Use Of A Therapeutically Effective Amount Of Bb-12 And LGG In The Preparation Of A Medicament For Preventing Or Treating Respiratory Infections And Acute Otitis Media In Infants

BACKGROUND OF THE INVENTION

[0001] This application claims priority to United States Provisional Application 60/584,830 filed July 1, 2004, which is incorporated by reference herein in its entirety.

(1) Field of the Invention

[0002] The present invention relates generally to a method for preventing or treating respiratory infections and acute otitis media in infants.

(2) Description of the Related Art

[0003] Respiratory tract infections are extremely common, especially among infants. In the first year of life, infants are prone to recurrent respiratory tract infections, often experiencing between three and six infections during that year alone. About 6% of infants less than one year of age are hospitalized for lower respiratory tract infections each year in the United States alone.

[0004] Respiratory infections and their symptoms can range from mild to severe, depending on the type of virus and the location of the infection. Upper respiratory infections often manifest themselves as common colds, causing inflammation and swelling of the lining of the nose, throat and sinuses. Influenza, commonly known as the flu, is a highly contagious viral infection of the upper respiratory tract. Symptoms of the flu include fever, chills, headache, muscle aches, dizziness, cough, sore throat, runny nose, nausea and diarrhea. Another upper respiratory infection, croup, causes a very deep cough and varying degrees of breathing difficulty, primarily when inhaling.

[0005] Lower respiratory infections are generally considered more serious than upper respiratory infections. Respiratory syncytial virus (RSV) is the most frequent cause of lower respiratory tract infections in infants and children younger than four years of age. Van Woensel, J., et

al., *Viral Lower Respiratory Tract Infection in Infants and Young Children*, BMJ 327:36-40 (2003). This is such a common virus that virtually all children have been infected with RSV by the age of three. In most infants and children, RSV is a mild respiratory infection that is indistinguishable from a common cold. It usually causes nasal stuffiness, nasal discharge and cough.

[0006] Protection against RSV involves both T- and B- cell responses, antibody responses (IgM, IgG, and IgA), as well as other immune system responses that are activated by bacterial and viral infections. A link between RSV infection in infancy and the development of recurrent wheezing, asthma and atopy later in childhood has been suggested. Thus, limiting RSV infections could prevent serious respiratory complications which extend well into childhood.

[0007] Bronchitis is a lower respiratory infection that affects the bronchial tubes, causing narrowing and swelling due to viral inflammation. Bronchiolitis is similar to bronchitis, but occurs primarily in infants. It is an inflammation of the smaller caliber tubes of the branching network of brochi. The infection causes labored breathing, frequent and dramatic coughing and wheezing and may require hospitalization.

[0008] The lower respiratory infection that is probably the most serious for infants is pneumonia. Pneumonia is caused by an infection in the alveoli, causing them to become filled with fluid, often of a thick purulent nature, that interferes with proper exchange of carbon dioxide. The severity of the pneumonia will depend on the amount of lung tissue involved.

[0009] Most upper and lower respiratory infections are caused by viruses for which no specific prevention or treatment is currently available. Some respiratory infections, including influenza, may be prevented with a vaccination. However, even when vaccinations are developed for specific respiratory infections, they are expensive and not universally available. Similarly, drugs to treat these infections have limited availability and are

expensive. Thus, it would be useful to provide a non-medicinal method for the treatment or prevention of respiratory infections in infants.

[00010] Frequent respiratory tract infections are often associated with acute otitis media (AOM), also known as middle ear infection. AOM is characterized by an acute, short course of inflammation and fluid in the middle ear. AOM can be accompanied by rhinitis, cough, fever, sore throat, ear ache, hypacusis, restlessness, irritability, loss of appetite, vomiting or diarrhea. Purulent otorrhea through a perforated tympanic membrane is also considered to constitute AOM.

[00011] Fifty percent of children have had at least one episode of AOM by one year of age. Eighty percent of children have had at least one episode by their third birthday. Between one and three years, 35% of children will have had recurrent episodes of AOM.

[00012] AOM can be caused by viruses or bacteria. The most common bacterial strains that cause AOM are *Streptococcus pneumoniae* (35% of cases), *Haemophilus influenzae* (30% of cases) and *Moraxella catarrhalis* (10% of cases). Because bacterial strains frequently cause the infection, AOM is commonly treated through the administration of antibiotics. In fact, more antibiotic prescriptions are written for AOM than for any other disease in infancy. The disadvantage to this widespread antibiotic treatment is the development of antibiotic resistance. For example, between 20% and 40% of *S. pneumoniae* strains are resistant to penicillins and cephalosporins. Similarly, between 30% and 40% of *H. influenzae* and about 90% of *M. catarrhalis* strains have developed antibiotic resistance.

[00013] Due to the prevalence of antibiotic resistance among pathogenic bacteria, the American Academy of Pediatrics and the American Academy of Family Physicians have developed guidelines suggesting a limited prescription of antibiotics for AOM. *American Academy of Pediatrics and the American Academy of Family Physicians, Subcommittee on the Management of Acute Otitis Media, Clinical Practice Guideline* (March 2004), available at

http://www.aafp.org/PreBuilt/final_aom.pdf. Therefore, as antibiotic therapies become more limited, it is important to provide alternative therapies to decrease the incidence of this painful and serious condition in infants and young children.

[00014] In a meta-analysis of data from multiple studies, results indicate that breastfeeding may have a positive effect on the frequency of both infant respiratory infection and AOM. Specifically, one study indicated that the feeding of many currently available infant formulas may be associated with a 3.6-fold increase in risk of infant hospitalization for respiratory infection when compared to at least four months of exclusive breastfeeding. Bachrach, V., *et al.*, Arch. Pediatr. Adolesc. Med. 57:237-43 (2003). Additionally, infants who are breastfed have been shown to have significantly fewer (about 50%) episodes of AOM than do infants who are exclusively formula-fed. Duffy, *et al.*, Pediatr. 100(4):E7 (1997). These differences may be attributed to the fact that human milk promotes the growth of beneficial bacteria such as *Lactobacilli* and *Bifidobacteria.* Duffy, *et al.*, Dig. Dis. Sci. 44(8):1499-1505 (1999).

[00015] It has been shown that the microflora of breast-fed infants contains predominantly *Bifidobacteria*. In contrast, the microflora of formula-fed infants is more diverse, containing *Bifidobacteria* and *Bacteroides* as well as the more pathogenic species, *Staphylococcus, Escherichia coli*, and *Clostridia*. The varied species of *Bifidobacteria* in the stools of breast-fed and formula-fed infants differ as well. A variety of factors have been proposed as the cause for the different fecal flora of breast-fed and formula-fed infants, including the lower content and different composition of proteins in human milk, a lower phosphorus content in human milk, the large variety of oligosaccharides in human milk, and numerous humoral and cellular mediators of immunologic function in breast milk. Agostoni, *et al.*, *Probiotic Bacteria in Dietetic Products for Infants: A Commentary by the ESPGHAN Committee on Nutrition*, J. Pediatr. Gastro. Nutr. 38:365-374 (Apr. 2004). Regardless of the cause for the differing bacterial populations, it is clear that breast milk has a

measurable benefit in the treatment or prevention of respiratory infections and AOM.

[00016] Both the American Academy of Pediatrics and World Health Organization advise mothers to breastfeed for between one and two years. In developed countries, however, these recommendations are sometimes difficult for working mothers to follow. In the United States, for example, 53% of lactating mothers introduce formula before their babies are a week old. By four months of age, 81% of infants receive formula on a regular basis. Fewer than 5% of American infants are being breastfed at twelve months of age. Wolf, J., Am. J. Pub. Health 93:2000-2010 (2003).

[00017] One way to encourage gut colonization with beneficial microorganisms in infants that are formula-fed is through the administration of probiotic bacteria. Probiotic bacteria are living microorganisms that exert beneficial effects on the health of the host. *Lactobacillus* spp. and *Bifidobacterium* spp. are among the common species of probiotics. Probiotics such as these have been shown effective in treating various gastrointestinal disorders.

[00018] For example, U.S. Patent No. 6,613,549 to Reid relates to the use of probiotic microorganisms such as *Lactobacillus* and *Bifidobacterium* in treating intestinal infection in infants. The patent does not, however, teach any treatment of infections outside the intestinal tract. While probiotics have been effective in decreasing the incidence of diarrheal disease and rotaviral shedding in hospitalized infants, a probiotic combination of *B. lactis* and *S. thermophilus* did not show a significant effect in terms of decreasing the frequency of overall incidents for which healthcare attention was sought. Saavedra, *et al.*, Am. J. Clin. Nutr. 79:261-67 (2004).

[00019] U.S. Patent Apps. Nos. 20040057965 and 20030180260 to Clancy, *et al.* describe the administration of an antigen and a probiotic to treat mucosal infections such as respiratory tract infections. Similarly, U.S. Patent App. No. 20040265291 to Drake, et al. relates to a method of inhibiting or reducing chronic or upper respiratory infection and ear

infection through the administration of a bacteria, a bacteria nutrient, and an antimicrobial agent. These references, however, relate solely to adult administration and do not disclose the administration of probiotics to infants to treat respiratory tract infections or AOM.

[00020] The gut microflora in infants is known to be far less developed than that of an adult. While the microflora of an adult human consists of more than 10^{13} microorganisms and nearly 500 species, some being harmful and some being beneficial, the microflora of an infant contains only a fraction of those microorganisms, both in absolute number but also in species diversity. Because the bacterial populations and species vary so immensely between the gut of an infant and the gut of an adult, it cannot be assumed that a probiotic administration designed for an adult would necessarily be beneficial for an infant.

[00021] It would be beneficial, therefore, to provide a probiotic remedy for the treatment and or prevention of respiratory infections and AOM in infants.

SUMMARY OF THE INVENTION

[00022] Briefly, the present invention is directed to a novel method for preventing or treating respiratory infections in infants, comprising administering to the infant a therapeutically effective amount of at least one *Bifidobacterium* species in conjunction with at least one probiotic bacterial species that promotes the growth and adherence of the selected strain of *Bifidobacteria* in the intestine. In one embodiment, the *Bifidobacterium* species can be chosen from among the *Bifidobacteria* that demonstrate immunomodulatory properties. These species may include, for example, *B. bifidum, B. adolescentis, B. animalis, B. lactis, B. infantis, B. longum,* and *B. thermophilum*. A particular species useful in the present invention is *B. lactis* Bb-12.

[00023] In one embodiment, the probiotic that promotes *Bifidobacteria* adherence is a member of the Lactobacillus species, such as, for example, *L. rhamnosus* GG (LGG), *L. delbrueckii* subsp. *bulgaricus*, or a combination of both.

[00024] The present invention is also directed to a novel method for preventing or treating acute otitis media in infants, comprising administering to the infant a therapeutically effective amount of at least one *Bifidobacterium* species and at least one probiotic that promotes the growth and adherence of the selected species of *Bifidobacteria* to intestinal mucosa.

[00025] The invention is also directed to a novel method for preventing or treating recurrent respiratory infections and recurrent AOM infections in infants. The method comprises administering to the infant a therapeutically effective amount of at least one *Bifidobacterium* species and at least one probiotic that promotes the growth and adherence of the selected species of *Bifidobacteria* to intestinal mucosa.

[00026] Among the several advantages found to be achieved by the present invention is that it provides a method for preventing or treating respiratory infections in infants without the necessity of administering unavailable or costly medications or vaccinations. The invention also provides a method for preventing or treating AOM without the necessity of administering antibiotics that may cause resistance among pathogenic bacterial species.

BRIEF DESCRIPTION OF THE DRAWINGS

[00027] Figure 1 is a graph indicating the effect of probiotic supplementation on the development of an AOM episode during the first year of life.

[00028] Figure 2 is a graph indicating the effect of probiotic supplementation on the development of a respiratory infection during the first year of life.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[00029] Reference now will be made in detail to the embodiments of the invention, one or more examples of which are set forth below. Each example is provided by way of explanation of the invention, not a limitation of the invention. In fact, it will be apparent to those skilled in the art that various modifications and variations can be made in the present invention

without departing from the scope or spirit of the invention. For instance, features illustrated or described as part of one embodiment, can be used on another embodiment to yield a still further embodiment.

[00030] Thus, it is intended that the present invention covers such modifications and variations as come within the scope of the appended claims and their equivalents. Other objects, features and aspects of the present invention are disclosed in or are obvious from the following detailed description. It is to be understood by one of ordinary skill in the art that the present discussion is a description of exemplary embodiments only, and is not intended as limiting the broader aspects of the present invention.

Definitions

[00031] As used herein, the term "treating" means ameliorating, improving or remedying a disease, disorder, or symptom of a disease or condition.

[00032] The term "preventing" means stopping or hindering a disease, disorder, or symptom of a disease or condition through some action.

[00033] The terms "therapeutically effective amount" refer to an amount that results in an improvement or remediation of the disease, disorder, or symptoms of the disease or condition.

[00034] The term "infant" means a human that is less than about 2 years old.

[00035] The terms "respiratory infection" or "respiratory illness" mean a disease or infection affecting the group of organs responsible for carrying oxygen from the air to the bloodstream and for expelling carbon dioxide.

[00036] The term "probiotic" means a microorganism that exerts beneficial effects on the health of the host. It can be a live microbial fed supplement that beneficially affects the host by improving its intestinal microbial balance, a microbial preparation that contains live or dead bacteria, or a combination of both. Live organisms are often preferred, as they produce a complete array of antigens, reproduce to increase the number of such organisms in the intestinal environment to promote

mucosal interaction, and may adhere to the intestinal tissues to better stimulate a mucosal immune response.

[00037] The term "prebiotic" means a non-digestible food ingredient that stimulates the growth and/or activity of probiotics.

[00038] The term "recurrent" means 3 or more occurrences of infection during one year.

[00039] As used herein, the term "infant formula" means a composition that satisfies the nutrient requirements of an infant by being a substitute for human milk. In the United States, the contents of an infant formula are dictated by the federal regulations set forth at 21 C.F.R. Sections 100, 106, and 107. These regulations define macronutrient, vitamin, mineral, and other ingredient levels in an effort to stimulate the nutritional and other properties of human breast milk.

Invention

[00040] In accordance with the present invention, a novel method for preventing or treating respiratory infections in infants has been developed. The method comprises administering to the infant a therapeutically effective amount of at least one species of *Bifidobacteria*, such as Bb-12, and at least one probiotic, such as LGG, that promotes the adherence of the selected *Bifidobacterium* strain to the intestinal mucosa.

[00041] *Bifidobacteria* are gram-positive anaerobes that operate in the lower part of the digestive system. They are non-motile, non-spore forming and catalase-negative. They have various shapes, including short, curved rods, club-shaped rods and bifurcated Y-shaped rods. They are classified as lactic acid bacteria due to their production of lactic acid during carbohydrate fermentation.

[00042] In one embodiment of the present invention, the *Bifidobacterium* species can be chosen from among the *Bifidobacteria* that demonstrate immunomodulatory properties. These species may include, for example, *B. bifidum, B. adolescentis, B. animalis, B. lactis, B. infantis, B. longum,* and *B. thermophilum*. A particular strain of *Bifidobacteria* that

is useful in the present invention is *B. lactis* Bb-12, available from Chr. Hansen Biosystems, located in Milwaukee, WI.

[00043] In one embodiment of the invention, the probiotic that promotes the adherence of *Bifidobacteria* to the intestinal musoca can be a member of the genus *Lactobacillus*. *Lactobacilli* are gram-positive facultative anaerobes. They are non-spore forming and non-flagellated rod or coccobacilli. Any species of *Lactobacilli* known in the art can be used in this embodiment. For example, the adherence-promoting probiotic can be LGG, *L. delbrueckii* subsp. *bulgaricus*, or a combination of both.

[00044] LGG is a *Lactobacillus* strain isolated from healthy human intestinal flora. It was disclosed in U.S. Patent No. 5,032,399 to Gorbach, *et al.*, which is incorporated herein in its entirety, by reference thereto. LGG is resistant to most antibiotics, stable in the presence of acid and bile, and attaches avidly to mucosal cells of the human intestinal tract. It survives for 1 to 3 days in most individuals and up to 7 days in 30% of subjects. In addition to its colonization ability, LGG also beneficially affects mucosal immune responses. LGG is deposited with the depository authority American Type Culture Collection under accession number ATCC 53103.

[00045] *L. delbrueckii* are Gram-positive, facultatively anaerobic, non-motile and non-spore-forming, rod-shaped microorganisms. Like other lactic acid bacteria, *L. delbrueckii* are acid tolerant, cannot synthesize porphyrins, and possess a strictly fermentative metabolism with lactic acid as the major metabolic end product. *L. delbrueckii* species contain three subspecies, *L. delbrueckii* subsp. *delbrueckii, L. delbrueckii* subsp. *lactis*, and *L. delbrueckii* subsp. *bulgaricus*.

[00046] Generally, the adherence rate of selected *Bifidobacteria* to intestinal mucosa is around 18%. Previous studies have indicated that certain bacterial species can promote the adherence of *Bifidobacteria* to the intestinal mucosa. Juntunen, M. *et al.* Clin. Diag. Lab. Immunol. 8:293-96 (2001). In the presence of two *Lactobacillus* species in particular, LGG (ATCC No. 53103) and *L. delbrueckii* subsp. *bulgaricus*

(available from Valio Ltd., Finland), the adherence of *Bifidobacteria* increased from 18% to 44% and 45%, respectively. *Id.* Though not wishing to be bound by this or any other theory, it is believed that these *Lactobacillus* strains coaggregate and thereby increase the adherence of *Bifidobacteria* to the intestinal mucosa and their residence time within the intestine.

[00047] According to the method of the present invention, when a strain of *Bifidobacteria* is provided in combination with at least one adherence-promoting probiotic, there is a decrease in the number of respiratory and AOM infections in infants to whom the combination is provided.

[00048] In the present invention, the form of administration of the *Bifidobacteria* strain and the adherence-promoting probiotic is not critical, as long as a therapeutically effective amount is administered to the infant. Most conveniently, the *Bifidobacteria* strain and adherence-promoting probiotic can be supplemented into an infant formula which can then be fed to an infant.

[00049] In an embodiment, the infant formula for use in the present invention is nutritionally complete and contains suitable types and amounts of lipid, carbohydrate, protein, vitamins and minerals. The amount of lipid or fat typically can vary from about 3 to about 7 g/100 kcal. The amount of protein typically can vary from about 1 to about 5 g/100 kcal. The amount of carbohydrate typically can vary from about 8 to about 12 g/100 kcal. Protein sources can be any used in the art, e.g., nonfat milk, whey protein, casein, soy protein, hydrolyzed protein, partially hydrolyzed protein, amino acids, and the like. In one embodiment, the protein is a combination of whey protein and casein in a ratio of 60:40. Carbohydrate sources can be any used in the art, e.g., lactose, glucose, corn syrup solids, maltodextrins, sucrose, starch, rice syrup solids, and the like. Lipid sources can be any used in the art, e.g., vegetable oils such as palm oil, soybean oil, palmolein, coconut oil, medium chain triglyceride oil, high oleic sunflower oil, high oleic safflower oil, and the like.

[00050] Conveniently, commercially available infant formula can be used. For example, Enfalac, Enfamil®, Enfamil® Premature Formula, Enfamil® with Iron, Lactofree®, Nutramigen®, Pregestimil®, and ProSobee® (available from Mead Johnson & Company, Evansville, IN, U.S.A.) may be supplemented with suitable levels of the *Bifidobacteria* strain and the adherence-promoting probiotic and used in practice of the method of the invention.

[00051] The infant formula of the present invention may contain ingredients designed to promote the growth of *Bifidobacteria* within the intestinal mucosa. For example, *Bifidobacteria* require ferrous iron, riboflavin and biotin for growth. These may be provided in combination with other ingredients in the infant formula.

[00052] As an alternative to an infant formula administration, the *Bifidobacteria* strain and the adherence-promoting probiotic can be administered as a supplement not integral to the formula feeding.

[00053] The present invention can be used to treat or prevent respiratory infections or AOM in infants that are exclusively formula-fed or in infants that are fed a combination diet of breast milk and infant formula.

[00054] In a particular embodiment of the invention, at least one prebiotic can be supplemented into the infant's diet in combination with the *Bifidobacteria* strain and adherence-promoting probiotic. In this embodiment, the prebiotic can be any prebiotic known in the art. In a particular embodiment, the prebiotic is selected from the group consisting of galacto-oligosaccharide, inulin, fructo-oligosaccharide, lactulose, neosugars, and combinations thereof.

[00055] In an embodiment of the present invention, the *Bifidobacteria* strain and the adherence-promoting probiotic are supplemented into the diet of the infant from birth until the infant reaches about one year of age. In another embodiment of the present invention, the *Bifidobacteria* strain and the adherence-promoting probiotic are supplemented into the diet of the infant from birth until the infant reaches about three years of age.

[00056] In an embodiment, a therapeutically effective amount of the *Bifidobacteria* strain and the adherence-promoting probiotic is between about 10^5 and 10^{11} colony forming units (cfu). In another embodiment, a therapeutically effective amount of the *Bifidobacteria* strain and the adherence-promoting probiotic is between about 10^6 and 10^8 cfu. In an embodiment, the therapeutically effective amount is administered daily. In other embodiments, the therapeutically effective amount can be administered every other day, weekly or monthly. The frequency and size of the probiotic dose will depend, for example, upon the microorganism chosen, the delivery vehicle and the infant to whom the dose is administered.

[00057] It is well within the level of knowledge of one of skill in the art to provide increased dosages, as determined by those of skill in the art to be safe and effective for an individual infant. Furthermore, minimum amounts may be varied based upon their combination with prebiotic compositions and other additives that may enhance the colonization of the *Bifidobacteria* strain.

[00058] In an embodiment of the present invention, the ratio of Bb-12 to LGG can be between about 10:1 and 1:10. In another embodiment of the invention, the ratio of Bb-12 to LGG can be between 5:1 and 1:5. In yet another embodiment of the invention, the ratio of Bb-12 to LGG can be between about 3:1 and 1:3. In a particular embodiment of the invention, the ratio of Bb-12 to LGG can be about 1:1.

[00059] The probiotic organisms of the present invention can be provided as a powder, in capsular form, as a component of an emulsion or a paste, or in any other suitable carrier determined by those of skill in the art to be an effective carrier for live microorganisms. Powder compositions containing probiotic microorganisms can be provided in individual pouches, for example, for admixing with infant formula or early foods. Capsules, for example, may be opened so that the contents can be mixed with infant formula, strained foods, milk, juice, or other foods for

providing a nutritional composition to an infant. An emulsion or paste can also be admixed into a variety of foods.

[00060] In an embodiment, the supplementation of a *Bifidobacteria* strain and an adherence-promoting probiotic prevents or treats the occurrence of upper respiratory infection, influenza, croup, respiratory syncytial virus, bronchitis, bronchiolitis and/or pneumonia. In another embodiment, the supplementation of a *Bifidobacteria* strain and an adherence-promoting probiotic prevents or treats the occurrence of AOM.

[00061] A significant reduction in the incidence of early and recurrent infections and the use of antibiotics during the first year of life was achieved by the particular probiotic combination of the present invention. The effect was most prominent with regard to respiratory infections and AOM, the most prevalent infection in infancy. This is shown in Figures 1 and 2. These figures illustrate that probiotic supplementation reduced the proportion of children that develop a respiratory infection or AOM episode during the first year of life. The present invention was also effective in reducing the occurrence of recurrent respiratory infections and recurrent AOM infections. Moreover, probiotics appeared to confer protection against early infections, the importance of which culminates in the fact that the children developing frequent infections, including AOM, experience their first infection early.

[00062] The following examples describe various embodiments of the present invention. Other embodiments within the scope of the claims herein will be apparent to one skilled in the art from consideration of the specification or practice of the invention as disclosed herein. It is intended that the specification, together with the examples, be considered to be exemplary only, with the scope and spirit of the invention being indicated by the claims which follow the examples. In the examples, all percentages are given on a weight basis unless otherwise indicated.

Example 1

[00063] This example describes the materials and methods necessary to show the effect of a strain of *Bifidobacteria* and an adherence-promoting probiotic on the frequency of respiratory infections and AOM.

[00064] The infants participating in the double-blind, placebo-controlled clinical study conducted by the inventors were recruited in well-baby clinics in Turku, Finland between September 2000 and May 2002. The sole inclusion criterion for the study was the need for infant formula before the age of two months. Infants with chronic disease were excluded.

[00065] In total, 81 infants were block-randomized with individual codes to receive either 1×10^{10} colony-forming units of both *Bifidobacterium lactis* Bb-12 and LGG or a microcrystalline cellulose placebo daily until the age of 12 months. The daily probiotics or placebo were provided in visually identical capsules, the contents of which were supplemented into infant formula (Enfamil®, Mead Johnson Nutritionals, Evansville, IN). The formula was to be used as the sole infant formula during this period. Maternal use of commercially available products containing probiotics was discouraged.

[00066] Clinical examination of the infants was performed at scheduled visits at the ages of 3, 7, and 12 months. The follow-up was completed by 72 of the 81 (89 %) infants enrolled. The mean age at the time of withdrawal amongst the 9 infants who did not complete the follow-up was 2.9 months (range 1.5 to 7.0) and, therefore, only the infants who completed the study were included in the analysis.

[00067] To ascertain that the probiotics remained viable during storage, microbiological analysis of a random sample of capsules was performed by microbiologist in a blinded fashion. Viable counts between 1×10^9 and 1×10^{10} per capsule were found for both LGG and Bb-12.

[00068] All infections during the study period were recorded in special diaries by the family or the family physician. Respiratory infections, doctor-diagnosed AOM, gastrointestinal infections, and the number of treatments with antibiotic agents were separately recorded in detail. The primary

outcome measure for the study was the incidence of early respiratory infections, doctor-diagnosed AOM, and gastrointestinal infections. The incidence of recurrent (defined as 3 or more occurrences) infections during the first year of life were considered secondary outcome measures. Early or recurrent need for antibiotics were interpreted to reflect suspected early or recurrent bacterial infections, respectively, and thus recorded. Tympanostomy before the age of 12 months was interpreted to indicate frequent ear infections. Tympanostomy is a surgical procedure that requires general anesthesia, but may be necessary to avoid structural damage to the ear which may occur as the result of AOM.

[00069] All health problems in the study population during the first year of life were recorded in detail to distinguish between symptoms of diseases with infectious and non-infectious etiology. Gastro-esophageal reflux disease as a possible noninfectious cause of cough, vomiting, or increased fussing was confirmed or excluded using a 24-hour esophageal pH probe study. The diagnosis of cow's milk allergy, a non-infectious cause of gastrointestinal or cutaneous symptoms, was confirmed by a double-blind, placebo controlled cow's milk challenge. Atopic eczema was diagnosed using the criteria introduced by Hanifin. Hanifin, J.M., *Atopic Dermatitis in Infants and Children*, Pediatr. Clin. Nutr. Am. 38:763-89 (1991). Atopic sensitization was assessed by skin prick testing at the ages of 7 and 12 months. The tested antigens included banana, potato, carrot, apple, wheat, rice, milk, egg, cod, soybean, and gliadin. The infant was considered sensitized in case of one or more positive reactions at either time point.

[00070] To ascertain compliance with the intervention, fecal samples were collected at enrollment prior to probiotic supplementation and again at the age of 3 months and stored at -86°C. Samples were available from 46 infants at enrollment and 45 infants at the age of 3 months. The fecal samples were thawed and serially diluted in phosphate buffered saline (pH 7.2, 10mM phosphate). For the detection of LGG, dilutions were spread on Rogosa agar (Oxoid, Basingstoke, UK) and incubated aerobically at

37°C for four days. Typical LGG colonies were purified and DNA was extracted from the colonies. Strain identity was verified by polymerase chain reaction.

[00071] The data were expressed as means with range or medians with IQR to give an estimate of the distribution of the data. The comparisons between the groups at baseline were conducted using the Mann-Whitney U test and the χ^2 test. Logistic regression analysis was used to compare the treatment groups with respect to early infections and recurrent infections and recurrent need for antibiotic treatment during the first 12 months of life. The analyses of recurrent infections and antibiotic treatment were performed with and without adjustment for other relevant factors. Stepwise regression analyses were performed in a forward manner in order to control for the relevant risk factors or confounding factors. The treatment group was forced to the model and the other factors introduced to the model were: gender, mode of birth, duration of exclusive breastfeeding, total duration of breastfeeding, older siblings, maternal smoking, pet ownership and family history of allergy. The criteria for entering and removing a variable were: probability of F-to-enter ≤ 0.10 and F-to-remove ≥ 0.15. The results are given in terms of relative risk, also known as risk ratio (RR), and 95% confidence interval (CI). Kaplan-Meier curves were applied for the time without a respiratory infection and time without an AOM and log rank test was used to compare the treatment groups. The data were analyzed using the SPSS (Version 11.5).

Example 2

[00072] This example illustrates the effects of Bb-12 and LGG on the frequency of respiratory infections and AOM. The baseline characteristics, shown in Table 1, were similar in infants receiving probiotics and placebo.

Table 1. Baseline Characteristics and History

	Probiotics (n= 32)	**Placebo (n=40)**
Boys	16 (50 %)	19 (48 %)
Gestational age mean (range)	39.8 weeks (36.7 to 42.1)	39.9 weeks (35.1 to 42.3)
Birthweight mean (range)	3440 g (2300 to 4100)	3540 g (2140 to 4580)
Older siblings	15 (47 %)	24 (60 %)
Parental smoking	18 (56 %)	22 (55 %)
Exclusive breastfeeding mean (range)	1.9 weeks (0.0 to 6.0)	1.9 weeks (0.0 to 6.0)
Total breastfeeding mean (range)	2.0 months (0.25 to 12.0)	2.4 months (0.25 to 7.5)
Age at start of intervention (range)	38 days (6 to 65)	35 days (2 to 59)

[00073] The mean age at start of intervention was 38 days (range 6-65) in infants receiving probiotics and 35 days in (range 2-59) in infants receiving placebo. The follow-up was completed by 72 of the 81 (89%) infants enrolled. The mean age at the time of withdrawal amongst the 9 infants who did not complete the follow-up was 2.9 months (range 1.5-7.0) and therefore only the infants who completed the study were included in the analysis.

[00074] The study probiotics reduced the risk of early respiratory infections and AOM as well as the need for antibiotic treatment during the first 7 months of life. These results are presented in detail in Table 2.

Table 2. The Incidence of Infections during the First 7 Months of Life

	Probiotics (n=32)	Placebo (n=40)	RR (95 % CI)	Adjusted RR (95 % CI)
Respiratory Infection	22 (69%)	31 (78%)	0.85 (0.44 to 1.19)	0.85 (0.44 to 1.19)
AOM	7 (22%)	20 (50%)	0.34 (0.10 to 0.90)	0.31* (0.09 to 0.85)
Gastrointestinal Infection	1 (3%)	6 (15%)	0.19 (0.003 to 1.64)	0.19 (0.003 to 1.64)
Antibiotic Use	10 (31%)	24 (60%)	0.39 (0.14 to 0.92)	0.36† (0.11 to 0.91)

[00075] During the first 7 months of life, 25/32 (78%) of infants receiving probiotics and 36/40 (90%) of infants receiving placebo had encountered at least one episode of acute infection. Specifically, 7/32 (22%) infants receiving probiotics and 20/40 (50%) of infants receiving placebo experienced AOM. Antibiotics were prescribed for 10/32 (31%) of infants receiving probiotics and 24/40 (60%) of infants receiving placebo. During the first 7 months of life, 22/32 (69%) of infants receiving probiotics and 31/40 (78%) of infants receiving placebo had encountered at least one episode of respiratory infection. Thus, probiotic supplementation decreased the risk for early AOM, respiratory infection and antibiotic use. The incidence of gastrointestinal infections during the first 7 months of life was low in both groups.

[00076] In addition to effects during the first 7 months of life, probiotics reduced significantly the incidence of recurrent infections during the first 12 months of life. These results are shown in Table 3.

* Adjusted for maternal allergies
† Adjusted for maternal allergies and mode of birth

Table 3. The Incidence of Recurrent Infections and Use of Medical Interventions during the First 12 Months of Life

	Probiotics (n=32)	Placebo (n=40)	RR (95 % CI)	Adjusted RR (95 % CI)
Any Infection	22 (69%)	31 (78%)	0.22 (0.05-0.98)	0.47 (0.16 to 1.20)
Respiratory Infection	9 (28%)	22 (55%)	0.40 (0.14 to 0.97)	0.48‡ (0.16 to 1.20)
AOM	4 (13%)	10 (25%)	0.47 (0.10 to 1.58)	0.54§ (0.11 to 1.88)
Antibiotic Use	10 (31%)	16 (40%)	0.76 (0.30 to 1.53)	0.71** (0.20 to 1.74)
Tympanostomy	0 (0%)	4 (10%)	0.23 (N/A to 1.91)	0.23 (N/A to 1.91)

[00077] During the first 12 months of life, a total of 53/72 (74%) of infants participating in the study experienced 3 or more infections. More specifically, 31/72 (43%) of infants suffered from recurrent respiratory infections and 14/72 (19%) from recurrent AOM during this period. Moreover, 26/72 (36%) of infants experienced a recurrent need for antibiotic treatment. Probiotics significantly reduced the incidence of recurrent respiratory infections during the first 12 months of life.

[00078] For example, 9/32 (28%) infants receiving probiotics and 22/40 (55%) of infants receiving placebo experienced three or more respiratory infections. Of the infant receiving probiotics, only 4/32 (13%) experienced three or more incidences of AOM. In contrast, of those infants receiving placebo, 10/40 (25%) experienced three or more incidences of AOM. In addition, the administration of probiotics tended to reduce the need for tympanostomy, performed either to prevent recurrent AOM or to treat secretory otitis media. None of the infants receiving probiotics required tympanostomy during the first year of life, whereas the procedure was performed on 4/40 (10%) of the infants receiving placebo.

[00079] Several factors were associated with the risk of recurrent infections and recurrent need for antibiotic treatment. Having older

‡ Adjusted for older siblings and paternal allergy
§ Adjusted for older siblings
** Adjusted for older siblings, maternal smoking, pet ownership, duration of exclusive breastfeeding and maternal allergy

siblings increased the risk for recurrent respiratory infections, the recurrent need for antibiotics, and tended to increase the risk for recurrent AOM during the first 12 months of life. A family history of allergy increased the risk for recurrent infections and maternal smoking was associated with recurrent antibiotic use. The duration of exclusive breastfeeding had an inverse association with recurrent antibiotic use. Pet ownership conferred protection against recurrent infections and recurrent need for antibiotics. Consequently, the effect of probiotic supplementation on the risk of recurrent infections during the first 12 months of life was adjusted for these factors.

[00080] Gastro-esophageal reflux disease was diagnosed in 1/32 (3%) infants receiving probiotics and 3/40 (8%) infants receiving placebo. None of the 32 infants receiving probiotics had cow's milk allergy as compared to 3/40 (8%) infants receiving placebo. In all, 4/32 (13%) infants receiving probiotics and 8/40 (20%) infants receiving placebo suffered from atopic eczema during the first year of life. Atopic sensitization was detected in 2/32 (6%) infants receiving probiotics and 3/40 (8%) infants receiving placebo. None of the probiotic-supplemented infants in the study experienced more than 2 gastrointestinal infections during the study period.

[00081] The relationship between fecal recovery of LGG at 3 months of age and the risk of infection during the first 7 months of life was also evaluated. These results are presented in Table 4.

Table 4. The Relationship of Fecal Recovery of LGG at 3 Months of Age and the Risk of Infections During the First 7 Months of Life

	Positive (n = 23)	Negative (n = 22)	RR (95% CI)
Respiratory Infection	15 (65%)	18 (82%)	0.68 (0.19 to 1.21) p = 0.31
AOM	4 (17%)	10 (45%)	0.30 (0.06 to 1.13) p = 0.057
Antibiotic Use	7 (30%)	12 (55%)	0.46 (0.12 to 1.27) p = 0.14
Recurrent Respiratory Infections	1 (4%)	2 (9%)	0.47 (0.01 to 6.95) p = 0.61
Recurrent AOM	0 (0%)	2 (9%)	0.38 (N/A to 5.05) p = 0.23
Recurrent Antibiotic Use	0 (0%)	5 (23%)	0.12 (N/A to 0.94) p = 0.022

[00082] Prior to probiotic supplementation, LGG was recovered in the feces of 12/46 infants, 8/28 (29%) in the probiotics group and 4/18 (22%) in the placebo group. Pre-intervention presence of LGG in feces was not associated with the incidence of infections in general, respiratory infections, AOM or gastrointestinal infections, nor was there an impact on antibiotic use at any age. At 3 months of age, i.e. after a minimum of 1 month of probiotic supplementation, LGG was recovered in 21/28 (75%) of infants receiving probiotics and 2/17 (12%) of infants receiving placebo, $p<0.0001$. Furthermore, the presence of LGG in feces at this time was associated with a reduced risk of having encountered at least one episode of AOM by the age of 7 months; 4/23 (17%) and 10/22 (45%) infants positive and negative for LGG, respectively. The presence of LGG in feces at 3 months of age was also indicative of protection against recurrent infections: 2/23 (9%) and 10/22 (45%) in infants positive and negative for LGG, respectfully, and recurrent need for antibiotics: 0/23 (0%) and 5/22 (23%) in infants positive and negative for LGG, respectively, by the age of 7 months.

[00083] All references cited in this specification, including without limitation, all papers, publications, patents, patent applications,

presentations, texts, reports, manuscripts, brochures, books, internet postings, journal articles, periodicals, and the like, are hereby incorporated by reference into this specification in their entireties. The discussion of the references herein is intended merely to summarize the assertions made by their authors and no admission is made that any reference constitutes prior art. Applicants reserve the right to challenge the accuracy and pertinence of the cited references

[00084] These and other modifications and variations to the present invention may be practiced by those of ordinary skill in the art, without departing from the spirit and scope of the present invention, which is more particularly set forth in the appended claims. In addition, it should be understood that aspects of the various embodiments may be interchanged in whole or in part. Furthermore, those of ordinary skill in the art will appreciate that the foregoing description is by way of example only, and is not intended to limit the invention so further described in such appended claims. Therefore, the spirit and scope of the appended claims should not be limited to the description of the preferred versions contained therein.

CLAIMS:

1. A nutritional formula, comprising an infant formula which comprises:

a lipid or fat;

a protein source selected from the group consisting of whey protein, casein, casein protein, nonfat milk, hydrolyzed protein and combinations thereof;

one or more *Bifidobacterium* species which comprises *Bifidobacterium lactis* Bb-12; and

one or more bacterial species which comprises *Lactobacillus rhamnosus* GG.

2. The nutritional formula of Claim 1, wherein the lipid or fat is present at a level of about 3 to about 7g per 100 kcal.

3. The nutritional formula of Claims 1 or 2, wherein the protein source is present at a level of about 1 to about 5g per 100 kcal.

4. The nutritional formula of any one of the preceding Claims, wherein the respiratory infection is selected from the group consisting of upper respiratory infection, influenza, croup, respiratory syncytial virus, bronchitis, bronchiolitis and pneumonia.

5. The nutritional formula of any one of the preceding Claims, wherein the amount of the one or more bacterial species which comprises *Lactobacillus rhamnosus* GG and the one or more *Bifidobacterium* species administered is between 10^5 and 10^{11} cfu per day.

6. The nutritional formula of any one of Claims 1 to 4, wherein the amount of the one or more bacterial species which comprises *Lactobacillus rhamnosus* GG and the one or more *Bifidobacterium* species administered is between 10^6 and 10^8 cfu per day.

7. The nutritional formula of any one of the preceding Claims, wherein the ratio of the one or more *Bifidobacterium* species and the one or more bacterial species which comprises *Lactobacillus rhamnosus* GG is between about 5:1 and 1:5.

8. The nutritional formula of any one of Claims 1 to 6, wherein the ratio of the one or more *Bifidobacterium* species and the one or more bacterial species which comprises *Lactobacillus rhamnosus* GG is between about 10:1 and 1:10.

9. The nutritional formula of any one of Claims 1 to 6, wherein the ratio of the one or more *Bifidobacterium* species and the one or more bacterial species which comprises *Lactobacillus rhamnosus* GG is about 1:1.

10. A nutritional formula, comprising an infant formula which comprises:

a lipid or fat;

a protein source selected from the group consisting of whey protein, casein, casein protein, nonfat milk, hydrolyzed protein and combinations thereof;

one or more *Bifidobacterium* species; and

one or more adherence-promoting probiotic.

11. The nutritional formula of Claim 10, wherein the lipid or fat is present at a level of about 3 to about 7g per 100 kcal.

12. The nutritional formula of Claims 10 or 11, wherein the protein source is present at a level of about 1 to about 5g per 100 kcal.

13. The nutritional formula of any one of Claims 10 to 12, wherein the respiratory infection is selected from the group consisting of upper respiratory infection, influenza, croup, respiratory syncytial virus, bronchitis, bronchiolitis and pneumonia.

14. The nutritional formula of any one of Claims 10 to 13, wherein the amount of the one or more bacterial species which comprises the one or more adherence-promoting probiotic and the one or more *Bifidobacterium* species administered is between 10^5 and 10^{11} cfu per day.

15. The nutritional formula of any one of Claims 10 to 13, wherein the amount of the one or more bacterial species which comprises the one or more adherence-promoting probiotic and the one or more *Bifidobacterium* species administered is between 10^6 and 10^8 cfu per day

16. The nutritional formula of any one of Claims 10 to 13, wherein the ratio of the one or more *Bifidobacterium* species and the one or more adherence-promoting probiotic is between about 5:1 and 1:5.

17. The nutritional formula of any one of Claims 10 to 13, wherein the ratio of the one or more *Bifidobacterium* species and one or more adherence-promoting probiotic is between about 10:1 and 1:10.

18. The nutritional formula of any one of Claims 10 to 13, wherein the ratio of the one or more *Bifidobacterium* species and the one or more adherence-promoting probiotic is about 1:1.

19. The nutritional formula of any one of Claims 10 to 18, wherein the one or more adherence-promoting probiotic comprises *Lactobacillus rhamnosus* GG.