic determinants are specific for at least one microbial pathogen that is pathogenic in the organ or tissue, extracting a quantifiable immune sample from the organ or tissue, measuring a characteristic of the immune response in the organ or tissue in the quantifiable immune sample following the administration of the medicament, and, comparing the characteristic of the immune response in the quantifiable immune sample to a corresponding characteristic of the immune response in a reference immune sample obtained from a corresponding organ or tissue. As used herein, an immune sample would contain sufficient biological material to determine a characteristic of an immune response. As used herein, a "characteristic" of an immune response can include, without limitation, the particular number of a particular immune cell type *(e.g.,* macrophage), or a particular cellular marker *(e.g.,* upregulation of an integrin) or gene product (*e.g.,* a cytokine). The foregoing is provided as an example and is non-limiting.

[0128] Optionally, the reference immune sample may be obtained from the corresponding organ or tissue in the animal prior to the step of administering the medicament. In another aspect, the reference immune sample may be obtained from the corresponding organ or tissue in a second animal such that it is specifically contemplated that at least two animals *(i.e.,* an animal from which a reference immune sample is obtained and a second animal from which a quantifiable immune sample) could be used in the methods described herein. Optionally, the animal may have a cancer situated in the organ or tissue.

[0129] Comparing the characteristic of the immune response may involve comparing, in the quantifiable and reference immune samples, an indication of the numbers of any one or more of the following cells as these cells are known to those skilled in the art: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or NK cells. Optionally, the macrophages may include any one or

more of the following: M1-like macrophages or M2-like macrophages.

[0130] Those persons skilled in the art will appreciate that macrophages can be defined as either "M1-like macrophages" or "M2-like macrophages". For example, M1-like macrophages are generally understood by those persons skilled in the art to promote a Th1 CD4+ T cell-mediated response (see, for *e.g.,* Biswas and Mantovani (2010), Nature Immunology 10:889-96). Moreover, M1-like macrophages are generally understood to have efficient antigen presentation capacity, and to be proficient at killing intracellular pathogens (for e.g., viruses). Moreover, M1-like macrophages are generally understood to be proficient, at least as compared with M2-like macrophages, in playing an immunological role in tumour destruction. Those skilled in the art will appreciate that there are numerous biological markers which can be employed to differentiate between M1-like macrophages and M2-like macrophages. For example, and as detailed herein, the expression of Nos2 is generally understood to correlate with an M1-like macrophage as compared with an M2-like macrophage (see, for *e.g.,* Laskin et al. (2010) Annual Rev. Pharmacol. Toxicol. 51: 267-288). Further, and for example, M1-like macrophages are generally understood to produce IL-12 and to be effectively activated by IFN-γ through the IFN- γR (Biswas and Mantovain, *supra*).

[0131] In contrast to M1-like macrophages, those persons skilled in the art will generally understand that M2-like macrophages promote a Th2 CD4+ T cell-mediated response (see, generally: Biswas and Mantovani (2010), Nature Immunology 10:889-96). Moreover, M2-like macrophages are generally understood to be effective and encapsulating and clearing extracellular parasites *etc.* Further, and in comparison to M1-like macrophages, M2-like macrophages are generally understood by those persons skilled in the art as playing a more significant role in immunoregulation both with respect to T_{reg} and B cells (Biswas and Mantovain, *supra)*.. Those persons skilled in the art will appreciate that there are numerous biological markers which can be employed to differentiate between M2-like macrophages and M1-like macrophages. For example, and as described herein, a diminished expression of Nos2 will generally be understood to correlate with M2-like macrophages as compared with higher expression being generally found in M1-like macrophages. Further, and as detailed in experiments herein, the expression of CD206 is generally understood as correlating with M2-like macrophages (see, for *e.g.,* Choi et al. (2010) Gastroenterology 138(7) 2399-409). Further, and as detailed in experiments herein, the expression of F4/80 is generally understood to correlate with M2-like macrophages. Further, and for example, M2-like macrophages are generally undersood to be effectively activated by IL-4 or by IL-13 through IL-4Rα (Biswas and Mantovain, *supra*).

[0132] Further, comparing the characteristic of the immune response may involve comparing a shift in an activation state of macrophages. The shift in the activation state of macrophages may optionally be characterized as a shift from M2-like macrophages to M1-like macrophages or vice versa. Those persons skilled in the art will appreciate that there are numerous biological markers that can be employed to monitor the activation of macrophages. As detailed herein, those skilled in the art will appreciate that defining a macrophage as being activated towards either a M1-like phenotype or a M2-like phenotype can be accomplished by chosing

markers that are known to associate with either of the respective phenotypes described herein. Diseases which have been associated with M1 and M2 macrophages include at least the following: atherosclerosis (see, for *e.g.,* Hirata et al. (2011) J. Am. Coll. Cardiol. 58(3): 248-255), allergic asthma (see, for *e.g.,* Moreira and Hogaboam (2011) J. Interferon. Cytokine Res. 31(6): 485-91), autoimmune prostatitis (see, for *e.g.,* Zhang and Schluesener (2011) Prostate), colitis (see, for *e.g.,* Waddell et al. (2011) J. Immunol. 186(10): 5993-6003), COPD (see, for *e.g.,* Kunz et al. (2011) Respir. Res. 22: 34), glomerulonephritis (see, for e.g., Fujita et al. (2010) Am. J. Pathol. 177(3): 1143-54), inflammatory bowel disease (see, for *e.g.,* Wendelsdorf et al. (2010) J. Theor. Biol. 264(4): 1225-39), chronic lung inflammation (see, for *e.g.,* Redente et al. (2010) J. Leukoc. Blol. 88(1): 159-68), steatohepatitis (see, for *e.g.,* Rensen et al. (2009) Am. J. Pathol. 175(4): 1473-82), pancreatitis (see, for *e.g.,* Gea-Sorli and Closa (2009) BMC Immunol. 31: 42), mycocarditis (see, for *e.g.,* Li et al. (2009) Circ. Res. 105(4): 353-64), liver fibrosis (see, for *e.g.,* Heymann et al. (2009) Inflamm. Allergy Drug Targets 8(4): 307-18), cystic fibrosis (see, for *e.g.,* Meyer et al. (2009) Am. J. Respir. Cell Mol. Biol. 41(5): 590-602), inflammatory renal disease (see, for *e.g.,* Wang et al. (2007) Kidney Int. 72(3): 290-299), and silicosis (see, for *e.g.,* Misson et al. (2004) J. Leukoc. Biol. 76(5): 926-232).

[0133] Optionally, comparing the characteristic of the immune response may involve identifying, in the quantifiable and reference immune samples, cellular markers on any one or more of the following cells as they are commonly understood to those persons skilled in the art: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or NK cells. The macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages. A person skilled in the art will appreciate that there are numerous cell markers (both extracellular and intracellular) that can be selected which can identify an immune response. For example, as described herein, the marker CD206 is generally understood as correlating with M2-like macrophages (see, for *e.g.,* Choi et al. (2010) Gastroenterology 138(7) 2399-409).

[0134] Optionally, comparing the characteristic of the immune response may involve identifying, in the quantifiable and reference immune samples, cytokines produced by any one or more of the following cells as they are commonly understood to those persons skilled in the art: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or NK cells. Those persons skilled in the art will appreciate that cytokines refer to small cell-signalling protein molecules and that there are numerous cytokines known in the art. For example, cytokines have been grouped into type 1 and type 2 classifications based on their role in immunological responses. Common type 1 cytokines include IFN-γ and TGF-β. Common type 2 cytokines include, but are not limited to IL-4 and IL-13. Cytokines can be detected by numerous methodologies known to those persons skilled in the art. For example, ELISA experiments were utilized to determine cytokine production from lung tissue.

[0135] As detailed herein, the macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages as has been defined herein. Optionally, the

cytokines are produced as a result of a shift in an activation state of the macrophages. Optionally, the macrophages shift from being M2-like macrophages to being M1-like macrophages. Further and optionally, the macrophages shift from being M1-like macrophages to being M2-like macrophages.

[0136] Optionally, comparing the characteristic of the immune response may involve identifying, in the quantifiable and reference immune samples, differential gene expression produced by any one or more of the following cells as they are commonly understood to those persons skilled in the art: inflammatory monocytes, macrophages, CD11b+ Gr-1+ cells, dendritic cells, CD11c+ MHC class II+ cells, CD4+ T cells, CD8+ T cells, or NK cells. The macrophages may include any one or more of the following: M1-like macrophages or M2-like macrophages. The term "differential gene expression" is understood to mean an appreciable difference between the expression of a particular gene of interest from at least two experimental conditions. For example, if under a first experimental condition a particular gene has a defined expression level as defined by gene expression methods used by those persons skilled in the art and if under a second experimental condition the same gene has an appreciable difference in its expression level, then there is differential expression of the gene of interest. Those persons skilled in the art will understand that there are numerous methodologies with which to detect differential gene expression. For example, commercially available quantitative PCR techniques can be used with respect to determining the relative Nos2/Arg1 ratios. Optionally, the differential gene expression is produced as a result of a shift in an activation state of the macrophages. Optionally, macrophages may shift from being M2-like macrophages to being M1-like macrophages as those terms have been defined herein.

[0137] In accordance with the present invention as defined by Claim 1, the antigenic composition comprising whole killed or attenuated *Escherichia coli* cells may be administered intradermally or subcutaneously at an administration site in successive doses given at a dosage interval of between one hour and one month, over a dosage duration of at least 1 week, 2 weeks, 2 months or 6 months. Optionally, the medicament may be administered in a dose so that each dose is effective to cause a visible localized inflammatory immune response at the administration site. Optionally, the medicament may be administered so that visible localized inflammation at the administration site occurs within 1 to 48 hours. However, a visable localized inflammatory immune
response may not always be present in all circumstances despite an immune response being initiated. Those skilled in the art will appreciate that there are other methods by which the mounting of an immune response can be monitored. For example, the profile (and relative change in characterization) of immune cells from a subject undergoing an immune reaction can be compared with those from a subject that is not undergoing an immune reaction.

[0138] In various aspects, embodiments of the invention relate to antigenic compositions comprising whole killed or attenuated *Escherichia coli* cells that may cause infections of the gastrointestinal tract, so that the organism may be characterized as a pathogen. However, an organism that is in some cases pathogenic may not always cause disease. Most animals are colonized to some degree by other organisms, such as bacteria, which generally exist in

symbiotic or commensal relationships with the host animal. Thus, many species of normally harmless bacteria are found in healthy animals, and are usually localized to the surface of specific organs and tissues. Often, these bacteria aid in the normal functioning of the body. For example, in humans, symbiotic *Escherichia coli* bacteria may be found in the intestine, where they promote immunity and reduce the risk of infection with more virulent pathogens.

[0139] Bacteria that are generally harmless, such as *Escherichia coli,* can cause infection in healthy subjects, with results ranging from mild to severe infection to death. Whether or not an organism, such as a bacterium, is pathogenic (i.e., causes infection) depends to some extent on factors such as the route of entry and access to specific host cells, tissues, or organs; the intrinsic virulence of the bacterium; the amount of the bacteria present at the site of potential infection; or the health of the host animal. Thus, organisms that are normally harmless can become pathogenic given favorable conditions for infection, and even virulent organisms may require specific circumstances to cause infection. Accordingly, organisms that are members of the normal flora can be pathogens when they move beyond their normal ecological role in the endogenous flora. For example, endogenous species can cause infection outside of their ecological niche in regions of anatomical proximity, for example by contiguous spread. When this occurs, and in the context of the present invention, these normally harmless endogenous organisms are considered pathogenic.

[0140] Specific organisms, such as bacterial species, viruses, worms, and protazoa are known to cause infections in specific regions of the GIT in otherwise healthy subjects. Examples of organisms that commonly cause infections in specific regions of the GIT are listed below; it will be understood that these examples are not intended to be limiting and that a skilled person would be able to readily recognize and identify infectious or pathogenic organisms that cause infections, or commonly cause infections, in various regions of the GIT in healthy adults, based for example on knowledge about particular patient populations, as represented for example by the following publications: Manual of Clinical Microbiology 8th Edition, Patrick Murray, Ed., 2003, ASM Press American Society for Microbiology, Washington DC, USA; Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases 5th Edition, G. L. Mandell, J.E. Bennett, R. Dolin, Eds., 2000, Churchill Livingstone, Philadelphia, PA, USA.

[0141] Infections of the mouth are commonly caused by the following bacterial species: Prevotella melaninogenicus, anaerobic streptococci, viridans streptococci, Actinomyces spp., Peptostreptococcus spp., or Bacteroides spp., or other oral anaerobes; or viral pathogens: herpes simplex, coxsackieviruses, or Epstein-Barr.

[0142] Infections of the esophagus are commonly caused by the following bacterial species: Actinomyces spp., Mycobacterium avium, Mycobacterium tuberculosis, or Streptococcus spp.; or viral pathogens: cytomegalovirus, herpes simplex, or varicella-zoster.

[0143] Infections of the stomach are commonly caused by the following bacterial species: Streptococcus pyogenes or Helicobacter pylori; or viral pathogens: cytomegalovirus, herpes simplex, Epstein-Barr, rotaviruses, noroviruses, or adenoviruses.

[0144] Infections of the small bowel are commonly caused by the following bacterial species: Escherichia coli, Clostridium difficile, Bacteroides fragilis, Bacteroides vulgatus, Bacteroides thetaiotaomicron, Clostridium perfringens, Salmonella enteriditis, Yersinia enterocolitica, or Shigella flexneri; or viral pathogens: adenoviruses, astroviruses, caliciviruses, noroviruses, rotaviruses, or cytomegalovirus.

[0145] Infections of the colon/rectum are commonly caused by the following bacterial species: Escherichia coli, Clostridium difficile, Bacteroides fragilis, Bacteroides vulgatus, Bacteroides thetaiotaomicron, Clostridium perfringens, Salmonella enteriditis, Yersinia enterocolitica, or Shigella flexneri; or viral pathogens: adenoviruses, astroviruses, caliciviruses, noroviruses, rotaviruses, or cytomegalovirus.

[0146] Infections of the anus are commonly caused by the following bacterial species: Streptococcus pyogenes, Bacteroides spp., Fusobacterium spp., anaerobic streptococci, Clostridium spp., Escherichia coli, Enterobacter spp., Pseudomonas aeruginosa, or Treponema pallidum; or viral pathogens: herpes simplex.

[0147] Organisms such as bacteria are often classified operationally as collections of similar strains (which generally refer to groups of presumed common ancestry with identifiable physiological but usually not morphological distinctions, and which may be identified using serological techniques against bacterial surface antigens). Thus, each bacterial species (e.g., Escherichia coli) has numerous strains (or serotypes), which may differ in their ability to cause infection or differ in their ability to cause infection in a particular organ/site. Certain strains of Escherichia coli are more likely to cause gastrointestinal infection/diarrhea, including enterotoxigenic E. coli (ETEC), enteropathogenic E. coli (EPEC), enterohemorrhagic E. coli (EHEC), Shiga toxin-producing E. coli (STEC), enteroaggregative E. coli (EAEC), enteroinvasive E. coli (EIEC) and diffuse adhering E. coli (DAEC). In accordance with the present invention, one or more of an ETEC, EPEC, EHEC, STEC, EAEC, EIEC or DAEC strains of E.coli (i.e., strains that cause colon infection), may be chosen for a formulation to treat and IBD.

[0148] Similarly, there may be numerous subtypes of specific viruses, worms, or protozoa, which are associated with disease in a particular population.

[0149] The compositions of the invention include whole killed or attenuated *Escherichia coli* cells that are pathogenic in a specific region of the GIT. The compositions may also include one or more isolated antigens from these organisms. Pathogenic organisms may be available commercially (for example from the American Type Culture Collection, Manassas, VA, USA), or may be clinical isolates from subjects having an infection.

[0150] The compositions of the invention derived from pathogens 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 subcutaneous or intradermal 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 pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the whole killed or attenuated *Escherichia coli cells,* use thereof in the pharmaceutical compositions of the invention is contemplated. Supplementary active compounds can also be incorporated into the compositions.

[0151] Methods well known in the art for making formulations are found in, for example, "Remington's Pharmaceutical Sciences" (20th edition), ed. A. Gennaro, 2000, Mack Publishing Company, Easton, PA. Formulations for parenteral administration may, for example, contain excipients, sterile water, or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated napthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the compounds. Other potentially useful parenteral delivery systems for include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. For therapeutic or prophylactic compositions, the formulations may be administered to an individual in an amount effective to stop or slow progression of Crohn's disease.

[0152] An "effective amount" of a whole killed or attenuated *Escherichia coli* cells according to the invention includes a therapeutically effective amount or a prophylactically effective amount. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result, such as reduction or elimination of symptoms of Crohn's disease. A therapeutically effective amount of a pathogenic species or antigen(s) thereof may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the compound to elicit a desired response in the individual. Dosage regimens may be adjusted to provide the optimum therapeutic response. A therapeutically effective amount may also be one in which any toxic or detrimental effects of the whole killed or attenuated *Escherichia coli* cells are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result, such as prevention of Crohn's disease. Typically, a prophylactic dose is used in subjects prior to or at an earlier stage of Crohn's disease, so that a prophylactically effective amount may be less than a therapeutically effective amount.

[0153] For administration by subcutaneous or intradermal injection, an exemplary range for therapeutically or prophylactically effective amounts of the whole killed or attenuated *Escherichia coli cells* may be about 1 million to 100,000 million organisms per ml, or may be

100 million to 7000 million organisms per ml, or may be 500 million to 6000 million organisms per ml, or may be 1000 million to 5000 million organisms per ml, or may be 2000 million to 4000 million organisms per ml, or any integer within these ranges. The total concentration of bacteria per ml may range from 1 million to 100,000 million organisms per ml, or may be 50 million to 7000 million organisms per ml, or may be 100 million to 6000 million organisms per ml, or may be 500 million to 5000 million organisms per ml, or may be 1000 million to 4000 million organisms per ml, or any integer within these ranges. The range for therapeutically or prophylactically effective amounts of antigens of a pathogenic bacterial species may be any integer from 0.1 nM-0.1 M, 0.1 nM-0.05M, 0.05 nM-15μM or 0.01 nM-10μM.

[0154] It is to be noted that dosage concentrations and ranges may vary with the severity of the condition to be alleviated, or may vary with the subject's immune response. In general, the goal is to achieve an adequate immune response. For administration by subcutaneous or intradermal infection, the extent of an immune response may be determined, for example, by size of delayed local immune skin reaction at the site of injection (e.g., from 0.25 inch to 4 inch diameter). The dose required to achieve an appropriate immune response may vary depending on the individual (and their immune system) and the response desired. Standardized dosages may also be used.

[0155] In the context of subcutaneous or intradermal administration, if the goal is to achieve a 2 inch local skin reaction, using an antigenic composition comprising whole killed or attenuated *Escherichia coli* cells, the total dose may, for example, range from 2 million bacteria (e.g., 0.001 ml of a vaccine with a concentration of 2,000 million organisms per ml) to more than 20,000 million bacteria (e.g., 1 ml of a vaccine with a concentration of 20,000 million organisms per ml). The concentrations of whole killed or attenuated *Escherichia coli* cells within a composition may also be considered. For example, if the concentration of whole killed or attenuated *Escherichia coli* cells, cell size of that species or antigenic load thereof is much higher relative to the other pathogenic bacterial species in the vaccine, then the local immune skin reaction of an individual may be likely due to its response to this specific bacterial species. In some embodiments, the immune system of an individual may respond more strongly to one bacterial species within a composition than another, depending for example on past history of exposure to infection by a particular species, so the dosage or composition may be adjusted accordingly for that individual.

[0156] For any particular subject, the timing and dose of treatments may be adjusted over time (e.g., timing may be daily, every other day, weekly, monthly) according to the individual need and the professional judgement of the person administering or supervising the administration of the compositions. For example, in the context of subcutaneous or intradermal administration, the compositions may be administered every second day. An initial dose of approximately 0.05 ml may be administered subcutaneously, followed by increases from 0.01-0.02 ml every second day until an adequate skin reaction is achieved at the injection site (for example, a 1 inch to 2 inch diameter delayed reaction of visible redness at the injection site). Once this adequate immune reaction is achieved, this dosing is continued as a maintenance dose. The maintenance dose may be adjusted from time to time to achieve the desired visible

skin reaction (inflammation) at the injection site. Dosing may be for a dosage duration, for example of at least 1 week, 2 weeks, 2 months, 6 months, 1, 2, 3, 4, or 5 years or longer.

[0157] In some embodiments, the invention may include antigenic compositions administered to one or more epithelial tissues by a intradermal or subcutaneous route. For example: to skin by intradermal or subcutaneous injection. Accordingly, in some embodiments the antigenic compositions of the invention are administered so as to provoke an immune response in a non-enteric tissue, such as an epithelial tissue. In some embodiments, one or more non-enteric routes of administration may be combined with one or more additional routes of administration, such as intramuscular or intravenous administration.

[0158] In various aspects of the invention, the antigenic compositions that are administered to a patient may be characterized as having an antigenic signature, i.e., a combination of antigens or epitopes, that is sufficiently specific that the antigenic composition is capable of eliciting an immune response that is specific to a particular *Escherichia coli,* such as an adaptive immune response.

[0159] The amount of whole killed or attenuated *Escherichia coli* cells in compositions of the invention may vary according to factors such as the disease state, age, sex, and weight of the individual. Dosage regimens may be adjusted to provide the optimum therapeutic response. For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It may be advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage.

[0160] In the case of antigenic formulations (analogous to a vaccine), an immunogenically effective amount of whole killed or attenuated *Escherichia coli* cells can be provided, alone or in combination with other compounds, with an immunological adjuvant. The whole killed or attenuated *Escherichia coli* cells may also be linked with a carrier molecule, such as bovine serum albumin or keyhole limpet hemocyanin to enhance immunogenicity. An antigenic composition ("vaccine") is a composition that includes materials that elicit a desired immune response. An antigenic composition may select, activate or expand memory B, T cells, neutrophils, monocytes or macrophages of the immune system to, for example, reduce or eliminate the symptoms of the Crohn's disease. In some embodiments, the whole killed or attenuated *Escherichia coli* cells, or compositions thereof of the invention are capable of eliciting the desired immune response in the absence of any other agent, and may therefore be considered to be an antigenic composition. In some embodiments, an antigenic composition includes a suitable carrier, such as an adjuvant, which is an agent that acts in a non-specific manner to increase the immune response to a specific antigen, or to a group of antigens, enabling the reduction of the quantity of antigen in any given vaccine dose, or the reduction of the frequency of dosage required to generate the desired immune response. A bacterial antigenic composition may include live or dead *Escherichia coli* bacteria capable of inducing an immune response against antigenic determinants normally associated with the bacteria. In some embodiments, an antigenic composition may include live *Escherichia coli* bacteria that

are of less virulent strains (attenuated), and therefore cause a less severe infection.

[0161] An antigenic composition comprising whole killed *Escherichia coli* cells for intradermal or subcutaneous administration by injection may be made as follows. The organism may be grown in suitable media, and washed with physiological salt solution. The organism may then be centrifuged, resuspended in saline solution, and killed with heat. The suspensions may be standardized by direct microscopic count, mixed in required amounts, and stored in appropriate containers, which may be tested for safety, shelf life, and sterility in an approved manner. In addition to the organism and/or antigens thereof, a killed preparation suitable for administration to humans may include phenol preservative (for example 0.4%) and/or sodium chloride (for example on the order of 0.9%). The composition may also for example include trace amounts of brain heart infusion (beef), peptones, yeast extract, agar, sheep blood, dextrose, sodium phosphate and/or other media components.

[0162] In general, the compositions of the invention should be used without causing substantial toxicity. Toxicity of the compounds of the invention can be determined using standard techniques, for example, by testing in cell cultures or experimental animals and determining the therapeutic index, i.e., the ratio between the LD50 (the dose lethal to 50% of the population) and the LD100 (the dose lethal to 100% of the population).

[0163] The rows of Table 1 list a number of bacterial species, together with the biological regions in which each species may form a part of the endogenous flora. For example, Abiotrophia spp. are typically members of the endogenous flora of the mouth.

Table 1: Human Bacterial Normal Flora (Endogenous Bacterial Human Pathogens)

Bacterial species	Mouth	Stomach	Duodenu m/ Jejunum	Ileum	Colon
CFL /mL	10^5	10^2	10^2	10^8	10^11
Abiotrophia spp	+				
Acholeplasma laidlawii	+				
Acidaminococcu s fermentans	+		+	+	+
Acinetobacter spp.	+		+	+	+
Actinobacillus spp.	+				
Actinobaculum spp.	+		+	+	+
Actinomyces spp.	+		+	+	+
Aeromonas spp.			+	+	+
Anaerorhabdus furcosus				+	+
Anaerococcus hydrogenalis				+	+
Anaerococcus lactolyticus				+	+
Anaerococcus prevotii				+	+

Bacterial species	Mouth	Stomach	Duodenu m/ Jejunum	Ileum	Colon
Atopobium spp.	+		+	+	+
Bacillus spp.				+	+
Bacteroides caccae				+	+
Bacteroides distasonis				+	+
Bacteroides eggerthii				+	+
Bacteroides fragilis				+	+
Bacteroides merdae				+	+
Bacteroides ovatus				+	+
Bacteroides splanchnicus				+	+
Bacteroides thetaiotaomicron				+	+
Bacteroides vulgatus				+	+
Bifidobacterium adolescentis			+	+	+
Bifidobacterium bifidum			+	+	+
Bifidobacterium breve			+	+	+
Bifidobacterium catenulatum			+	+	+
Bifidobacterium dentium	+		+	+	+
Bifidobacterium longum			+	+	+
Bilophila wadsworthia	+		+	+	+
Burkholderia cepacia			+	+	+
Butyrivibrio fibrisolvens			+	+	+
Campylobacter concisus			+	+	+
Campylobacter curvus			+	+	+
Campylobacter gracilis			+	+	+
Campylobacter jejuni			+	+	+
Campylobacter rectus			+	+	+
Campylobacter showae	+		+	+	+
Campylobacter sputorum	+				
Capnocytophaga granulosum	+				
Capnocytophaga gingivalis	+				
Campylobacter haemolytica	+				

Bacterial species	Mouth	Stomach	Duodenu m/ Jejunum	Ileum	Colon
Capnocytophaga ochracea	+		+	+	+
Capnocytophaga sputigena	+				
Cardiobacterium hominis	+				
Cedecea spp					+
Centipeda periodontii	+				
Citrobacter freundii			+	+	+
Citrobacter koseri			+	+	+
Clostridium spp.			+	+	+
Corynebacterium accolens	+				
Corynebacterium afermentans	+				
Desulfomonas pigra			+	+	+
Dysgonomonas spp.			+	+	+
Eikenella corrodens	+		+	+	+
Enterobacter aerogenes			+	+	+
Enterobacter cloacae			+	+	+
Enterobacter gergoviae			+	+	+
Enterobacter sakazakii			+	+	+
Enterobacter taylorae			+	+	+
Enterococcus spp.			+	+	+
Escherichia coli			+	+	+
Escherichia fergusonii			+	+	+
Escherichia hermannii			+	+	+
Escherichia vulneris			+	+	+
Eubacterium spp.	+		+	+	+
Ewingella americana	+				
Finegoldia magnus			+	+	+
Fusobacterium alocis	+				
Fusobacterium gonidiaformans			+	+	+
Fusobacterium mortiferum			+	+	+
Fusobacterium naviforme			+	+	+

Bacterial species	Mouth	Stomach	Duodenu m/ Jejunum	Ileum	Colon
Fusobacterium necrophorum	+		+	+	+
Fusobacterium nucleatum	+				
Fusobacterium sulci	+				
Fusobacterium russii			+	+	+
Fusobacterium varium			+	+	+
Gardnerella vaginalis			+	+	+
Gemella haemolysans	+				
Gemella morbillorum	+		+	+	+
Globicatella spp.	+				+
Granulicatella spp.	+				
Haemophilus spp.	+				
Hafnia alvei			+	+	+
Helcococcus kunzii					
Helicobacter spp.			+	+	+
Kingella spp.	+				
Klebsiella spp.	+		+	+	+
Lactobacillus acidophilus	+	+	+	+	+
Lactobacillus breve	+				
Lactobacillus casei	+				
Lactobacillus fermentum	+	+	+	+	+
Lactobacillus reuteri		+	+	+	+
Lactobacillus salivarius	+	+	+	+	+
Leclercia adecarboxylata			+	+	+
Leminorella spp.			+	+	+
Leptotrichia buccalis	+				
Megasphaera elsdenii			+	+	+
Micrococcus luteus	+				
Micrococcus lylae	+				
Micromonas micros	+				
Mitsuokella multiacidus			+	+	+
Mobiluncus curisii			+	+	+
Mobiluncus mulieris			+	+	+
Moellerella wisconsensis			+	+	+

Bacterial species	Mouth	Stomach	Duodenu m/ Jejunum	Ileum	Colon
Moraxella catarrhalis	+				
other Moraxella spp.	+				
Morganella morganii			+	+	+
Mycoplasma buccale	+				
Mycoplasma fermentans	+				
Mycoplasma hominis	+				
Mycoplasma lipophilum	+				
Mycoplasma orale	+				
Mycoplasma pneumoniae	+				
Mycoplasma salivarium	+				
Pantoea agglomerans			+	+	+
Pasteurella multocida	+				
Pediococcus spp.	+				+
Peptoniphilus asaccharolyticus			+	+	+
Peptostreptococc us anaerobus	+		+	+	+
Peptostreptococc us productus			+	+	+
Porphyromonas asaccharolytica	+		+	+	+
Porphyromonas catoniae	+		+		
Porphyromonas endodontalis	+		+		
Porphyromonas gingivalis	+		+		
Prevotella buccae	+		+		
Prevotella buccalis	+		+		
Prevotella corporis	+		+		
Prevotella dentalis	+		+		
Prevotella denticola	+		+		
Prevotella enoeca	+		+		
Prevotella heparinolytica	+		+		
Prevotella intermedia	+		+		
Prevotella loescheii	+		+		
Prevotella melaninogenica	+		+		

Bacterial species	Mouth	Stomach	Duodenu m/ Jejunum	Ileum	Colon
Prevotella nigrescens	+		+		
Prevotella oralis	+		+		
Prevotella oris	+		+		
Prevotella oulorum	+		+		
Prevotella tannerae	+		+		
Prevotella veroralis	+		+		
Prevotella zoogleoformans	+		+		
Propionibacteriu m propionicum	+				
Proteus mirabilis				+	+
Proteus penneri				+	+
Proteus vulgaris				+	+
Providencia rettgeri				+	+
Providencia stuartii			+	+	+
Pseudomonas aeruginosa			+	+	+
Retortamonas intestinalis			+	+	+
Rothia dentocariosa	+				
Rothia mucilaginosa	+				
Ruminococcus productus			+	+	+
Selenomonas spp.	+				
Serratia liquefaciens				+	+
Serratia marcescens				+	+
Serratia odorifera				+	+
Staphylococcus aureus	+				
Staphylococcus epidermidis	+				
Streptococcus agalactiae			+	+	+
Streptococcus anginosus	+		+	+	+
Streptococcus bovis			+	+	+
Streptococcus constellatus	+		+	+	+
Streptococcus criceti	+				
Streptococcus crista	+				

Bacterial species	Mouth	Stomach	Duodenu m/ Jejunum	Ileum	Colon
Streptococcus equisimilis	+				
Streptococcus gordonii	+				
Streptococcus intermedius	+			+	+
Streptococcus mitis	+	+			
Streptococcus mutans	+				
Streptococcus oralis	+				
Streptococcus parasanguis	+				
Streptococcus pyogenes	+	+			
Streptococcus salivarius	+	+			
Streptococcus sanguis	+	+			
Streptococcus sobrinus	+				
Streptococcus vestibularis	+				
Group C + G Streptococci	+				+
Succinivibrio dextrinosolvens			+	+	+
Sutterella spp.	+			+	+
Suttonella indologenes	+				
Tissierella praeacuta			+	+	+
Treponema denticola	+				
Treponema maltophilum	+				
Treponema socranskii	+				
Treponema vincentii	+				
Ureaplasma urealyticum	+				
Veillonella spp.	+		+	+	+

[0164] Endogenous microbial flora, such as bacteria, have access to tissues for pathogenesis either through contiguous spread or bacteremic spread. Under favorable conditions, all endogenous organisms can become pathogenic and invade locally and spread by contiguous spread to adjacent tissues and organs. Endogenous bacterial flora of the skin, mouth and colon are the species that are understood to also be amenable to bacteremic spread. Bacteria that are members of a particular endogenous flora domain may therefore cause infection in tissues or organs to which these bacteria may spread. Accordingly, one aspect of the invention involves the use of endogenous whole killed or attenuated *Escherichia coli* cells to treat an

Crohn's disease having symptoms localized to a region of the GIT in which the endogenous bacteria may spread to cause infection. The columns of Table 2 list domains for endogenous flora. The rows of Table 2 list regions of the GIT within which IBD may be situated. Accordingly, one aspect of the invention involves the use of endogenous whole killed or attenuated *Escherichia coli* cells to formulate antigenic compositions, or the selection of existing formulations having the endogenous whole killed or attenuated *Escherichia coli* cells, for treating an Crohn's disease situated in the region of the GIT to which the pathogen may spread to cause an infection. Accordingly, in alternative embodiments, Crohn's disease that is symptomatic in the region listed in the first column of Table 2 may be treated with antigenic compositions comprising whole killed or attenuated *Escherichia coli* cells that are members of the endogenous flora of one or more of the endogenous flora domains listed in the first row of Table 2 and indicated with an X or a check mark in the appropriate row.

Table 2: Tissue/Organ Pathogenicity of Endogenous Flora

Tissue/ organ site	Mouth	Stomach	Duo-denum/ Jejunum	Ileum	Colon
Oral	x				
Tonsil	x				
Nasopharynx/Sinus	x				
Esophagus		x			
Stomach		x			
Small bowel			x	x	
Colon/ Rectum					x
Anus					x

[0165] In accordance with the combined information in Tables 1 and 2, Crohn's disease manifest in a particular region of the GIT set out in column 1 of Table 2 may be treated with antigenic compositions comprising whole killed or attenuated *Escherichia coli* species of Table 1, so that the column headings in Table 2 are in effect replaced with the bacterial species of Table 1.

[0166] In some embodiments, whole killed or attenuated *Escherichia coli* cells for use in the invention may be exogenous bacterial pathogens. In some embodiments, both endogenous and exogenous whole killed or attenuated *Escherichia coli* species targeted to a specific tissue or organ may be used in combination.

Table 3 Exogenous Bacterial Human Pathogens, and their Sites of Infection in the GIT

Bacterial Species	**Region of the GIT**
Aerobacter spp.	small bowel, colon,
Bacillus anthracis	oral, small bowel, colon, hematological
Bacillus cereus	colon,
other Bacillus spp.	colon, stomach, small bowel

Bacterial Species	Region of the GIT
Brucella spp.	small bowel, colon
Campylobacter coli	small bowel, colon
Campylobacter jejuni	colon
Campylobacter sputorum	small bowel, colon
Clostridium bifermentans	small bowel, colon, stomach
Clostridium botulinum	colon, small bowel
Clostridium difficile	colon
Clostridium indolis	small bowel, colon, stomach,
Clostridium mangenolii	small bowel, colon, stomach
Clostridium perfringens	small bowel, colon, stomach
Clostridium sordellii	small bowel, colon, stomach
Clostridium sporogenes	small bowel, colon, stomach
Clostridium subterminale	small bowel, colon, stomach
Edwarsiella tarda	small bowel, colon
Francisella tularensis	small bowel
Helicobacter pylori	stomach
Leptospirosis spp.	oral
Listeria monocytogenes	small bowel, colon
Mycobacterium bovis	colon, small bowel
Mycobacterium tuberculosis	small bowel, colon
Pediococcus spp.	colon
Plesiomonas shigelloides	small bowel, colon
Rickettsia rickettsiae	small bowel
Salmonella spp.	stomach, small bowel, colon
Shigella boydii	colon
Shigella dysenteriae	colon
Shigella flexneri	colon
Shigella sonnei	colon
other Spirillum spp. Streptococcus zooepidemicus	colon
	small bowel
Treponema pallidum	oral, anus
Tropheryma whipplei	small bowel, colon
Vibrio cholerae	colon, small bowel
Vibrio fluvialis	small bowel, colon

Bacterial Species	Region of the GIT
Vibrio furnissii	small bowel, colon
Vibrio hollisae Vibrio parahaemolyticus	small bowel, colon colon, small bowel
Yersinia enterocolitica	small bowel, colon
Yersinia pseudotuberculosis	small bowel, colon

[0167] In some embodiments, pathogens for use in the invention may be viral pathogens. Table 4 provides an exemplary list of viral pathogens together with the tissue and organ sites for which each viral species is reportedly a pathogen. Accordingly, one aspect of the invention involves utilizing immunogenic compositions that are specific for the named viruses to treat an IBD situated in the region of the GIT that is identified adjacent to the name of the virus in Table 4.

Table 4 Viral Human Pathogens and Their Sites of Infection

Virus	Region of the GIT
Herpes Simplex virus (1 and 2)	rectum, anus
Cytomegalovirus	small bowel, colon/rectum
Epstein-Barr virus	oral
Adenovirus	oral, small bowel, colon
Human papillomavirus	anus, oral
Orthoreoviruses	small bowel, colon, oral
Coltiviruses	oral
Rotaviruses	small bowel, colon
Alphaviruses	small bowel, colon,
Coronaviruses	oral, small bowel, colon
Toroviruses	small bowel, colon
Parainfluenza viruses	oral
Respiratory syncytial virus	oral
Human metapneumovirus	oral, small bowel, colon
Vesicular stomatitis virus	oral, small bowel, colon
Rabies virus	oral
Influenza virus	oral
Hantaviruses	oral
Machupo virus	small bowel, colon
Junin virus	small bowel, colon
Poliovirus	small bowel, colon
Coxsackieviruses	small bowel, colon

Virus	Region of the GIT
Echoviruses	oral, small bowel, colon
Hepatitis A virus	small bowel, colon
Rhinoviruses	oral
Noroviruses and other Caliciviruses	small bowel, colon
Astroviruses	small bowel, colon
Picobirnaviruses	small bowel, colon
Hepatitis E virus	small bowel, colon

[0168] The cumulative information in Tables 1 through 4 provides an extensive identification of pathogens that may be used in the formulation of antigenic compositions of the invention, together with an identification of the region of the GIT in which these organisms are pathogenic, and accordingly identifies the region of the GIT in which an IBD is situated that may be treated with an antigenic formulation of the invention.

[0169] In some embodiments, the *Escherichia coli* pathogen selected for use in antigenic compositions of the invention may be one that is a common cause of acute infection in the region of the GIT in which the Crohn's disease to be treated is situated. Table 5 identifies bacterial and viral pathogens of this kind, together with the region of the GIT in which they commonly cause infection. Accordingly, in selected embodiments, Crohn's disease residing in a region of the GIT identified in the first column of Table 5 may be treated with an antigenic composition that comprises whole killed or attenuated *Escherichia coli* cells listed in the second column of Table 5.

Table 5: Common causes of acute infection (bacteria and viruses) for selected regions of the GIT

Selected regions of the GIT	Common Bacterial or Viral Pathogens
Oral	Prevotella melaninogenicus, anaerobic streptococci, viridans streptococci, Actinomyces spp., Peptostreptococcus spp., Bacteroides spp., and other oral anaerobes
	herpes simplex, coxsackieviruses, Epstein-Barr
Stomach	Streptococcus pyogenes, Helicobacter pylori
	cytomegalovirus, herpes simplex, Epstein-Barr, rotaviruses, noroviruses, adenoviruses
Small bowel	Escherichia coli, Clostridium difficile, Bacteroides fragilis, Bacteroides vulgatus, Bacteroides thetaiotaomicron, Clostridium perfringens, Salmonella enteriditis, Yersinia enterocolitica, Shigella flexneri
	adenoviruses, astroviruses, caliciviruses, noroviruses,

Selected regions of the GIT	Common Bacterial or Viral Pathogens
	rotaviruses, cytomegalovirus
Colon/Rectum	Escherichia coli, Clostridium difficile, Bacteroides fragilis, Bacteroides vulgatus, Bacteroides thetaiotaomicron, Clostridium perfringens, Salmonella enteriditis, Yersinia enterocolitica, Shigella flexneri
	adenoviruses, astroviruses, caliciviruses, noroviruses, rotaviruses, cytomegalovirus
Anus	Streptococcus pyogenes, Bacteroides spp., Fusobacterium spp., anaerobic streptococci, Clostridium spp., E. coli, Enterobacter spp., Pseudomonas aeruginosa, Treponema pallidum
	herpes simplex

[0170] The specific organisms which commonly cause infection in a specific region of the GIT may vary by geographical location. Table 5 is thus not an exhaustive list of common pathogens for all geographic locations and population groups. It is understood that a clinical microbiologist skilled in the art could determine the common pathogenic species in a particular geographic area or population group for a specific region of the GIT in accordance with the invention.

[0171] Humans are hosts to a wide range of gastrointestinal parasites, including various protozoa and helminths, which for purposes of the present invention constitute pathogens of the GIT (Schafer, T.W., Skopic, A. Parasites of the small intestine. Curr Gastroenterol Reports 2006;8:312-20; Jernigan, J., Guerrant, R.L., Pearson, R.D. Parasitic infections of the small intestine. Gut 1994;35:289-93; Sleisenger & Fordtran's Gastrointestinal and liver disease. 8th ed. 2006; Garcia, L.S. Diagnostic medical parasitology. 5th ed. 2007). Antigenic components of various protozoa, including for example: *Giardia lamblia, Cryptosporidium parvum, Cryptosporidium hominus, Isospora belli,* Sarcocystis species, Coccidian like bodies (Cyclospora species), *Enterocytozoon bieneusi, Entamoeba histolytica, Entamoeba dispar, Entamoeba coli, Entamoeba hartmanni, Endolimax nana, Iodamoeba bütschlii, Dientameoba fragilis, Blastocystis hominus, Cyclospora cayetanensis,* Microsporidia, *Trypanosoma cruzi, Chilomastix mesnili, Pentatrichomonas hominis, Balantidium coli.* Similarly, antigenic components of various helminths, include for example: Cestodes (tapeworms), *Taenia saginata, Taenia solium,* Diphyllobothrium species, *Hymenolepis nana, Hymenolepis diminuta, Dipylidium caninum,* Nematodes (round worms), *Ascaris lumbricoides, Strongyloides stercoralis, Necator americanus, Ancylostoma duodenale, Ancylostoma caninum, Tichuris trichiura, Capillaria philippinensis,* Trichostrongylus species, Trichinella species, *Necator americanus,* Anisakis and related species, *Angiostrongylus costaricensis, Enterobius vermicularis,* Trematodes (flukes), *Fasciolopsis buski,* Heterophyes speicies, Echinostoma species, *Clonorchis sinensis,* Opisthorchis species, Fasciola species, *Metagonimus yokogawi, Schistosoma mansoni, Schistosoma japonicum, Schistosoma mekongi, Schistosoma intercalatum,* Echinostoma species and Paragonimus species.

[0172] In selected embodiments, the invention involves diagnostic steps to assess a patient's previous exposure to an organism. For example, the diagnostic steps may include taking a medical history of exposure to selected pathogens, and/or evaluating a patient's immune response to a selected pathogen. For example, a serology test may be conducted to detect antibodies to selected pathogens in a patient's sera. In connection with this aspect of the invention, antigenic determinants of a selected pathogen may be chosen for use in an immunogenic composition on a selected patient based on a diagnostic indication that the patient has had one or more prior exposure(s) to the pathogen, for example by virtue of the presence of antibodies to antigenic determinants of that pathogen in the patient's sera.

[0173] In further selected embodiments, the invention involves diagnostic steps to assess a patient's immunological response to treatment with a selected immunogenic composition. For example, the diagnostic steps may include evaluating a patient's immune response to the antigenic determinants of that immunogenic composition, for example using a serological test to detect antibodies to those antigenic determinants. In connection with this aspect of the invention a treatment with a selected immunogenic composition may be continued if the evaluation indicates that there is an active immunological response to the antigenic determinants of that composition, and the vaccine treatment may be discontinued, and an alternative treatment with a different immunogenic composition may be initiated, if the evaluation indicates that there is not a sufficiently active immunological response to the antigenic determinants of the immunogenic composition.

[0174] The word "comprising" is used herein as an open-ended term, substantially equivalent to the phrase "including, but not limited to", and the word "comprises" has a corresponding meaning. As used herein, the singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a thing" includes more than one such thing. Citation of references herein is not an admission that such references are prior art to the present invention.

[0175] The following example illustrates an embodiment of the invention.

EXAMPLE 1: Inflammatory bowel disease studies

[0176] This example provides an illustration of the clinically effective use of an antigenic formulation comprising killed *E. coli* in the treatment of a patient with Crohn's disease, over a three-month course of treatment. During the course of treatment, the patient became symptom free and ceased using anti-inflammatory medications.

[0177] The patient initially presented reporting pain in the area of the large intestine, while under treatment with prednisone and Imuran™.

[0178] Treatment was initiated with a subcutaneous inoculum of a killed formulation of whole

E. coli, derived from an E. coli strain collected from a patient with an E. coli colonic infection. The dosing schedule involved a subcutaneous dose every second day, beginning with a 0.05 ml dose, gradually increasing in volume until a 2 inch diameter light pink/red skin response was achieved within 24 hours after injection at the injection site. The dose eventually required to achieve this skin response was 0.09-0.11 ml in this patient, and this dose has been continued as a maintenance dose every second day.

[0179] One week following the initiation of treatment with the antigenic preparation of whole killed E. coli, the patient reported that the pain was gone. Within about two months, the patient discontinued treatment with prednisone while continuing on a daily does of 150mg of Imuran™. Subsequently, the patient also discontinued use of Imuran™.

[0180] By two-months following the initiation of treatment with the *E. coli* composition, the patient was self-administering 0.09-0.11 ml of the E.coli preparation every other day. The patient self-adjusted this dosage so as to provoke a local inflammatory reaction evidenced by a pink spot of approximately 2 inches in diameter that persisted at the site of administration for about two days.

OTHER EMBODIMENTS

[0181] Numeric ranges are inclusive of the numbers defining the range. In the specification, the word "comprising" is used as an open-ended term, substantially equivalent to the phrase "including, but not limited to", and the word "comprises" has a corresponding meaning. Citation of references herein shall not be construed as an admission that such references are prior art to the present invention.

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Patentkrav

1. Antigen sammensætning til anvendelse i behandling af en human patient for Crohn's sygdom, hvor den antigene sammensætning omfatter hele dræbte eller svækkede patogene *Escherichia coli*-celler, og hvor den antigene sammensætning administreres intradermalt eller subkutant ved et administrationssted i på hinanden følgende doser givet ved et doseringsinterval på mellem en time og en måned, over en doseringsvarighed på mindst 1 uge.

2. Antigen sammensætning til anvendelse ifølge krav 1, hvor den antigene sammensætning administreres over en doseringsvarighed på mindst 2 uger.

3. Antigen sammensætning til anvendelse ifølge krav 1, hvor den antigene sammensætning administreres over en doseringsvarighed på mindst 2 måneder.

4. Antigen sammensætning til anvendelse ifølge krav 1, hvor den antigene sammensætning administreres over en doseringsvarighed på mindst 6 måneder.

5. Antigen sammensætning til anvendelse ifølge et hvilket som helst af de foregående krav, hvor den antigene sammensætning administreres i en dosis således at hver dosis effektiv forårsager et synligt lokaliseret inflammatorisk immunrespons ved administrationsstedet.

6. Antigen sammensætning til anvendelse ifølge et hvilket som helst af de foregående krav, hvor den antigene sammensætning administreres således at den synligt lokaliserede inflammation ved administrationsstedet sker indenfor 1 til 48 timer.

7. Antigen sammensætning til anvendelse ifølge et hvilket som helst af kravene 1 til 4, hvor den antigene sammensætning producerer en lokaliseret immunreaktion synlig i huden ved administrationsstedet, og hvor den synligt lokaliserede inflammation er et inflammationsområdet på fra 2mm til 100mm i diameter.

8. Antigen sammensætning til anvendelse ifølge krav 7, hvor den synligt lokaliserede inflammation indtræder 2 til 48 timer efter administration.

9. Antigen sammensætning til anvendelse ifølge krav 7 eller 8, hvor den synligt lokaliserede inflammation varer fra 2 til 72 timer.

10. Antigen sammensætning til anvendelse ifølge et hvilket som helst af de foregående krav, hvor den humane patient yderligere administreres med en effektiv mængde af et anti-inflammatorisk eller et immunundertrykkende medikament.

11. Antigen sammensætning til anvendelse ifølge krav 10, hvor medikamentet er sulfasalazin, mesalamin, et corticosteroid, azathioprin, mercaptopurin, infliximab, adalimumab, certolizumabpegol, methotrexat, cyclosporin eller natalizumab.

12. Antigen sammensætning til anvendelse ifølge et hvilket som helst af de foregående krav, hvor patienten er diagnosticeret som havende lidt af en tidligere patogen eksponering til *Escherichia coli.*

13. Antigen sammensætning til anvendelse ifølge et hvilket som helst af de foregående krav, hvor de hele dræbte eller svækkede patogene *Escherichia coli*-celler er en *E. coli*-stamme indsamlet fra en patient med en *E. coli*-tarminfektion.

14. Antigen sammensætning til anvendelse ifølge et hvilket som helst af de foregående krav, hvor de hele dræbte eller svækkede patogene *Escherichia coli*-celler er en *E. coli*-stamme der er patogen i fordøjelseskanalen.