Title: NUTRACEUTICAL BLEND FOR THE ENHANCEMENT OF THE IMMUNE SYSTEM

Abstract: The present invention relates to compositions of matter including a combination of a prebiotic compound and a probiotic compound. More particularly, the present invention relates to the combination of the probiotic Active Hexose Correlated Compound (AHCC) and the prebiotic Bifidobacterium lactis 12 (BB-12). The compositions of the present invention are useful for enhancing the immune system of a subject. The compositions of the present invention are also useful for enhancing the immune response to vaccination in a subject. The compositions of the present invention are also useful for enhancing the immune response to cancer in a subject.

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NUTRACEUTICAL BLEND FOR THE ENHANCEMENT OF THE IMMUNE SYSTEM

BACKGROUND OF THE INVENTION

Probiotics are microorganisms that are believed to provide some health benefits when consumed. They may be ingested by humans and animals. Prebiotics are non-digestible food ingredients that stimulate the growth and/or activity of bacteria in the digestive system in ways claimed to be beneficial to health. Many prebiotic and probiotic compounds have been studied, including the prebiotic Active Hexose Correlated Compound (AHCC) and the probiotic Bifidobacterium lactis 12 (BB12).

AHCC is an alpha-glucan-rich dietary supplement extracted from the mycelia of Basidiomycota mushrooms, such as shitake (LATINULINA EDODES). It contains a mix of oligosaccharides (comprising around 74% of AHCC, approximately 20% of which is the alpha-1,4-glucantype), amino acids, lipids, and minerals. AHCC is water soluble, water stable, and microcoated with candelilla wax to improve solubility in the intestines. AHCC is predominantly used for its purported ability to stimulate the immune system and as an adjuvant therapy to reduce the adverse side effects of chemotherapy. AHCC was developed in Japan in 1992, with several studies reporting a variety of therapeutic effects, including antioxidant and anticancer activity when combined with one or more other compounds, prevention of diabetes onset and liver injury, and improvement of immune response.

In Japan, various mushrooms and tree fungi have a long history of medicinal use. AHCC is made from the mycelia (vegetative portion) of various mushrooms in the general family of basidiomycete. It has been used to treat cancer with some apparent success.

Bifidobacterium is a type of gram-positive, non-motile anaerobic bacteria. It is an endosymbiotic inhabitant within the body, such as in the gastrointestinal tract of mammals, including humans. Bifidobacteria are one of the major genera of bacteria that make up the colon flora in mammals. Some Bifidobacterium strains are considered as important probiotics and used in the food industry. Different species and/or strains of Bifidobacteria may exert a range of
beneficial health effects, including the regulation of intestinal microbial homeostasis, the inhibition of pathogens and harmful bacteria that colonize and/or infect the gut mucosa, the modulation of local and systemic immune responses, the repression of procarcinogenic enzymatic activities within the microbiota, the production of vitamins, and the bioconversion of a number of dietary compounds into bioactive molecules. It is believed that Bifidobacterium improve the gut mucosal barrier and lowers levels of lipopolysaccharide in the intestine.

The genus Bifidobacterium possesses a unique fructose-6-phosphate phosphoketolase pathway employed to ferment carbohydrates. Much metabolic research on Bifidobacteria has focused on oligosaccharide metabolism as these carbohydrates are available in their otherwise nutrient-limited habitats.

Studies have shown that BB12 alone or in combination with other compounds has a beneficial effect within gastrointestinal and immune functions. Specifically, it relieves constipation, improves fecal properties and microbiota, it has a positive effect against acute diarrhea and restores the intestinal microbiota after antibiotic treatments. Studies have further shown an advantage in treating colic and reducing the risk of rotavirus diarrhea in infants.

There is a need in the art for effective therapy to augment a patient's immune system. The present invention addresses this need.

**SUMMARY OF THE INVENTION**

In one aspect, the present invention provides for a composition comprising a prebiotic and a probiotic bacteria that is capable of modulating the immune system. In one embodiment, the prebiotic is Active Hexose Correlated Compound (AHCC). In one embodiment, the probiotic bacteria is Bifidobacterium lactis BB12 (BB12). In one embodiment, the composition further comprises one or more pharmaceutically acceptable carriers. In one embodiment, the composition is retrained in a pharmaceutically acceptable carrier that is rapid release, immediate release, slow release, or delayed release.

In one embodiment, the composition further comprises one or more prebiotics. In one embodiment, the composition further comprises one or more
probiotic bacteria selected from the group consisting of: Lactobacillus plantarum, Lactobacillus acidophilus, Lactobacillus paracasei, Leuconostoc mesenteroides, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus salivarius, Pediococcus pentosaceus, Streptococcus thermophiles, Bacillus subtilis, Bacillus coagulans, Enterococcus faecium, Bifidobacterium bifidum, Bifidobacterium lactis, Bifidobacterium longum, and Bifidobacterium infantis.

In another aspect, the present invention provides for a method of enhancing the immune system of a subject, the method comprising administering to the subject a therapeutically effective amount of a composition comprising AHCC and BB12. In one embodiment, the composition is administered in combination with another therapeutic agent. In one embodiment, the composition is administered orally, parenterally, intravenously, intradermally, intramuscularly, or subcutaneously. In one embodiment, the composition is administered with food or drink. In one embodiment, the subject is a human. In one embodiment, the subject is a veterinary subject.

In another aspect, the present invention provides for a method of enhancing the immune response to a vaccine in a subject, said method comprising administering to the subject the vaccine and a therapeutically effective amount of a composition comprising AHCC and BB12. In one embodiment, the composition is administered prior to the administration of the vaccine. In one embodiment, the composition is administered simultaneously with the vaccine. In one embodiment, the composition is administered after the administration of the vaccine. In one embodiment, the composition is administered in combination with another therapeutic agent. In one embodiment, the composition is administered orally, parenterally, intravenously, intradermally, intramuscularly, or subcutaneously. In one embodiment, the composition is administered with food or drink. In one embodiment, the subject is a human. In one embodiment, the subject is a veterinary subject.

In another aspect, the present invention provides for a method of enhancing the immune response to cancer in a subject, said method comprising administering to the subject a therapeutically effective amount of a cancer-specific antigen and a composition comprising AHCC and BB12. In one embodiment, the
composition is administered in combination with another therapeutic agent. In one embodiment, the composition is administered orally, parenterally, intravenously, intradermally, intramuscularly, or subcutaneously. In one embodiment, the composition is administered with food or drink. In one embodiment, the subject is a human. In one embodiment, the subject is a veterinary subject.

**BRIEF DESCRIPTION OF THE DRAWINGS**

The following detailed description of preferred embodiments of the invention will be better understood when read in conjunction with the appended drawings. For the purpose of illustrating the invention, there are shown in the drawings embodiments which are presently preferred. It should be understood, however, that the invention is not limited to the precise arrangements and instrumentalities of the embodiments shown in the drawings.

Figure 1 depicts the results of an experiment comparing the hematocrit results between female human subjects who ingested the composition of the present invention and female human subjects who ingested a placebo. The improvement from baseline in the group that ingested the composition of the present invention was statistically significant after only 4 weeks.

Figure 2 depicts the results of an experiment comparing the number of CD11c+ myeloid dendritic cells between human subjects who ingested the composition of the present invention and human subjects who ingested a placebo. The group that ingested the composition of the present invention showed over 20% increase in myeloid dendritic cells at the end of 4 weeks, while no change was seen in the placebo group over the same period. This increase reached statistical significance in the group that ingested the composition at the end of 8 weeks.

Figure 3 depicts the results of an experiment comparing the number of monocyte cells between human subjects who ingested the composition of the present invention and human subjects who ingested placebo. At the end of 8 weeks, a statistically significant increase of 30% was seen in the group ingesting the composition, while only a minor, insignificant change was seen in the placebo group (p<0.0001).
Figure 4 depicts the results of an experiment comparing the number of CD14+CD16+ subset of monocyte cells between human subjects who ingested the composition of the present invention and human subjects who ingested placebo. A highly significant increase was seen in the group ingesting the composition for both the initial 4 week phase and for the phase following vaccine administration, while no change was seen in the placebo group.

Figure 5 depicts the results of an experiment comparing the level of vaccine-specific IgG3 between human subjects who ingested the composition of the present invention and human subjects who ingested placebo. After administering the vaccine, only the group ingesting the composition showed a statistically significant increase in the serum levels of vaccine-specific IgG3.

DETAILED DESCRIPTION

The present invention relates to compositions of matter including a combination of a prebiotic compound and a probiotic compound. More particularly, the present invention relates to the combination of the prebiotic Active Hexose Correlated Compound (AHCC) and the probiotic Bifidobacterium lactis 12 (BB12).

The present invention also relates to methods of treatment by administering the compositions of the invention to a subject in need thereof. In one embodiment, the present invention relates to methods of enhancing a subject's immune system by administering the compositions of the invention. In one embodiment, the present invention relates to methods of enhancing a subject's immune response to vaccination by administering the compositions of the invention.

Definitions

It is to be understood that the figures and descriptions of the present invention have been simplified to illustrate elements that are relevant for a clear understanding of the present invention, while eliminating, for the purpose of clarity, many other elements found in typical microscope devices. Those of ordinary skill in the art may recognize that other elements and/or steps are desirable and/or required in implementing the present invention. However, because such elements and steps are
well known in the art, and because they do not facilitate a better understanding of the present invention, a discussion of such elements and steps is not provided herein. The disclosure herein is directed to all such variations and modifications to such elements and methods known to those skilled in the art.

Unless defined elsewhere, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described.

As used herein, each of the following terms has the meaning associated with it in this section.

The articles "a" and "an" are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

"About" as used herein when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of ±20%, ±10%, ±5%, ±1%, and ±0.1% from the specified value, as such variations are appropriate.

The term "abnormal" when used in the context of organisms, tissues, cells or components thereof, refers to those organisms, tissues, cells or components thereof that differ in at least one observable or detectable characteristic (e.g., age, treatment, time of day, etc.) from those organisms, tissues, cells or components thereof that display the "normal" (expected) respective characteristic. Characteristics which are normal or expected for one cell or tissue type, might be abnormal for a different cell or tissue type.

The term "antigen" or "ag".as used herein is defined as a molecule that provokes an immune response. This immune response may involve either antibody production, or the activation of specific immunologically-competent cells, or both. The skilled artisan will understand that any macromolecule, including virtually all proteins or peptides, can serve as an antigen. Furthermore, antigens can be derived from
recombinant or genomic DNA. A skilled artisan will understand that any DNA, which comprises a nucleotide sequences or a partial nucleotide sequence encoding a protein that elicits an immune response therefore encodes an "antigen" as that term is used herein. Furthermore, one skilled in the art will understand that an antigen need not be encoded solely by a full length nucleotide sequence of a gene. It is readily apparent that the present invention includes, but is not limited to, the use of partial nucleotide sequences of more than one gene and that these nucleotide sequences are arranged in various combinations to elicit the desired immune response. Moreover, a skilled artisan will understand that an antigen need not be encoded by a "gene" at all. It is readily apparent that an antigen can be generated synthesized or can be derived from a biological sample. Such a biological sample can include, but is not limited to a tissue sample, a tumor sample, a cell or a biological fluid.

"An antigen presenting cell" (APC) is a cell that are capable of activating T cells, and includes, but is not limited to, monocytes/macrophages, B cells and dendritic cells (DCs).

The term "anti-tumor effect" as used herein, refers to a biological effect which can be manifested by a decrease in tumor volume, a decrease in the number of tumor cells, a decrease in the number of metastases, an increase in life expectancy, or amelioration of various physiological symptoms associated with the cancerous condition.

The term "B cell" as used herein is defined as a cell derived from the bone marrow and/or spleen. B cells can develop into plasma cells which produce antibodies.

The phrase "biological sample" is used herein in its broadest sense. A sample may be of any biological tissue or fluid from which biomarkers of the present invention may be detected, extracted, isolated, characterized or measured. Examples of such samples include but are not limited to blood, lymph, urine, gynecological fluids, biopsies, amniotic fluid and smears. Samples that are liquid in nature are referred to herein as "bodily fluids." Biological samples may be obtained from a patient by a variety of techniques including, for example, by scraping or swabbing an area or by
using a needle to aspirate bodily fluids. Methods for collecting various biological samples are well known in the art. Frequently, a sample will be a "clinical sample," i.e., a sample derived from a patient. Such samples include, but are not limited to, bodily fluids which may or may not contain cells, e.g., blood (e.g., whole blood, serum or plasma), urine, saliva, tissue or fine needle biopsy samples, and archival samples with known diagnosis, treatment and/or outcome history. Biological samples also include tissues, such as, frozen sections taken for histological purposes. The sample also encompasses any material derived by processing a biological sample. Derived materials include, but are not limited to, cells (or their progeny) isolated from the sample, proteins or nucleic acid molecules extracted from the sample. Processing of a biological sample may involve one or more of: filtration, distillation, extraction, concentration, inactivation of interfering components, addition of reagents, and the like.

The term "cancer" as used herein is defined as a hyperproliferation of cells whose unique trait—loss of normal control—results in unregulated growth, lack of differentiation, local tissue invasion, and/or metastasis. Examples include but are not limited to, breast cancer, prostate cancer, ovarian cancer, cervical cancer, skin cancer, pancreatic cancer, colorectal cancer, renal cancer, liver cancer, brain cancer, lymphoma, leukemia, lung cancer, germ-cell tumors, and the like.

As used herein, the term "container" includes any receptacle for holding the pharmaceutical composition. For example, in one embodiment, the container is the packaging that contains the pharmaceutical composition. In other embodiments, the container is not the packaging that contains the pharmaceutical composition, i.e., the container is a receptacle, such as a box or vial that contains the packaged pharmaceutical composition or unpackaged pharmaceutical composition and the instructions for use of the pharmaceutical composition. Moreover, packaging techniques are well known in the art. It should be understood that the instructions for use of the pharmaceutical composition may be contained on the packaging containing the pharmaceutical composition, and as such the instructions form an increased functional relationship to the packaged product. However, it should be understood that
the instructions may contain information pertaining to the compound's ability to perform its intended function, e.g., treating or preventing a disease in a subject.

A "disease" is a state of health of an animal wherein the animal cannot maintain homeostasis, and wherein if the disease is not ameliorated then the animal's health continues to deteriorate. In contrast, a "disorder" in an animal is a state of health in which the animal is able to maintain homeostasis, but in which the animal's state of health is less favorable than it would be in the absence of the disorder. Left untreated, a disorder does not necessarily cause a further decrease in the animal's state of health.

A disease or disorder is "ameliorated" if the severity or frequency of at least one sign or symptom of the disease or disorder experienced by a patient is reduced.

As used herein "endogenous" refers to any material from or produced inside an organism, cell, tissue or system.

As used herein, the term "exogenous" refers to any material introduced from or produced outside an organism, cell, tissue or system.

"Instructional material," as that term is used herein, includes a publication, a recording, a diagram, or any other medium of expression which can be used to communicate the usefulness of components of the invention in the kit for identifying or alleviating or treating the various diseases or disorders recited herein. Optionally, or alternately, the instructional material may describe one or more methods of identifying or alleviating the diseases or disorders in a cell or a tissue of a subject. The instructional material of the kit may, for example, be affixed to a container that contains the compositions of the invention or be shipped together with a container that contains the compositions of the invention. Alternatively, the instructional material may be shipped separately from the container with the intention that the recipient uses the instructional material and the compound cooperatively.

By the term "modulating," as used herein, is meant mediating a detectable increase or decrease in the level of a response in a subject compared with the level of a response in the subject in the absence of a treatment or compound, and/or compared with the level of a response in an otherwise identical but untreated subject.
The term encompasses perturbing and/or affecting a native signal or response thereby mediating a beneficial therapeutic response in a subject, preferably, a human.

The term "nutritional composition" may be a food product intended for human consumption, for example, a beverage, a drink, a bar, a snack, an ice cream, a dairy product, for example a chilled or a shelf-stable dairy product, a fermented dairy product, a drink, for example a milk-based drink, an infant formula, a growing-up milk, a confectionery product, a chocolate, a cereal product such as a breakfast cereal, a sauce, a soup, an instant drink, a frozen product intended for consumption after heating in a microwave or an oven, a ready-to-eat product, a fast food or a nutritional formula.

The terms "patient," "subject," "individual," and the like are used interchangeably herein, and refer to any animal, or cells thereof whether in vitro or in situ, amenable to the methods described herein. In certain non-limiting embodiments, the patient, subject or individual is a human. The term "patient" as used herein is meant to include a human or a veterinary patient. Within the context of the present invention, veterinary patients include both mammalian and non-mammalian veterinary patients, the latter including such veterinary patients as, for example, lizards and birds.

The term "prebiotic" includes any substance or combination of substances that may be utilized as a nutrient by a microorganism, may induce the growth and/or activity of a microorganism, may induce the replication of a microorganism, may be utilized as an energy source by the microorganism, and/or may be utilized by the microorganism for the production of biomolecules (i.e. RNA, DNA, and proteins). Non-limiting examples of prebiotics include mucopolysaccharides, oligosaccharides, polysaccharides, amino acids, vitamins, nutrient precursors, harvested metabolic products of biological organisms, microbial lysates, lipids, and proteins.

The terms "probiotic", "probiotic organisms" and the like include live microorganisms that beneficially affect the health of a host. The benefits to the health of the host include, but are not limited to, improving the microbial balance of the intestines. Other beneficial effects to the host include, for example, enhancing the immune system, stimulation of phagocytotic activity, stimulation of interferon, reduction of hypertension, decrease in the risk of cancer, increase in antimicrobial
activity and immunomodulating effects, reduction of hypercholesterolemia, and treatment of cancer.

As used herein, the term "pharmaceutical composition" refers to a mixture of at least one compound of the invention with other chemical components and entities, such as carriers, stabilizers, diluents, dispersing agents, suspending agents, thickening agents, and/or excipients. The pharmaceutical composition facilitates administration of the compound to an organism. Multiple techniques of administering a compound exist in the art including, but not limited to, intravenous, oral, aerosol, parenteral, ophthalmic, pulmonary and topical administration.

"Pharmaceutically acceptable" refers to those properties and/or substances which are acceptable to the patient from a pharmacological/toxicological point of view and to the manufacturing pharmaceutical chemist from a physical/chemical point of view regarding composition, formulation, stability, patient acceptance and bioavailability. "Pharmaceutically acceptable carrier" refers to a medium that does not interfere with the effectiveness of the biological activity of the active ingredient(s) and is not toxic to the host to which it is administered.

As used herein, the term "pharmaceutically acceptable carrier" means a pharmaceutically acceptable material, composition or carrier, such as a liquid or solid filler, stabilizer, dispersing agent, suspending agent, diluent, excipient, thickening agent, solvent or encapsulating material, involved in carrying or transporting a compound useful within the invention within or to the patient such that it may perform its intended function. Typically, such constructs are carried or transported from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation, including the compound useful within the invention, and not injurious to the patient. Some examples of materials that may serve as pharmaceutically acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil,
cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; surface active agents; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and other non-toxic compatible substances employed in pharmaceutical formulations. As used herein, "pharmaceutically acceptable carrier" also includes any and all coatings, antibacterial and antifungal agents, and absorption delaying agents, and the like that are compatible with the activity of the compound useful within the invention, and are physiologically acceptable to the patient. Supplementary active compounds may also be incorporated into the compositions. The "pharmaceutically acceptable carrier" may further include a pharmaceutically acceptable salt of the compound useful within the invention. Other additional ingredients that may be included in the pharmaceutical compositions used in the practice of the invention are known in the art and described, for example in Remington's Pharmaceutical Sciences (Genaro, Ed., Mack Publishing Co., 1985, Easton, PA), which is incorporated herein by reference.

The term "T cell" as used herein is defined as a thymus-derived cell that participates in a variety of cell-mediated immune reactions.

The term "T-helper" as used herein with reference to cells indicates a sub-group of lymphocytes (a type of white blood cell or leukocyte) including different cell types identifiable by a skilled person. In particular, T-helper cell according to the present disclosure include effector Th cells (such as Th1, Th2 and Th17). These Th cells secrete cytokines, proteins or peptides that stimulate or interact with other leukocytes.

The terms "treatment", "treating" and the like are used herein to generally mean obtaining a desired pharmacological and/or physiological effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of partially or completely curing a disease and/or adverse effect attributed to the disease. The term "treatment" as used herein covers any treatment of a disease in a subject and includes: (a) preventing a
disease related to an undesired immune response from occurring in a subject which may be predisposed to the disease; (b) inhibiting the disease, i.e. arresting its development: or (c) relieving the disease, i.e. causing regression of the disease.

The term "therapeutic" as used herein means a treatment and/or prophylaxis. A therapeutic effect is obtained by suppression, remission, or eradication of a disease state.

"Effective amount" or "therapeutically effective amount" are used interchangeably herein, and refer to an amount of a compound, formulation, material, or composition, as described herein effective to achieve a particular biological result. Such results may include, but are not limited to, the inhibition of virus infection as determined by any means suitable in the art.

Throughout this disclosure, various aspects of the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6, etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, 6, and any whole and partial increments therebetween. This applies regardless of the breadth of the range.

Description

The present invention relates to the unexpected discovery that certain compositions comprising prebiotics and probiotic bacteria are effective in enhancing a subject's immune system and immune response. In particular, the present invention relates to the unexpected discovery that the composition comprising AHCC and BB12 provides surprising results in the enhancement of a subject's immune system, such as through an increase in red blood cell count, hemoglobin content, and hematocrit. The results presented herein demonstrate that the compositions of the present invention have
particular benefits in augmenting a subject's immune response by improving antigen presentation and immunoglobulin secretion.

In one embodiment, the present invention provides compositions and methods to enhance the immune system. Therefore the present invention includes compositions and methods to provide a therapeutic benefit by enhancing the immune system.

In one embodiment, the composition of the invention comprises a combination of a prebiotic and a probiotic bacteria. In one embodiment, the composition of the present invention comprises AHCC and BB12.

In one embodiment, the invention relates to generically treating diseases or disorders associated with dysfunctional immune response whereby enhancing the immune system is a desired therapy. Accordingly, the invention includes the use of AHCC and BB12 in combination with a therapeutic agent such as an anti-tumor agent, a chemotherapeutic agent, an anti-cell proliferation agent, an anti-tumor vaccine and the like.

Compositions

In one embodiment, the present invention provides a generic concept for administering a prebiotic and probiotic bacteria as a therapy for enhancing a subject's immune system or immune response. In one embodiment, administering a prebiotic and probiotic bacteria enhances the immune system thereby providing an advantageous scenario for more effective vaccinations.

Prebiotic

In various embodiments, a prebiotic of the invention can be a saccharide that is difficult to digest, non-digestible or essentially non-digestible by a human and acts to encourage the growth of probiotic bacteria in the gut, increase adhesion of probiotic bacteria in the gut, displace pathogens, or provide a fermentable dose of carbohydrate to probiotic bacteria (symbiotic) or selected commensal bacteria and increase the levels of those microbial populations (notably lactobacilli and bifidobacteria) in the gastrointestinal tract. A prebiotic can be a saccharide that is non-
digestible by the human host and can act as a non-digestible fiber in the diet. This non-
digestibility is because humans lack the enzymes to break down some or all of the
prebiotic oligosaccharide as it travels through the digestive tract. When a prebiotic
reaches the small intestine and colon, bacteria encoding an enzyme or enzymes capable
of digesting the prebiotic can break down the prebiotic into simple sugars that the
bacteria can use. Non-limiting examples of prebiotics can include one or more of a
carbohydrate, carbohydrate monomer, carbohydrate oligomer, or carbohydrate polymer.

In one embodiment, the prebiotic is AHCC. AHCC, as described
elsewhere herein, refers to a mixture of polysaccharides, amino acids, lipids and
minerals derived from cocultured mycelia of several species of Basidiomycete
mushrooms. AHCC has been implicated with immunomodulation and protection against
infection. AHCC can enhance tumor immune surveillance by regulating both innate and
adaptive immune responses (Gao, Y. et al., Cancer Immunol. Immunother., 55(10):
commercially provided by Amino Up Chemical Co. Ltd, Japan. AHCC may increase
macrophage antigen presentation activity and inhibition of tumor-derived immune
suppressive factors, enhance macrophage proliferation and activation, promote
differentiation of Th1 cells; increase macrophage production of IL-12, increase NK
activity; promote apoptosis of cancer cells. AHCC in cancer patients has been reported
to increase TNF-a, γ-interferon, interleukin-12 and decrease immunosuppressive acidic
protein (LAP) and tumor growth factor (TGF)-a.

Probiotic

In various embodiments, a probiotic of the invention relates to
microorganisms (e.g., bacteria, yeast, fungus and/or virus) which may form a portion of
host flora, for example transient flora, and/or which may confer a therapeutic benefit to
a host, for example when administered in adequate amounts. Non-limiting examples of
probiotic bacteria include Lactobacillus plantarum, Lactobacillus acidophilus,
Lactobacillus paracasei, Leuconostoc mesenteroides, Lactobacillus bulgaricus,
Lactobacillus sakei, Lactobacillus salivarius, Pediococcus pentosaceus, Streptococcus
thermophiles, Bacillus subtilis, Bacillus coagulans, and Enterococcus faecium.
In one embodiment, the probiotic bacteria is BB12. BB12, as described elsewhere herein, refers to a particular strain of Bifidobacterium probiotic bacteria. In various embodiments, the AHCC and BB12 composition may be administered with additional probiotic bacteria. In one embodiment, the probiotic bacteria is a Bifidobacterium selected from the group consisting of Bifidobacterium adolescentis, Bifidobacterium animalis, Bifidobacterium asteroidis, Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium catenulatum, Bifidobacterium infantis, Bifidobacterium longum and Bifidobacterium pseudocatenulatum.

The probiotic bacteria of the present invention may also include mutant, variant, and genetically modified mutants of probiotic bacteria strains whose genetic and/or phenotypic properties are altered compared to the parent strain. Naturally occurring variants of probiotic bacteria strains include the spontaneous alterations of targeted properties selectively isolated while deliberate alteration of parent strain properties is accomplished by conventional genetic manipulation technologies, such as gene disruption, conjugative transfer, etc.

The general state of probiotic bacteria is in the form of viable cells, or freeze-dried cells (which was used to generate the data herein). However, it can also be extended to non-viable cells such as killed cultures or compositions containing beneficial factors expressed by the probiotic bacteria. This could include thermally killed micro-organisms or micro-organisms killed by exposure to altered pH or subjection to pressure. With non-viable cells product preparation is simpler, cells may be incorporated easily into pharmaceuticals and storage requirements are much less limited than viable cells.

Methods of Treatment

In various embodiments, the present invention provides methods of treatment using the compositions of the invention. In one embodiment, the methods relate to methods of enhancing the immune system. In one embodiment, the methods relate to methods of enhancing the immune response to vaccination. In one embodiment, the method relates to enhancing the immune response to treat a disease or disorder that is associated with dysfunctional immune response. In one embodiment,
the methods relate to methods of enhancing the immune response to treat cancer. In one embodiment, the methods relate to methods of enhancing the immune response to instances where the immune system is challenged or compromised, such as in therapy, surgery and the likes.

5 In one embodiment, the methods relate to the administration of a therapeutic amount of the compositions of the invention. For example, in certain instances, a subject may be administered from 0.5mg to 100g of the compositions of the invention. Preferably, subject may be administered from 1mg to 12g of the compositions of the invention.

10 Method of Enhancing Immune System

In one embodiment, the present invention includes methods of enhancing the immune system by administering the compositions of the invention to a subject. The methods include methods of potentiating an immune response in normal subjects, in subjects who are immunocompromised, or in subjects who are at risk of infection due to disease, hospitalization, age or other predisposing medical factors.

The methods of the present invention are effective in generally boosting the immune system in normal subjects. Normal subjects have a normally functioning immune system but may wish to enhance their immune system. For instance, normal subjects may use the methods of the present invention to maintain their health or as prophylaxis against possible immune system challenges.

The methods of the present invention are effective in boosting the immune response, for example, of subjects who are injured, immunocompromised or protein malnourished. Immunocompromised subjects generally exhibit an attenuated or reduced ability to mount a normal cellular or humoral defense to challenge by infectious agents, e.g., viruses, bacteria, fungi and protozoa. Protein malnourished subjects generally have a serum albumin level of less than about 3.2 grams per deciliter (g/dl) and/or unintentional weight loss of greater than 10% of usual body weight.

The methods of the present invention can be used to therapeutically or prophylactically treat subjects who are at a heightened risk of infection due to imminent surgery, injury, illness, radiation or chemotherapy, or other condition which
deleteriously affects the immune system. The method is useful to treat subjects who have a disease or disorder which causes the normal metabolic immune response to be reduced or depressed, such as HIV infection (AIDS). For example, the method can be used to pre-initiate the metabolic immune response in subjects who are undergoing chemotherapy or radiation therapy, or who are at a heightened risk for developing secondary infections or post-operative complications because of a disease, disorder or treatment resulting in a reduced ability to mobilize the body's normal metabolic responses to infection. Treatment with the compositions of the invention has been shown to be particularly effective in mobilizing the host's normal immune defenses, thereby engendering a measure of protection from infection in the treated host.

Method of Enhancing Immune Response to Vaccination

In one embodiment, the present invention includes methods of enhancing a subject's immune response to vaccination by administering the compositions of the present invention, such as an adjuvant. Adjuvant activity is manifested by a significant increase in immune-mediated protection by development of an immune response in an individual who otherwise would not respond at all to a vaccine. Enhancement of humoral immunity is typically manifested by a significant increase in the titer of antibody raised to the antigen.

The methods of the present invention, providing the administration of the compositions of the invention in conjunction with a vaccine, have the following advantages. The total antigenic load of vaccine to be administered may be reduced since less antigen in the presence of the compositions of the invention would elicit an immunologic response at least equivalent to that achieved by the administration of the normal amount of the vaccine. Since less antigen would be required per vaccination by administering the compositions of the invention in accordance with the present invention, the probability of undesirable side-effects associated with some vaccines currently in use would be reduced.

The immune response of certain types of individuals who respond poorly to vaccination would be enhanced by administering the compositions of the invention in conjunction with a vaccine. Types of individual who should benefit from the methods of
the present invention include (1) those types having impaired immune responsiveness, (2) those individuals who appear normal but who are nevertheless nonresponsive to certain vaccines as well as (3) individuals undergoing immunosuppressive therapies such as radiation and chemotherapy.

The vaccines contemplated for use in accordance with the present invention include but are not limited to bacterial vaccines, toxoid vaccines (inactivated toxins), and viral vaccines, or mixtures thereof used for active immunization. See for example chapter 75 entitled "Immunizing Agents" in Remington's Pharmaceutical Sciences 14th Edition 1990 Mack Publishing Co. p 1426-1441 and the antitoxins, toxoids, vaccines and live vaccines approved by the U.S. Food and Drug Administration and listed on page 208-209 (Product Category Index) of the Physician's Desk Reference, 46th Ed. 1992. Suitable bacterial vaccines include bacterial vaccines against the following disease entities or states: cholera, pertussis, plague, typhoid fever, meningitis, pneumococcal pneumonia, H. influenza type B, leprosy, gonorrhea, Group B meningococcus, and Group B streptococcus, Gram-negative sepsis, E. coli sepsis, and Pseudomonas aeruginosa. Suitable toxoids include diphtheria toxoid, botulism toxoid, and tetanus toxoid. Suitable viral vaccines include live and inactivated viral vaccines against the following disease entities or states: poliomyelitis, measles rubella, yellow fever, mumps, hepatitis B, hepatitis C and viral influenza.

In addition, the compositions of the invention may be used to enhance the protection afforded by animal or human vaccines that are considered "weak" (i.e., provide diminished protection in terms of level, extent, and/or duration). Examples of such vaccines are bacterins such as Bordetella bacterin, Escherichia coli bacterins, Haemophilus bacterins, Leptospirosis vaccines, Moraxella bovis bacterin, Pasteurella bacterin and Vibrio fetus bacterin, pneumococcal vaccines and attenuated live or killed virus products or recombinant antigenic viral products such as hepatitis B, influenza A & B, bovine respiratory disease vaccine, infectious bovine rhinotracheitis, parainfluenza-3, respiratory syncytial virus, bovine virus diarrhea vaccine, equine influenza vaccine, feline leukemia vaccine, feline respiratory disease vaccine
rhinotracheitis, calicivirus, pneumonitis, canine parovovirus vaccine, transmissible gastroenteritis vaccine, pseudorabies vaccine, and rabies vaccine.

Method of Enhancing Immune Response to Treat a Disease or Disorder

In one embodiment, the invention is applicable to treating a disease or disorder that is associated with a dysfunctional immune response. In one embodiment, the dysfunctional immune response includes autoimmune diseases. An autoimmune disease is the result of an inappropriate and excessive response to a self-antigen. Examples of autoimmune diseases include but are not limited to, Addison's disease, alopecia areata, ankylosing spondylitis, autoimmune hepatitis, autoimmune parotitis, Crohn's disease, diabetes (Type I), dystrophic epidermolysis bullosa, epididymitis, glomerulonephritis, Graves' disease, Guillain-Barré syndrome, Hashimoto's disease, hemolytic anemia, systemic lupus erythematosus, multiple sclerosis, myasthenia gravis, pemphigus vulgaris, psoriasis, rheumatic fever, rheumatoid arthritis, sarcoidosis, scleroderma, Sjogren's syndrome, spondyloarthropathies, thyroiditis, vasculitis, vitiligo, myxedema, pernicious anemia, ulcerative colitis, among others. The invention should not be limited to only the diseases listed herein. Rather, the invention is applicable to any disease associated with dysfunctional immune response.

Method of Enhancing Immune Response to Cancer

In one embodiment, the invention is applicable to tumor vaccines. In one embodiment, the subject has a type of cancer which expresses a tumor-specific antigen. In accordance with the present invention, an antigenic composition can be made which comprises a tumor-specific antigen sequence component. In such cases, the combination a tumor-specific antigen is administered in combination with an immunostimulatory agent (e.g., AHCC and BB12) to a patient in need thereof, resulting in an improved therapeutic outcome for the patient, evidenced by, e.g., a slowing or diminution of the growth of cancer cells or a solid tumor which expresses the tumor-specific antigen, or a reduction in the total number of cancer cells or total tumor burden.
The present invention provides a means to increase immunogenicity of a cell to generate an induced immune response to the tumor-associated antigen in the patient.

In another embodiment, the compounds of the present invention may be used in combination with existing therapeutic agents used to treat cancer. In some instances, the compounds of the invention may be used in combination these therapeutic agents to enhance the antitumor effect of the therapeutic agent.

In order to evaluate potential therapeutic efficacy of the compounds of the invention in combination with the antitumor therapeutics described elsewhere herein, these combinations may be tested for antitumor activity according to methods known in the art.

In one aspect, the present invention contemplates that the immunostimulatory agent (e.g., AHCC and BB12) of the invention may be used in combination with a therapeutic agent such as an anti-tumor agent including but not limited to a chemotherapeutic agent, an anti-cell proliferation agent or any combination thereof.

In one aspect, the present invention contemplates that the immunostimulatory agent of the invention may be used in combination with a targeted anti-cancer agent, such as monoclonal antibodies, signal transduction inhibitors, gene expression modulators, and the like.

The invention should not be limited to any particular chemotherapeutic agent. Rather, any chemotherapeutic agent can be linked to the antibodies of the invention. For example, any conventional chemotherapeutic agents of the following non-limiting exemplary classes are included in the invention: alkylating agents; nitrosoureas; antimetabolites; antitumor antibiotics; plant alkyloids; taxanes; hormonal agents; and miscellaneous agents.

Alkylating agents are so named because of their ability to add alkyl groups to many electronegative groups under conditions present in cells, thereby interfering with DNA replication to prevent cancer cells from reproducing. Most alkylating agents are cell cycle non-specific. In specific aspects, they stop tumor growth
by cross-linking guanine bases in DNA double-helix strands. Non-limiting examples include busulfan, carboplatin, chlorambucil, cisplatin, cyclophosphamide, dacarbazine, ifosfamide, mechlorethamine hydrochloride, melphalan, procarbazine, thiotepa, and uracil mustard.

5 Anti-metabolites prevent incorporation of bases into DNA during the synthesis (S) phase of the cell cycle, prohibiting normal development and division. Non-limiting examples of antimetabolites include drugs such as 5-fluorouracil, 6-mercaptopurine, capecitabine, cytosine arabinoside, floxuridine, fludarabine, gemcitabine, methotrexate, and thioguanine.

There are a variety of antitumor antibiotics that generally prevent cell division by interfering with enzymes needed for cell division or by altering the membranes that surround cells. Included in this class are the anthracyclines, such as doxorubicin, which act to prevent cell division by disrupting the structure of the DNA and terminate its function. These agents are cell cycle non-specific. Non-limiting examples of antitumor antibiotics include dactinomycin, daunorubicin, doxorubicin, idarubicin, mitomycin-C, and mitoxantrone.

Plant alkaloids inhibit or stop mitosis or inhibit enzymes that prevent cells from making proteins needed for cell growth. Frequently used plant alkaloids include vinblastine, vincristine, vindesine, and vinorelbine. However, the invention should not be construed as being limited solely to these plant alkaloids.

The taxanes affect cell structures called microtubules that are important in cellular functions. In normal cell growth, microtubules are formed when a cell starts dividing, but once the cell stops dividing, the microtubules are disassembled or destroyed. Taxanes prohibit the microtubules from breaking down such that the cancer cells become so clogged with microtubules that they cannot grow and divide. Non-limiting exemplary taxanes include paclitaxel and docetaxel.

Hormonal agents and hormone-like drugs are utilized for certain types of cancer, including, for example, leukemia, lymphoma, and multiple myeloma. They are often employed with other types of chemotherapy drugs to enhance their effectiveness.

Sex hormones are used to alter the action or production of female or male hormones and
are used to slow the growth of breast, prostate, and endometrial cancers. Inhibiting the production (aromatase inhibitors) or action (tamoxifen) of these hormones can often be used as an adjunct to therapy. Some other tumors are also hormone dependent. Tamoxifen is a non-limiting example of a hormonal agent that interferes with the activity of estrogen, which promotes the growth of breast cancer cells.

Miscellaneous agents include chemotherapeutics such as bleomycin, hydroxyurea, L-asparaginase, and procarbazine that are also useful in the invention.

An anti-cell proliferation agent can further be defined as an apoptosis-inducing agent or a cytotoxic agent. The apoptosis-inducing agent may be a granzyme, a Bcl-2 family member, cytochrome C, a caspase, or a combination thereof. Exemplary granzymes include granzyme A, granzyme B, granzyme C, granzyme D, granzyme E, granzyme F, granzyme G, granzyme H, granzyme I, granzyme J, granzyme K, granzyme L, granzyme M, granzyme N, or a combination thereof. In other specific aspects, the Bcl-2 family member is, for example, Bax, Bak, Bcl-Xs, Bad, Bid, Bik, Hrk, Bok, or a combination thereof.

In additional aspects, the caspase is caspase-1, caspase-2, caspase-3, caspase-4, caspase-5, caspase-6, caspase-7, caspase-8, caspase-9, caspase-10, caspase-11, caspase-12, caspase-13, caspase-14, or a combination thereof. In specific aspects, the cytotoxic agent is TNF-α, gelonin, Prodigiosin, a ribosome-inhibiting protein (RIP), Pseudomonas exotoxin, Clostridium difficile Toxin B, Helicobacter pylori VacA, Yersinia enterocolitica YopT, Violacein, diethylenetriaminepentaacetic acid, irofulven, Diptheria Toxin, mitogillin, ricin, botulinum toxin, cholera toxin, saporin 6, or a combination thereof.

In some embodiments, an effective amount of an immunostimulatory agent (e.g., AHCC and BB12) of the invention and a therapeutic agent is a synergistic amount. As used herein, a "synergistic combination" or a "synergistic amount" of a compound of the invention and a therapeutic agent is a combination or amount that is more effective in the therapeutic or prophylactic treatment of a disease than the incremental improvement in treatment outcome that could be predicted or expected from a merely additive combination of (i) the therapeutic or prophylactic benefit of the
compound of the invention when administered at that same dosage as a monotherapy and (ii) the therapeutic or prophylactic benefit of the therapeutic agent when administered at the same dosage as a monotherapy.

5

Method of Enhancing Immune Response to Therapy

In one embodiment, the invention includes methods of enhancing a subject's immune response to therapy. In various embodiments, the methods of the present invention relate to the administration of the compositions of the invention to a subject that is exposed to any number of relevant treatment modalities, including but not limited to treatment with agents such as natalizumab, efalizumab, antiviral agents, chemotherapy, cryotherapy, radiation, immunosuppressive agents, such as cyclosporin, azathioprine, methotrexate, mycophenolate, and FK506, antibodies, or other immunoablative agents such as CAMPATH, anti-CD3 antibodies, cytoxin, fludaribine, cyclosporin, FK506, rapamycin, mycophenolic acid, steroids, FR901228, and irradiation.

In this regard, it has been observed that following certain treatments and therapies, in particular treatments and therapies that damage the immune system, shortly after treatment during the period when patients would normally be recovering from the treatment, the quality of the immune system is impaired. Thus, it is contemplated within the context of the present invention to administer the compositions of the present invention to a subject during this recovery phase. The compositions of the present invention are especially useful to create a condition in a subject wherein repopulation, recirculation, regeneration, and/or expansion of particular cell types is favored, especially during a defined window of time following therapy. Illustrative cell types include T cells, B cells, dendritic cells, and other cells of the immune system.

In certain embodiments, the methods of the present invention comprise the administration of the compositions of the present invention to a subject in conjunction with (e.g., before, simultaneously or following) any number of relevant treatment modalities, including but not limited to treatment with agents such as antiviral
therapy, cidofovir and interleukin-2, Cytarabine (also known as ARA-C) or natalizumab treatment for MS patients or efalizumab treatment for psoriasis patients or other treatments for PML patients. In further embodiments, the compositions of the invention may be used in combination with chemotherapy, radiation, immunosuppressive agents, 5 such as cyclosporin, azathioprine, methotrexate, mycophenolate, and FK506, antibodies, or other immunoablative agents such as CAM PATH, anti-CD3 antibodies or other antibody therapies, cytoxin, fludaribine, cyclosporin, FK506, rapamycin, mycophenolic acid, steroids, FR901228, cytokines, and irradiation. These drugs inhibit either the calcium dependent phosphatase calcineurin (cyclosporine and FK506) or 10 inhibit the p70S6 kinase that is important for growth factor induced signaling (rapamycin). (Liu et al, Cell 66:807-815, 1991; Henderson et al., Immun. 73:316-321, 1991; Bierer et al., Curr. Opin. Immun. 5:763-773, 1993; Isoniemi (supra)). In a further embodiment, the compositions of the present invention are administered to a patient in conjunction with (e.g., before, simultaneously or following) bone marrow transplantation, T cell ablative therapy using either chemotherapy agents such as, fludarabine, external-beam radiation therapy (XRT), cyclophosphamide, or antibodies such as OKT3 or CAMPATH. In another embodiment, the compositions of the present invention are administered following B-cell ablative therapy such as agents that react with CD20, e.g., Rituxan. For example, in one embodiment, subjects may undergo standard treatment with high dose chemotherapy followed by peripheral blood stem cell transplantation. In certain embodiments, following the transplant, subjects receive an infusion of the compositions of the present invention. In an additional embodiment, compositions of the present invention are administered before or following surgery. 20

In another embodiment, the methods of the present invention are useful in pain therapy, such as therapy for chronic pain, acute pain, as well as pre-, intra-, and postoperative pain. In various embodiments, the methods of the present invention comprise the administration (i.e., pre-administration, co-administration, and/or post-administration) of other treatments and/or agents to modify (e.g., enhance) the effectiveness of the compositions of the present invention. In one embodiment, the 25 other agents include anti-pain/anti-inflammatory agents.
In one embodiment, the additional anti-pain/anti-inflammation agents may include one or more agents selected from the following classes of receptor antagonists and agonists and enzyme activators and inhibitors, each class acting through a differing molecular mechanism of action for pain and/or inflammation inhibition: (1) serotonin receptor antagonists; (2) serotonin receptor agonists; (3) histamine receptor antagonists; (4) bradykinin receptor antagonists; (5) kallikrein inhibitors; (6) tachykinin receptor antagonists, including neurokinin, and neurokinin2 receptor subtype antagonists; (7) calcitonin gene-related peptide (CGRP) receptor antagonists; (8) interleukin receptor antagonists; (9) inhibitors of enzymes active in the synthetic pathway for arachidonic acid metabolites, including (a) phospholipase inhibitors, including PLA2 isoform inhibitors and PLCγ isoform inhibitors, (b) cyclooxygenase inhibitors, including non-selective cyclooxygenase inhibitors and cyclooxygenase-2 (COX-2) selective inhibitors, and (c) lipoygenase inhibitors; (10) prostanoid receptor antagonists including eicosanoid EP-1 and EP-4 receptor subtype antagonists and thromboxane receptor subtype antagonists; (11) leukotriene receptor antagonists including leukotriene B4 receptor subtype antagonists and leukotriene D4 receptor subtype antagonists; (12) opioid receptor agonists, including µ-opioid, δ-opioid, and κ-opioid receptor subtype agonists; (13) purinoceptor agonists and antagonists including P2X receptor antagonists and P2Y receptor agonists; (14) adenosine triphosphate (ATP)-sensitive potassium channel openers; (15) mitogen-activated protein kinase (MAPK) inhibitors; (16) neuronal nicotinic acetylcholine receptor agonists; and (17) soluble receptors. Each of the above agents functions either as an anti-inflammatory agent and/or as an anti-nociceptive, i.e., anti-pain or analgesic, agent.

Methods of Administration

In the various methods of treatment, the administration of the composition of the invention may be for either "prophylactic" or "therapeutic" purpose. When provided prophylactically, the composition of the present invention is provided in advance of any symptom, although in particular embodiments the vaccine aspect of the invention is provided following the onset of one or more symptoms to prevent further symptoms from developing or to prevent present symptoms from becoming worse. The
prophylactic administration of composition serves to prevent or ameliorate any subsequent infection or disease. When provided therapeutically, the pharmaceutical composition is provided at or after the onset of a symptom of infection or disease. Thus, the present invention may be provided either prior to the anticipated exposure to a disease-causing agent or disease state or after the initiation of the infection or disease.

An effective amount of the composition would be the amount that achieves this selected result of enhancing the immune response, and such an amount could be determined as a matter of routine by a person skilled in the art. For example, an effective amount of for treating an immune system deficiency against cancer or pathogen could be that amount necessary to cause activation of the immune system, resulting in the development of an antigen specific immune response upon exposure to antigen. The term is also synonymous with "sufficient amount."

In order to impart their therapeutic effect, the compositions of the invention may be delivered into a subject. In one embodiment, the subject is a mammal. In another embodiment, the subject is a cell. One mechanism for delivery is by any of the methods mentioned above which physically or chemically permeabilize the cell membrane. Another embodiment of the invention for transferring the AHCC and BB12 composition into cells may involve particle bombardment. This method depends on the ability to accelerate microprojectiles carrying the short chain fatty acids to a high velocity allowing them to pierce cell membranes and enter cells without killing them. Several devices for accelerating small particles have been developed. One such device relies on a high voltage discharge to generate an electrical current, which in turn provides the motive force. The microprojectiles used have consisted of biologically inert substances such as tungsten or gold beads.

In a further embodiment of the invention, the compositions of the invention may be entrapped in a liposome. Liposomes are vesicular structures characterized by a phospholipid bilayer membrane and an inner aqueous medium. Multilamellar liposomes have multiple lipid layers separated by aqueous medium. They form spontaneously when phospholipids are suspended in an excess of aqueous
solution. The lipid components undergo self-rearrangement before the formation of closed structures and entrap water and dissolved solutes between the lipid bilayers.

In another embodiment of the invention, the compositions of the invention may be immobilized on the surface of a substrate. The substrate surface may be any surface capable of having an agent/ligand bound thereto or integrated into and that is biocompatible. The biocompatible surface may be biodegradable or non-biodegradable. The surface may be natural or synthetic, and a synthetic surface may be a polymer. The surface may comprise collagen, purified proteins, purified peptides, polysaccharides, glycosaminoglycans, or extracellular matrix compositions. A polysaccharide may include for example, cellulose, agarose, dextran, chitosan, hyaluronic acid, or alginate. Other polymers may include polyesters, polyethers, polyanhydrides, polyalkylcyanoacrylates, polyacrylamides, polyorthoesters, polyphosphazenes, polyvinylacetates, block copolymers, polypropylene, polytetrafluorethylene (PTFE), or polyurethanes. The polymer may be lactic acid or a copolymer. A copolymer may comprise lactic acid and glycolic acid (PLGA). Non-biodegradable surfaces may include polymers, such as poly(dimethylsiloxane) and poly(ethylene- vinyl acetate). Biocompatible surfaces include for example, glass (e.g., bioglass), collagen, metal, hydroxyapatite, aluminate, bioceramic materials, hyaluronic acid polymers, alginate, acrylic ester polymers, lactic acid polymer, glycolic acid polymer, lactic acid/glycolic acid polymer, purified proteins, purified peptides, or extracellular matrix compositions. Other polymers comprising a surface may include glass, silica, silicon, hydroxyapatite, hydrogels, collagen, acrolein, polyacrylamide, polypropylene, polystyrene, nylon, or any number of plastics or synthetic organic polymers, or the like.

The compositions of the present invention and the pharmaceutical compositions containing said compounds, may be administered orally, and thus be formulated in a form suitable for oral administration, i.e. as a solid or a liquid preparation. Suitable solid oral formulations include tablets, capsules, pills, granules, pellets and the like. Suitable liquid oral formulations include solutions, suspensions, dispersions, emulsions, oils and the like. If formulated in form of a capsule, the
compositions of the present invention comprise, in addition to the active compound and the inert carrier or diluent, a hard gelating capsule.

The compositions of the present invention and the pharmaceutical compositions containing said compounds may be further administered intranasally, i.e. by inhalation and thus may be formulated in a form suitable for intranasal administration, i.e. as an aerosol or a liquid preparation.

The compositions of the present invention may also, for example, be formulated as suppositories, containing conventional suppository bases for use in human or veterinary medicine or as pessaries, for example, containing conventional pessary bases.

The compositions of the present invention may, for example, be formulated for parenteral administration (e.g., by injection, for example, bolus injection or continuous infusion) and may be presented in unit dose form in ampules, pre-filled syringes, small volume infusion containers or in multi-dose containers with an added preservative. The injection formulations may be suitable for intradermal, subcutaneous, intramuscular, or intravenous injection. The active ingredients may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredients may be in powder form, obtained by aseptic isolation of sterile solid or by lyophilization from solution, for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water, before use.

The amount, frequency and period of administration will vary depending upon factors such as the level of the specific antibody titers, the class of antibody to be induced, the vaccine type as well as the age of the patient and general physical condition. The compositions of the invention can be administered before, concurrently with, or after the vaccine is administered.

In one embodiment, the compositions of the invention are administered separately from the vaccine, although it may be administered in combination with the vaccine. For instance, when the compositions of the invention are combined with the vaccine, the composition administered may contain an immunogen that is effective in
eliciting a specific response to a given pathogen or antigen, a pharmaceutically acceptable vaccine carrier, and an immunopotentiating amount of the compositions of the invention. In one embodiment, the compositions of the invention are administered prior to the administration of the vaccine and at the same site where the vaccine is to be administered. The formulations and pharmaceutical compositions contemplated by the above dosage forms can be prepared with conventional pharmaceutically acceptable excipients and additives, using conventional techniques. Other adjuvants may be administered either with the vaccine or together with the compositions of the invention.

If multiple doses of the vaccine are to be administered over a period of time, additional doses of the compositions of the invention may be administered in conjunction with each subsequent dose of the vaccine. The amount of the compositions of the invention so administered with each subsequent dose of the vaccine may be more, the same or less than the amount of the compositions of the invention administered in conjunction with the initial dose of the vaccine. The amount of the compositions of the invention administered with each subsequent dose of the vaccine will depend upon the antibody response of the patient after the first dose of the vaccine.

In various embodiments, the immunostimulatory agent of the invention may be co-administered with various other compounds (cytokines, chemotherapeutic and/or antiviral drugs, among many others). Alternatively, the immunostimulatory agent of the invention may be administered an hour, a day, a week, a month, or even more, in advance of an immunogenic composition, or any permutation thereof. Further, the immunostimulatory agent may be administered an hour, a day, a week, or even more, after administration of an immunogenic composition, or any permutation thereof. The frequency and administration regimen will be readily apparent to the skilled artisan and will depend upon any number of factors such as, but not limited to, the type and severity of the disease being treated, the age and health status of the animal, the identity of the compound or compounds being administered, the route of administration of the various immunostimulatory agent and the immunogenic composition, and the like.

Pharmaceutical Compositions
The present invention includes pharmaceutical compositions comprising one or more compositions of the present invention. The formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with a carrier or one or more other accessory ingredients, and then, if necessary or desirable, shaping or packaging the product into a desired single- or multi-dose unit.

Said compositions may comprise additional medicinal agents, pharmaceutical agents, carriers, buffers, adjuvants, dispersing agents, diluents, and the like depending on the intended use and application.

Examples of suitable pharmaceutical carriers, excipients and/or diluents are well known in the art and include, but are not limited to, a gum, a starch (e.g., corn starch, pregeletanized starch), a sugar (e.g., lactose, mannitol, sucrose, dextrose), a cellulose material (e.g. microcrystalline cellulose), an acrylate (e.g. polymethylacrylate), calcium carbonate, magnesium oxide, talc, or mixtures thereof.

Pharmacologically acceptable carriers for liquid formulations are aqueous or non-aqueous solutions, suspensions, emulsions or oils. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, and injectable organic esters such as ethyl oleate. Examples of oils are those of animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, olive oil, sunflower oil, turmeric oil, fish-liver oil, another marine oil, or a lipid from milk or eggs.

Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media such as phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions etc. Compositions comprising such carriers can be formulated by well-known conventional methods. Suitable carriers may comprise any material which, when combined with the biologically active compound of the invention, retains the biological activity. Preparations for parenteral administration may include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive
oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles may include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles may include fluid and nutrient replenishes, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present including, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like, in addition, the pharmaceutical composition of the present invention might comprise proteinaceous carriers, e.g., serum albumin or immunoglobulin, preferably of human origin.

The pharmaceutical compositions provided herein may also be administered as controlled-release compositions, i.e. compositions in which the active ingredient is released over a period of time after administration. Controlled- or sustained-release compositions include formulation in lipophilic depots (e.g. fatty acids, waxes, oils). In another embodiment, the composition is an immediate-release composition, i.e. a composition in which all the active ingredient is released immediately after administration.

Further, the pharmaceutical compositions according to the invention and as described herein in the various embodiments may or a composition comprising said compound may be administered admixed to food, functional food, drinks, medicinal food.

Although the description of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions which are suitable for ethical administration to humans, it will be understood by the skilled artisan that such compositions are generally suitable for administration to animals of all sorts. Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and perform such modification with merely ordinary, if any, experimentation. Subjects to which administration of the pharmaceutical compositions of the invention is contemplated
include, but are not limited to, humans and other primates, mammals including commercially relevant mammals such as non-human primates, cattle, pigs, horses, sheep, cats, and dogs.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in bulk, as a single unit dose, or as a plurality of single unit doses. As used herein, a "unit dose" is discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient which would be administered to a subject or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage.

The relative amounts of the active ingredient, the pharmaceutically acceptable carrier, and any additional ingredients in a pharmaceutical composition of the invention will vary, depending upon the identity, size, and condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100% (w/w) active ingredient.

In addition to the active ingredient, a pharmaceutical composition of the invention may further comprise one or more additional pharmaceutically active agents. Controlled- or sustained-release formulations of a pharmaceutical composition of the invention may be made using conventional technology.

Compositions of the present invention may also comprise a prebiotic component. "Prebiotic" includes substances or compounds that are fermented by the intestinal flora of the pet and hence promote the growth or development of lactic acid bacteria in the gastro-intestinal tract of the pet at the expense of pathogenic bacteria. The result of this fermentation can be a release of fatty acids, in particular short-chain fatty acids in the colon. This release can have the effect of reducing the pH value in the colon. Non-limiting examples of suitable prebiotics include oligosaccharides, such as inulin and its hydrolysis products commonly known as fructooligosaccharides, galacto-oligosaccharides, xylo-oligosaccharides, or oligo derivatives of starch (such as pectin, beta-glucan, and resistant starch). The prebiotics may be provided in any suitable form.
For example, the prebiotic may be provided in the form of plant material that contains the fiber. Suitable plant materials include asparagus, artichokes, onions, wheat or chicory, or residues of these plant materials. Alternatively, the prebiotic fiber may be provided as an inulin extract, for example extracts from chicory are suitable. Suitable inulin extracts may be obtained from Orafti SA of Tirlemont 3300, Belgium under the trade mark "Raftiline". For example, the inulin may be provided in the form of Raftiline (g) ST which is a fine white powder, which contains about 90 to about 94% by weight of inulin, up to about 4% by weight of glucose and fructose, and about 4 to 9% by weight of sucrose. Alternatively, the fiber may be in the form of a fructooligosaccharide such as obtained from Orafti SA of Tirlemont 3300, Belgium under the trade mark "Raftilose". For example, the inulin may be provided in the form of Raftilose (g) P95. Otherwise, the fructooligosaccharides may be obtained by hydrolyzing inulin, by enzymatic methods, or by using micro-organisms.

Pharmaceutical compositions also include nutritional compositions, such as oral nutritional compositions for oral consumption and optionally for enteral adsorption, wherein the nutritional composition includes the compounds of the present invention.

If the nutritional compositions are formulated to be administered orally, the compositions may be a liquid oral nutritional supplement (e.g., incomplete feeding) or a complete feeding. In this manner, the nutritional compositions may be administered in any known form including, for example, tablets, capsules, liquids, chewables, soft gels, sachets, powders, syrups, liquid suspensions, emulsions and solutions in convenient dosage forms.

A nutritional formula encompasses any nutritionally complete or supplementary formulation (a nutritional supplement, for example). As used herein, "nutritionally complete" are preferably nutritional products that contain sufficient types and levels of macronutrients (protein, fats and carbohydrates) and micronutrients to be sufficient to be a sole source of nutrition for the subject to which it is being administered to. Patients can receive 100% of their nutritional requirements from such complete nutritional compositions. According to one embodiment, the nutritional
formula is a supplementary formulation providing supplementary nutrition. A
"supplementary formula" may not be nutritionally complete, but preferably contains
specific nutrients that are supportive, for example in combination with physical
exercise, with further of the beneficial effects of the invention, and which address
specific or additional needs of the subject.

The nutritional formula may be a generally applicable nutritional
formula, for example adapted to subjects of a specific age, for example a formula for
children, but it may also be a formula for elderly patients, for intensive care patients, or
a specially adapted formula for patients suffering from a specific disease, for example.

Any nutritional formula may be reconstitutable, that is, present in a substantially dried,
for example powdered form, or ready-to-drink, in the form of liquid formulas, for
example.

Kits of the Invention

The invention also includes a kit comprising compounds useful within
the methods of the invention and an instructional material that describes, for instance,
the method of administering AHCC and BB12 as described elsewhere herein, or the
method of administering prebiotics and probiotics as described elsewhere herein.
Formulations of a pharmaceutical composition suitable for parenteral administration
comprise the active ingredient combined with a pharmaceutically acceptable carrier,
such as sterile water or sterile isotonic saline. Such formulations may be prepared,
packaged, or sold in a form suitable for bolus administration or for continuous
administration. Injectable formulations may be prepared, packaged, or sold in unit
dosage form, such as in ampules or in multi dose containers containing a preservative.
Formulations for parenteral administration include, but are not limited to, suspensions,
solutions, emulsions in oily or aqueous vehicles, pastes, and implantable sustained-
release or biodegradable formulations. Such formulations may further comprise one or
more additional ingredients including, but not limited to, suspending, stabilizing, or
dispersing agents. In one embodiment of a formulation for parenteral administration,
the active ingredient is provided in dry (i.e., powder or granular) form for reconstitution
with a suitable vehicle (e.g., sterile pyrogen free water) prior to parenteral administration of the reconstituted composition.

The pharmaceutical compositions may be prepared, packaged, or sold in the form of a sterile injectable aqueous or oily suspension or solution. This suspension or solution may be formulated according to the known art, and may comprise, in addition to the active ingredient, additional ingredients such as the dispersing agents, wetting agents, or suspending agents described herein. Such sterile injectable formulations may be prepared using a non-toxic parenterally acceptable diluent or solvent, such as water or 1,3 butane diol, for example. Other acceptable diluents and solvents include, but are not limited to, Ringer's solution, isotonic sodium chloride solution, and fixed oils such as synthetic mono or di-glycerides. Other parentally-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form, in a liposomal preparation, or as a component of a biodegradable polymer system. Compositions for sustained release or implantation may comprise pharmaceutically acceptable polymeric or hydrophobic materials such as an emulsion, an ion exchange resin, a sparingly soluble polymer, or a sparingly soluble salt.

**EXPERIMENTAL EXAMPLES**

The invention is further described in detail by reference to the following experimental examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless otherwise specified. Thus, the invention should in no way be construed as being limited to the following examples, but rather, should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the compounds of the present invention and practice the claimed methods. The following working examples therefore, specifically point out the preferred embodiments of the present invention, and are not to be construed as limiting in any way the remainder of the disclosure.
Example 1: Effects of a nutraceutical blend on selected immune parameters

A randomized, double-blinded, placebo controlled study was conducted, where 52 healthy subjects ingested either a placebo or a composition comprising AHCC (1,800 mg AHCC-FD/day) with a probiotic product BB12 (12 billion CFU/day) for 8 weeks. At the 4 week time point, the subjects were administered a flu vaccine. The ingredients of the composition were blended, then encapsulated. A color-matched placebo was made using inert coloring substances and the same excipients as used in the active product. Blood was drawn at the 0, 4, and 8 week time points.

Distinct changes were found in the numbers of various subsets of circulating immune cells during the consumption of AHCC/BB12 in the absence of an immune challenge (loading phase), as well as after the vaccine challenge. General observations throughout the study include: a significant improvement in the red blood cell numbers, hemoglobin content, and hematocrit in the female population (Figure 1); and a continuous increase in CD14+ CD16+ monocytes (Figure 4) and CD14+ myeloid dendritic cells (Figure 2) throughout the 8-week study.

Observations made during the loading phase include: an increase in the numbers of myeloid dendritic cells; and an increase in CD14+ CD16+ monocytes (Figures 2 and 4). These changes may have led to more effective antigen presentation during the vaccine challenge, thus preparing the immune system for an immune challenge.

Observations made after the vaccine challenge include: a continued increase in CD14+ monocytes (Figure 3); a continued increase in CD14+ CD16+ monocytes (Figure 4); and a specific increase in the IgG3 subclass of vaccine-specific IgG (Figure 5). Data suggests a protection from the reduction in several cell types otherwise seen after the vaccine challenge: CD56++ NK cells, CD4+ T cells, and CD8+ T cells.

Following the vaccine challenge, several effector cell types were increased in the blood circulation in the AHCC/BB12 group compared to the placebo group, suggestive of an improved alertness of the immune system. In addition, the
reduction in multiple cell types seen after the vaccine challenge in the placebo group was attenuated in the group consuming AHCC/BB12.

The multitude of phenotypic data and numbers of immune cell subsets presented here only represents one of several aspects of what can be measured in the blood circulation, pertaining to immune function. Several other aspects of the immune status are relevant, and material has been banked so that such testing can be performed at a later time, without repeating the clinical phase, but allowing direct correlations to the existing data sets.

The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety.

While this invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such embodiments and equivalent variations.
CLAIMS

What is claimed is:

1. A composition comprising a prebiotic and a probiotic bacteria that is capable of modulating the immune system.

2. The composition of claim 1, wherein the prebiotic is Active Hexose Correlated Compound (AHCC).

3. The composition of claim 1, wherein the probiotic bacteria is Bifidobacterium lactis BB12 (BB12).

4. The composition of claim 1, further comprising one or more pharmaceutically acceptable carriers.

5. The composition of claim 1, wherein the composition is retained in a pharmaceutically acceptable carrier that is rapid release, immediate release, slow release, or delayed release.

6. The composition of claim 1, further comprising one or more prebiotic.

7. The composition of claim 1, further comprising one or more probiotic bacteria selected from the group consisting of: Lactobacillus plantarum, Lactobacillus acidophilus, Lactobacillus paracasei, Leuconostoc mesenteroides, Lactobacillus bulgaricus, Lactobacillus sasei, Lactobacillus salivarius, Pediococcus pentosaceus, Streptococcus thermophiles, Bacillus subtilis, Bacillus coagulans, Enterococcus faecium, Bifidobacterium bifidum, Bifidobacterium lactis, Bifidobacterium longum, and Bifidobacterium infantis.
8. A method for enhancing the immune system of a subject, the method comprising administering to the subject a therapeutically effective amount of a composition comprising AHCC and BB12.

9. The method of claim 8, wherein the composition is administered in combination with another therapeutic agent.

10. The method of claim 8, wherein the composition is administered orally, parenterally, intravenously, intradermally, intramuscularly, or subcutaneously.

11. The method of claim 8, wherein the composition is administered with food or drink.

12. The method of claim 8, wherein the subject is a human.

13. The method of claim 8, wherein the subject is a veterinary subject.

14. A method for enhancing the immune response to a vaccine in a subject, said method comprising administering to the subject the vaccine and a therapeutically effective amount of a composition comprising AHCC and BB12.

15. The method of claim 14, wherein the composition is administered prior to the administration of the vaccine.

16. The method of claim 14, wherein the composition is administered simultaneously with the vaccine.

17. The method of claim 14, wherein the composition is administered after the administration of the vaccine.
18. The method of claim 14, wherein the composition is administered in combination with another therapeutic agent-

19. The method of claim 14, wherein the composition is administered orally, parenterally, intravenously, intradermally, intramuscularly, or subcutaneously.

20. The method of claim 14, wherein the composition is administered with food or drink.

21. The method of claim 14, wherein the subject is a human.

22. The method of claim 14, wherein the subject is a veterinary subject.

23. A method for enhancing the immune response to cancer in a subject, said method comprising administering to the subject a therapeutically effective amount of a cancer-specific antigen and a composition comprising AHCC and BB12.

24. The method of claim 23, wherein the composition is administered in combination with another therapeutic agent.

25. The method of claim 23, wherein the composition is administered orally, parenterally, intravenously, intradermally, intramuscularly, or subcutaneously.

26. The method of claim 23, wherein the composition is administered with food or drink.

27. The method of claim 23, wherein the subject is a human.
28. The method of claim 23, wherein the subject is a veterinary subject.

29. A kit for enhancing the immune system in a subject, said kit comprising a composition comprising at least AHCC and BB12.

30. A kit for enhancing the immune response to a vaccine in a subject, said kit comprising the vaccine and a composition comprising at least AHCC and BB12.
Figure 1

Hematocrit, AHCC/BB12, Females

Hematocrit, Placebo, Females
# Myeloid Dendritic Cells (CD11c+)

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<th>AHCC/BB12 Blend</th>
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<th>Group StDev (cells/μl)</th>
<th>% Change Avg</th>
<th>% Change StDev</th>
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**Compare Placebo and Blend**

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</tr>
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<td>D56</td>
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</table>

**Figure 2**
Figure 3

Monocyte Cell Numbers

AHCC/BB12 Blend

Placebo

Group Avg (cells/μl) | Group SD (cells/μl) | % Change Avg | % Change SD | P-values | Paired t-test | 2-tailed | 2-tailed
---|---|---|---|---|---|---|---

DO | 143.35 | 47.54 | 47.68 | | | | |
D28 | 164.87 | 62.44 | 62.16 | | | | |
D56 | 195.38 | 71.86 | 71.62 | | | | |

Compare Placebo and Blend

P-values | Paired t-test | 2-tailed | 2-tailed
---|---|---|---

D0-D28 | 0.0278 | 0.0001 | 0.0001 |
D0-D56 | 0.0001 | 0.0001 | 0.0001 |
D28-D56 | 0.0085 | 0.0085 | 0.0085 |
### CD14+/CD15+ Monocyte Cell Numbers

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<th>% Change StDev</th>
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<td>% Change StDev</td>
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<td>42.2113</td>
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</table>

**Paired t-test** 1-tailed: D0-D28 0.4038 0.8077
Paired t-test 2-tailed: D0-D28 0.1666 0.3331
Paired t-test 1-tailed: D28-D56 0.0055 0.1010
Paired t-test 2-tailed: D28-D56 0.0055 0.1010

**Unpaired t-test**
D0     0.0343
D28    0.9652
D56    0.5633

**Figure 4**
**Vaccine-Specific IgG₃ (absorbance units)**

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<tr>
<td>1-tailed</td>
<td>2-tailed</td>
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| D0-D28    | 0.1243        | 0.2486        |
| D0-D56    | 0.0226        | 0.0453        |
| D28-D56   | 0.0155        | 0.0251        |

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<tbody>
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<td>1-tailed</td>
<td>2-tailed</td>
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| D0-D28    | 0.1584        | 0.3168        |
| D0-D56    | 0.1539        | 0.3079        |
| D28-D56   | 0.1697        | 0.2760        |

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**Figure 5**
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K35/74 A61P37/02 A61P37/04

ADD.

According to International Patent Classification (IPC) onto both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, FSTA, PAJ, WPI, Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<td>X</td>
<td>CHOWDHURY A H ET AL: &quot;PP043-M0N EFFECT OF BI FIDUS BB536 AND ACTIVE HEXOSE CORRELATED COMPOUND ON IMMUNE FUNCTION IN HEALTHY INDIVIDUALS&quot;, CLINICAL NUTRITION, vol. 32, 30 August 2013 (2013-08-30), XP028699614, ISSN: 0261-5614, DOI: 10.1016/j0261-5614(13)60355-6 abstract</td>
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<td>DE 202 02 562 UI (ORTHOMOL PHARMAZETUTSCHEN VERTR [DE]) 23 May 2002 (2002-05-23) pages 13, 14</td>
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[X] Further documents are listed in the continuation of Box C.  [X] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) on which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"S" document member of the same patent family

Date of the actual completion of the international search

9 July 2015

Date of mailing of the international search report

15/07/2015

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Thalmai r, Michael a
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<td>ANASTASIA N. VLASOVA ET AL: &quot;Lactobacilli and Bifidobacteria a Promote Immune</td>
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<td>Homeostasis by Modulating Innate Immune Responses to Human Rotavirus in Neonatal</td>
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<td>Gnotobiotic Pigs&quot;, PLOS ONE, vol. 8, no. 10, 2 October 2013 (2013-10-02), pages</td>
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