# METHOD OF USING β-HYDROXY-β-METHYLBUTYRATE

## TECHNICAL FIELD

**[0001]** The present invention relates to a method of treating individuals with β-hydroxy-β-methylbutyrate (HMB) to modulate cytokine production.

## BACKGROUND OF THE INVENTION

**[0002]** Allergies and asthma in the industrialized world have increased in prevalence and severity over recent years. Asthma is now, in fact, the most common chronic illness among children.

**[0003]** Much is known about the pathogenesis of allergies and asthma. Both are immune-based diseases. Both are associated with an imbalance in the relative levels of type-1 and type-2 cytokines in the body. It has been observed that individuals with allergies or asthma have a higher relative ratio of type 2 to type1 cytokines. It is believed that this skewed ratio then contributes to the pathogenesis of allergies and asthma.

**[0004]** In general, cytokines are cell-produced regulatory proteins that influence, in paracrine or autocrine fashion, cell function. They are produced by immune cells and are therefore categorized by their inducible function and the cell types involved in the response.

**[0005]** Type 1 cytokines, for example, elicit or augment primarily cell-mediated immune responses against pathogens. Type 1 cytokines are involved in inflammatory responses, viral immunity, intracellular parasite immunity and allograft rejection. Type 1 cytokines include interleukin 2 (IL-2), interleukin 12 (IL-12), and interferon γ (IFNγ). Type 1 cytokines can suppress the production of type 2 cytokines.

**[0006]** Type 2 cytokines, by contrast, elicit or augment primarily antibody-mediated immune responses against pathogens. Type 2 cytokines are involved in humoral responses, helminth immunity, and allergic responses. Type 2 cytokines include interleukin 4 (IL-4), interleukin 5 (IL-5), interleukin 10 (IL-10), and interleukin 13 (IL-13). Type 2 cytokines can suppress the production of type 1 cytokines.

**[0007]** Given the association between cytokine imbalance in allergies and asthma, it is believed that therapies directed to the normalization of the ratio of type 1 to type 2 cytokine levels will help treat or even prevent such diseases. To that end, it has now been discovered herein that β-hydroxy-β-methylbutyrate (HMB) exposure (*in vitro*) increases the relative ratio of type-1 to type-2 cytokines in stimulated peripheral blood mononuclear cells (PBMC), thus providing a potential new therapy for treating individuals having or at risk for developing allergies and asthma.

**[0008]** As a commercially available ingredient, HMB is found in a variety of nutritional products. It is also a metabolite of the essential amino acid leucine and is therefore found naturally in the human body. HMB is also found in a variety of plants, including citrus fruits and alfalfa, as well as in catfish. It is also known and used for a variety of purposes, including to build or maintain muscle mass in appropriate individuals and to enhance overall immune function.

**[0009]** To date, however, there have been no reports on the effect of HMB in modulating type 1 and type 2 cytokine production nor any disclosure of the use of HMB to affect cytokine imbalance in treating conditions responsive thereto, including allergies and asthma.

## Summary of the Invention

**[0010]** The methods of the present invention are directed to the modulation of type 1 to type 2 cytokine levels in the body in those individuals afflicted with conditions characterized by a corresponding cytokine imbalance, to thus provide treatment of the underlying condition. Most notable among such conditions are allergies and asthma.

**[0011]** A first embodiment of the present invention is a method of treating an individual having a condition characterized by a relative imbalance of type 1 to type 2 cytokine levels in the body, wherein the method comprises the administration to the individual of an effective amount of β-hydroxy-β-methylbutyrate to thus modulate the imbalance, typically by increasing the relative levels or production of type 1 to type 2 cytokines. The present invention includes those embodiments in which the condition is asthma, allergies, or both.

**[0012]** A second embodiment of the present invention is a method of treating an individual having or at risk for developing allergies, the method comprising the administration to the individual an effective amount of β-hydroxy-β-methylbutyrate (HMB).

**[0013]** A third embodiment of the present invention is a method of treating individuals having or at risk for developing asthma, the method comprising the administration to the individual an effective amount of β-hydroxy-β-methylbutyrate (HMB).

**[0014]** A fourth embodiment of the present invention is a method of treating elderly individuals at risk for developing age-related infections, the method comprising the administration to such individuals an effective amount of β-hydroxy-β-methylbutyrate (HMB).

**[0015]** The present invention is based upon the discovery that peripheral blood mononuclear cells (PBMC) stimulated with the T cell stimulus CD3/CD28 and simultaneously exposed to HMB exhibit a shift in type 1 and type 2 cytokine production, favoring type 1 cytokine production. The shift occurs as HMB exposure increases the production of type 1 cytokines such as interferon-γ (IFNγ), interleukin 12 (IL-12), and interleukin 2 (IL-2), without a corresponding increase in the production of type 2 cytokines such as interleukin 4 (IL-4), interleukin 5 (IL-5), interleukin 10 (IL-10), and interleukin 13 (IL-13).

## Brief Description of the Drawings

**[0016]** Fig. 1 summarizes experimental data showing the effect of HMB on type 1 cytokine (IL-2, IL-12, IFNγ, TNFα, GM-CSF) production from peripheral blood mononuclear cells simultaneously stimulated with CD3/CD28 for 24 hours (*$p<0.05$, paired sample, 2 tailed t-test, comparison to no HMB).

**[0017]** Fig. 2 summarizes experimental data showing the effect of HMB on type 2 cytokine (IL-4, IL-5, IL-10, IL-13) production from peripheral blood mononuclear cells simultaneously stimulated with CD3/CD28 for 24 hours (*$p<0.05$, paired sample, 2 tailed t-test, comparison to no HMB).

**[0018]** Fig. 3 summarizes experimental data showing the effect of HMB on the ratio cytokine type 1 (IL-2) to type 2 cytokines (IL-4, IL-5, IL-13, IL-10) from peripheral

blood mononuclear cells simultaneously stimulated with CD3/CD28 for 24 hours (*p<0.05, paired sample, 2 tailed t-test, comparison to no HMB)

**[0019]** Fig. 4 summarizes experimental data showing the effect of HMB on the ratio cytokine type 1 (IL-12) to type 2 cytokines (IL-4, IL-5, IL-13, IL-10) from peripheral blood mononuclear cells simultaneously stimulated with CD3/CD28 for 24 hours (*p<0.05, paired sample, 2 tailed t-test, comparison to no HMB)

**[0020]** Fig. 5 summarizes experimental data showing the effect of HMB on the ratio cytokine type 1 (IFNγ) to type 2 cytokines (IL-4, IL-5, IL-13, IL-10) from peripheral blood mononuclear cells simultaneously stimulated with CD3/CD28 for 24 hours (*p<0.05, paired sample, 2 tailed t-test, comparison to no HMB)

## Detailed Description

**[0021]** The methods of the present invention comprise the administration of an effective amount of β-hydroxy-β-methylbutyrate (HMB) to an individual in need thereof in the manner and for the purposes described herein. These and other essential or optional elements or features of the methods of the present invention are described in detail hereinafter.

**[0022]** The terms "treating" and "treatment" as used herein, unless otherwise specified, includes preventing a condition, delaying the onset of a condition, reducing the severity of symptoms of a condition, or eliminating some or all of the symptoms of a condition.

**[0023]** The term "ameliorate" as used herein, unless otherwise specified, means to eliminate, delay, or reduce the prevalence or severity of symptoms associated with a condition.

**[0024]** The term "condition" as used herein, unless otherwise specified, includes pathological and non-pathological conditions, all of which are characterized by an aberration or imbalance in the relative amounts of type 1 to type 2 cytokines.

**[0025]** The term "elderly individual" as used herein, unless otherwise specified, means someone more than 60 years old, preferably more than 70 years old.

**[0026]** The term "modulate" as used herein, unless otherwise specified, means to reduce the imbalance (i.e., imbalance associated with a condition) of type 1 to type 2

cytokine levels in the body, or to otherwise increase the ratio of type 1 to type 2 cytokines, including an increase in type 1 cytokine levels without a corresponding increase in type 2 cytokine levels.

**[0027]** All percentages, parts and ratios as used herein are by weight of the total composition, unless otherwise specified. All such weights as they pertain to listed ingredients are based on the active level and, therefore, do not include solvents or by-products that may be included in commercially available materials, unless otherwise specified.

**[0028]** All numerical ranges as used herein, whether or not expressly preceded by the term "about", are intended and understood to be preceded by that term, unless otherwise specified.

**[0029]** All references to singular characteristics or limitations of the present invention shall include the corresponding plural characteristic or limitation, and vice versa, unless otherwise specified or clearly implied to the contrary by the context in which the reference is made.

**[0030]** All combinations of method or process steps as used herein can be performed in any order, unless otherwise specified or clearly implied to the contrary by the context in which the referenced combination is made.

**[0031]** The methods of the present invention may also be substantially free of any optional or selected essential feature described herein, provided that the remaining method still contains all of the required limitations as described herein.

## Embodiments

**[0032]** We now turn to the first embodiment of the present invention. Conditions included within the first embodiment of the present invention include allergy, asthma, solid tumors, cancers including advanced ovarian cancer and melanoma, kidney tumors, and stress, including psychological stress after a burn injury, surgical stress and pre-surgical stress. The methods are especially useful in treating allergy, asthma, or both.

**[0033]** With respect to allergy and asthma, elevated levels of IL-4, a type 2 cytokine, have been associated with the promotion or aggravation of allergy and asthma.

Therefore, the first embodiment of the present invention, which is directed to a method of treating an individual having a condition comprising administering to the individual an amount of HMB effective to modulate or otherwise cause an increase in type 1 cytokine levels without a corresponding increase in type 2 cytokine levels, can treat individuals suffering from the symptoms of allergy and asthma because the increase in type 1 cytokines will serve to promote a balanced type 1 to type 2 cytokine profile.

**[0034]** With respect to cancers, including advanced ovarian cancer, studies have shown that the direct injection into the abdominal cavity of the type 1 cytokine IFN-γ may prolong the survival time for women with advanced ovarian cancer. This treatment has been shown to be effective both during the initial chemotherapy as well as after chemotherapy for individuals in whom chemotherapy has failed. Therefore, the first embodiment of the present invention, which is directed to a method of treating an individual having a condition comprising administering to the individual an amount of HMB effective to modulate or otherwise cause an increase in type 1 cytokine levels without a corresponding increase in type 2 cytokine levels, potentiates treatment of individuals with cancer, including advanced ovarian cancer, because the method has been discovered to raise levels of type 1 cytokines, including IFNγ.

**[0035]** With respect to kidney tumors and melanoma, studies have shown that interleukin 2 given as an injection under the skin can treat some kidney tumors and melanoma. When used as a cancer treatment, it is thought that IL-2 strengthens the body's natural defense mechanism and causes some cancer cells to be recognized and eliminated by immune cells. Therefore, the first embodiment of the present invention, which is directed to a method of treating an individual having a condition comprising administering to the individual an amount of HMB effective to modulate or otherwise cause an increase in type 1 cytokine levels without an increase in type 2 cytokines, potentiates treatment of individuals having kidney tumors or melanoma because the inventors have discovered that the method of the first embodiment of the present invention can raise levels of type 1 cytokines, including IL-2.

**[0036]** With respect to psychological stress after a burn injury, surgical stress and pre-surgical stress, studies have shown that stress increases type 2 and suppresses type 1 cytokine production. The immune system is compromised when individuals experience stress due to the production of type 2 cytokines and the suppression of type 1 cytokines that accompany periods of stress. Therefore, the first embodiment

of the present invention, which is directed to a method of treating an individual having a condition comprising administering to the individual an amount of HMB effective to modulate or otherwise cause an increase in type 1 cytokine levels without an increase in type 2 cytokines, can treat stress after a burn injury, surgical stress and pre-surgical stress because administering to individuals an amount of HMB effective to increase type 1 cytokine levels without increasing type 2 cytokine levels accommodates for the cytokine imbalance associated with stress. The increase in type 1 cytokines promotes a balanced type1 to type 2 cytokine profile in the individual.

**[0037]** The type 1 cytokines included within the first embodiment of the present invention include interferon-γ, interleukin 2, and interleukin 12. The type 2 cytokines included within the first embodiment of the present invention include interleukin 4, interleukin 5, interleukin 10, and interleukin 13.

**[0038]** Some of the protective functions of IFNγ include inhibition of viral replication, stimulation of macrophages and enhancement of cell surface molecules necessary for self-recognition in an immune response. Additionally, adequate levels of IFNγ are required for protection against infection and disease. IFNγ also antagonizes several actions of type 2 cytokine IL-4 and inhibits the proliferation of IL-4 producing cells. Therefore, the ability to induce production of IFNγ aids in the treatment of individuals with conditions such as those discussed herein. The inventors have discovered that HMB can induce the production of IFNγ without affecting the production of type 2 cytokines and therefore the present method is effective in treating conditions of the type discussed herein.

**[0039]** Some of the protective functions of IL-2 include inducing proliferation of all T cells, activated B cells, and natural killer cell and enhancing killing of tumor cells by the induction of tumoricidal cytokines from T cells and natural killer cells. Adequate levels of IL-2 are also required for protection against infection and disease. Therefore, the ability to induce production of IL-2 aids in the treatment of individuals with conditions such as those discussed herein. The inventors have discovered that administration of HMB induces the production of IL-2 without increasing the type 2 cytokine levels and therefore the present method is effective in treating conditions of the type discussed herein.

**[0040]** While adequate levels of type 2 cytokine IL-4 are also required for protection against infection and disease, elevated levels of IL-4 have been associated with the promotion of allergies, asthma and stress. Therefore, the ability to treat an individual having a condition as described herein is dependent upon both the ability to induce the production of type 1 cytokines such as IFNγ and IL-2, but also the ability to not simultaneously increase the production of type 2 cytokines, and particularly IL-4, as increased levels of IL-4 are known to promote allergies, asthma and stress. The first embodiment of the present invention is directed to a method of treating an individual having a condition wherein the administration of HMB induces the production of IL-2 and IFNγ without a corresponding increase in IL-4 levels.

**[0041]** Another aspect of the first embodiment of the present invention is directed to a method of treating an individual having a condition characterized by an imbalance in type 1 and type 2 cytokines, comprising administering to the individual an amount of HMB effective to modulate or otherwise cause an increase in type 1 cytokine levels without a corresponding increase in type 2 cytokine levels, wherein the amount of HMB administered is an amount effective to ameliorate allergic symptoms. Elevated levels of type 2 cytokine IL-4 are associated with the promotion of allergies. But type 1 cytokines such as IFNγ antagonize several actions of IL-4 and inhibit the proliferation of IL-4 producing cells. Therefore, the present method is capable of ameliorating the symptoms of allergies when the amount of HMB administered to an individual is an effective amount to promote a balanced type 1 to type 2 cytokine profile.

**[0042]** Similarly, the present invention is directed to a method of treating an individual having a condition characterized by an imbalance in type 1 and 2 cytokines, comprising administering to the individual an amount of HMB effective to modulate or otherwise cause an increase in type 1 cytokine levels without a corresponding increase in type 2 cytokine levels, wherein the amount of HMB administered is an amount effective to ameliorate asthmatic symptoms. Elevated levels of type 2 cytokine IL-4 are associated with the promotion of asthma. But type 1 cytokines such as IFNγ antagonize several actions of IL-4 and inhibits the proliferation of IL-4 producing cells. Therefore, the present method is capable of ameliorating the symptoms of asthma when the amount of HMB administered to an individual is an effective amount to promote a balanced type1 to type 2 cytokine profile.

**[0043]** Still another aspect of the first embodiment of the present invention is directed to a method of treating an individual having a condition characterized by an imbalance in type 1 and type 2 cytokines, comprising administering to the individual an amount of HMB effective to modulate or otherwise cause an increase in type 1 cytokine levels without a corresponding increase in type 2 cytokine levels, wherein the amount of HMB administered is an amount effective to prevent a decrease in $FEV_1$, or the forced expiratory volume in 1 second. Individuals suffering from severe and persistent asthma display a low $FEV_1$ percentage value, while those who suffer from only mild and intermittent asthma display a higher percentage value. Thus, in administering an amount of HMB effective to induce the production of type 1 cytokines without inducing the production of type 2 cytokines in order to decrease the promotion of asthma associated with imbalanced type1 to type 2 cytokine profiles, the present method is capable of preventing a decrease in $FEV_1$.

**[0044]** Individuals who experience only mild and intermittent symptoms of asthma display a $FEV_1$ value of greater than or equal to 80%. Therefore, another aspect of the first embodiment of the present invention is directed to a method of treating an individual having a condition where cytokine production is induced, comprising administering to the individual an amount of HMB effective to cause an increase in type 1 cytokine levels without a corresponding increase in type 2 cytokine levels, wherein the amount of HMB administered is an amount effective to maintain basal $FEV_1$ above 80%. In altering the imbalanced type 1 to type 2 cytokine profiles associated with asthma through the method of the first embodiment of the present invention, the method is capable of maintaining basal $FEV_1$ above 80%.

**[0045]** We now turn to the second embodiment of the present invention. The present invention includes a method of treating allergy in an individual having or at risk for developing allergy, the method comprising the administration to the individual an amount of β-hydroxy-β-methylbutyrate effective to prevent or ameliorate symptoms of allergies. Individuals who are at risk for allergy include those who are already suffering from allergies and those who are genetically or otherwise predisposed to having allergies.

**[0046]** The term "allergies" as used herein includes hay fever, food allergies, allergic conjunctivitis, atopic dermatitis, inhalant (air born allergens) allergy, and other common allergies. Such allergies are often associated with exposure to allergens

such as animal danders, pollens, insect stings or bites, house dust, house dust mites, molds, some drugs, and foods, especially fish, eggs, milk and nuts.

**[0047]** We now turn to the third embodiment of the present invention. The present invention includes a method of treating asthma in an individual having or at risk for asthma, the method comprising the administration to the individual of an amount of β-hydroxy-β-methylbutyrate effective to prevent or ameliorate asthmatic symptoms. Individuals who are at risk for asthma include those who are already suffering from asthma and those who are genetically or otherwise predisposed to having asthma.

**[0048]** We now turn to the fourth embodiment of the present invention. The present invention includes a method of treating elderly individuals at risk of developing age-related infections, including both bacterial and viral infections, respiratory and non-respiratory, the method comprising the administration to such individuals of an amount of β-hydroxy-β-methylbutyrate effective to reduce the risk or prevalence of such infections.

**[0049]** An effective amount of HMB, for the purposes of the methods described herein, most typically ranges from 0.1 g to 10 g, including from 0.5 g to 5.0 g, and also including from 1.0 g to 3.5 g, of HMB per day. The total daily dose may be administered as a single, divided, or continuous (or semi-continuous) dose (e.g., enteral feeding), every day or on selected intermittent days.

**[0050]** The methods of the present invention are preferably directed to oral administration.

**Product Forms**

**[0051]** The methods of the present invention may be directed to any product form suitable for the safe administration of an effective amount of HMB to the targeted population or selected individual, all in accordance with the methods herein. Such products include pharmaceutical dosage forms (e.g., capsules, tablets, liquids, topicals, etc.) as well as nutritional products.

**[0052]** Nutritional products for use herein further comprise one or more (preferably all) of fat, protein, carbohydrate, minerals, and vitamins. Such products include solids, liquids, powders, and gels.

**[0053]** Non-limiting examples of solid nutritional product forms suitable for use herein include snack and meal replacement products, including those formulated as bars, sticks, cookies or breads or cakes or other baked goods, frozen liquids, candy, breakfast cereals, powders or granulated solids or other particulates, snack chips or bites, and so forth.

**[0054]** Non-limiting examples of liquid nutritional product forms suitable for use herein include snack and meal replacement products such as those formulated as juices or other acidified beverages, milk or soy-based beverages, shakes, coffees, teas, carbonated beverages, non-carbonated beverages, enteral feeding compositions, and so forth. These liquid compositions are most typically formulated as suspensions or emulsions, but can also be formulated in any other suitable form such as solutions, liquid gels, and so forth.

**[0055]** Many different sources and types of proteins, lipids, and carbohydrates are known and can be used in the various nutritional products described herein, provided that the selected nutrients are safe and effective for oral administration and are compatible with the essential and other added ingredients.

**[0056]** Carbohydrates suitable for use in the nutritional products may be simple, complex, or variations or combinations thereof. Non-limiting examples of suitable carbohydrates include hydrolyzed or modified starch or cornstarch, maltodextrin, glucose polymers, sucrose, corn syrup, corn syrup solids, rice-derived carbohydrate, glucose, fructose, lactose, high fructose corn syrup, indigestible oligosaccharides (e.g., fructooligosaccharides), honey, sugar alcohols (e.g., maltitol, erythritol, sorbitol), and combinations thereof.

**[0057]** Carbohydrates suitable for use herein also include soluble dietary fiber, non-limiting examples of which include gum arabic, sodium carboxymethyl cellulose, guar gum, citrus pectin, low and high methoxy pectin, oat and barley glucans, carrageenan, psyllium and combinations thereof. Soluble dietary fiber is also suitable as a carbohydrate source herein, non-limiting examples of which include oat hull fiber, pea hull fiber, soy hull fiber, soy cotyledon fiber, sugar beet fiber, cellulose, corn bran, and combinations thereof.

**[0058]** Proteins suitable for use in the nutritional products include hydrolyzed, partially hydrolyzed or non-hydrolyzed proteins or protein sources, and can be derived from any known or otherwise suitable source such as milk (e.g., casein,

whey), animal (e.g., meat, fish), cereal (e.g., rice, corn), vegetable (e.g., soy), or combinations thereof. The proteins for use herein can also include, or be entirely or partially replaced by, free amino acids known for use in nutritional products, non-limiting examples of which include tryptophan, glutamine, tyrosine, methionine, cysteine, arginine, and combinations thereof.

**[0059]** Fats suitable for use in the nutritional products include coconut oil, fractionated coconut oil, soy oil, corn oil, olive oil, safflower oil, high oleic safflower oil, MCT oil (medium chain triglycerides), sunflower oil, high oleic sunflower oil, palm and palm kernel oils, palm olein, canola oil, marine oils, cottonseed oils, and combinations thereof.

**[0060]** The concentration or amount of carbohydrate, protein, and carbohydrate in the nutritional compositions of the present invention can vary considerably depending upon the particular product form and the various other formulations and targeted dietary needs. These macronutrients are most typically formulated within any of the caloric ranges (embodiments A, B, or C) described in the following table.

| Nutrients | Nutritional Embodiments | | |
|---|---|---|---|
| | A | B | C |
| Carbohydrate - % total calories | 1-98 | 10-75 | 30-50 |
| Fat - % total calories | 1-98 | 20-85 | 35-55 |
| Protein - % total calories | 1-98 | 5-70 | 15-35 |

**[0061]** The nutritional compositions for use herein may further comprise other optional components that may modify the physical, chemical, aesthetic or processing characteristics of the products or serve as pharmaceutical or additional nutritional components when used in the targeted population. Many such optional ingredients are known or otherwise suitable for use in medical food or other nutritional products or pharmaceutical dosage forms and may also be used in the compositions herein, provided that such optional ingredients are safe for oral administration and are compatible with the essential and other ingredients in the selected product form.

**[0062]** Non-limiting examples of such optional ingredients include preservatives, anti-oxidants, emulsifying agents, buffers, additional pharmaceutical actives, additional nutrients as described herein, sweeteners including artificial sweeteners (e.g., saccharine, aspartame, acesulfame K, sucralose) colorants, flavors, thickening agents and stabilizers, emulsifying agents, lubricants, and so forth.

**[0063]** The nutritional compositions for use herein may further comprise any of a variety of other vitamins or related nutrients, non-limiting examples of which include vitamin A, vitamin D, vitamin E, vitamin K, thiamine, riboflavin, pyridoxine, vitamin $B_{12}$, carotenoids (e.g., beta-carotene, zeaxanthin, lutein, lycopene), niacin, folic acid, pantothenic acid, biotin, vitamin C, choline, inositol, salts and derivatives thereof, and combinations thereof.

**[0064]** The nutritional compositions for use herein may further comprise any of a variety of other additional minerals, non-limiting examples of which include calcium, phosphorus, magnesium, iron, zinc, manganese, copper, sodium, potassium, molybdenum, chromium, chloride, and combinations thereof.

Experiment

**[0065]** The following experiment is conducted to determine the relationship between HMB exposure and cytokine production.

**[0066]** To induce production of cytokines, PBMCs, isolated from peripheral blood of 10 normal healthy donors, are stimulated for 24 hours with the T cell stimulant CD3/CD28. Cytokine production is analyzed using a Bio-plex Cytokine Assay. The Bio-Plex technology is based on antibody-antigen interactions, wherein fluorescently labeled beads conjugated with antibody directed against the cytokine of interest bind target cytokine to the bead. This bead-cytokine complex is then exposed to a biotinylated detection antibody and a streptavidin–PE (phycoerythrin) reporter molecule. The signal from the reporter molecule is directly proportional to the amount of cytokine present, thus enabling cytokine quantification.

**[0067]** Each of the T cell-derived cytokines quantified in the experiment is described in the following table:

| Type 1 cytokines | |
|---|---|
| Interleukin 2 (IL-2) | Growth factor for all subpopulations of T cells and also promotes the proliferation of activated B cells |
| Interleukin 12 (IL-12) | Induces the synthesis of IFNγ, IL-2, and Tumor necrosis factor α (TNFα) from helper T cells committed to the production of type 1 cytokines (Th1 cells), promotes the generation of lymphokine activated killer cells, inhibits the synthesis of IgE production |
| Interferon γ (IFNγ) | Influences cell mediated mechanisms of cytotoxicity, has antiviral and antiparasitic activities and inhibits the proliferation of transformed cells |

| | |
|---|---|
| Granulocyte Macrophage colony stimulating factor (GM-CSF) | Stimulates the proliferation and differentiation of neutrophilic, eosinophilic, and monocytic lineages and activates the mature forms of these cell types |
| Tumor necrosis factor α (TNFα) | Induces cytolysis and cytostasis of tumor cells, enhances the proliferation of T cells, promotes the proliferation and differentiation of B cells in the presence of IL-2 |
| Type 2 cytokines | |
| Interleukin 4 (IL-4) | Promotes the proliferation and differentiation of activated B cells |
| Interleukin 5 (IL-5) | Promotes growth and differentiation of eosinophils |
| Interleukin 13 (IL-13) | Down-modulates macrophage activity, reduces pro-inflammatory cytokine production, induces human monocyte differentiation and B cell differentiation and proliferation |
| Interleukin 10 (IL-10) | Suppressive cytokine which down-regulates type 1 cytokine production |

A t-test (paired sample, two-tailed) is performed in which a broad range of type 1 and type 2 cytokines are evaluated from cultures with HMB compared to cultures and without HMB.

**[0068]** Significant dose response increases are then observed in the production of the following type 1 cytokines: IL-2 (5mM and 10mM HMB), IL-12 (5 mM and 10 mM HMB), and IFNγ, (5 mM and 10 mM HMB); the results of which are summarized in Fig. 1.

**[0069]** Concerning type 2 cytokine production, a significant decrease is seen in IL-10 production following 10 mM exposure to HMB (see Figure 2), while HMB does not significantly affect the production of GC-CSF, TNFα, IL-4, IL-5, and IL-13. These results are summarized in Fig. 1 and Fig. 2.

**[0070]** The shifts in cytokine production favoring type 1 are summarized in Figs. 3, 4, and 5. Increases in IL-2 production relative to IL-4 and IL-10 production are demonstrated at HMB concentrations of 5 mM and 10 mM, while increased in IL-2 production relative to IL-5 and IL-13 production are demonstrated at HMB concentrations of 1 mM, 5 mM, and 10 mM (Fig. 3). Increases in IL-12 production relative to IL-4, IL-5, IL-13, and IL-10 are demonstrated at HMB concentrations of 5 mM and 10 mM (Fig. 4). Increases in IFNγ production relative to IL-4 and IL-10 are

demonstrated at an HMB concentration of 10 mM, while relative to IL-5 increases are demonstrated at HMB concentrations of 5mM and 10 mM (Fig. 5)

**[0071]** The data show that HMB exposure increases type 1 cytokine production (IL-2, IL-12, IFNγ) while reducing production of certain type 2 cytokines (IL-10) and not significantly affecting the production of other type 2 cytokines (GC-CSF, TNFα, IL-4, IL-5, IL-13). The net result, therefore, is a shift in type 1 and type 2 cytokine production in favor of type 1 cytokine production.

## EXAMPLES

**[0072]** The following examples illustrate specific embodiments of the methods of the present invention, including some nutritional and other product forms suitable for use therein. The examples are given solely for the purpose of illustration and are not to be construed as limitations of the present invention, as many variations thereof are possible without departing from the spirit and scope of the invention.

**[0073]** The nutritional compositions described below are representative examples of nutritional products suitable for use in the methods of the present invention. Each may be prepared by conventional methods of making nutritional emulsion, some examples of which are described in U.S. Patent Publication 20050215640A1, which description is incorporated herein by reference.

**Liquid Nutritional #1 (Weight Gain Formula)**

| Ingredient | Amt (kg) | Ingredient | Amt (kg) |
|---|---|---|---|
| Water | 316 | Vitamin DEK premix | 0.04 |
| Ultratrace/trace min. premix | 0.06 | Carrageenan | 0.03 |
| Potassium chloride | 0.072 | Soy lecithin | 0.6 |
| Na citrate | 2.89 | Sodium caseinate | 15.5 |
| Potassium iodide | 0.0001 | Calcium caseinate | 4.2 |
| Potassium citrate | 1.5 | Ca HMB monohydrate | 2.6 |
| Corn syrup | 7.68 | Milk protein isolate | 14 |
| Maltodextrin | 53.6 | Sardine oil | 6.9 |
| Mg phosphate dibasic | 0.26 | Ascorbic acid | 0.12 |
| Ca phosphate tribasic | 0.99 | KOH 45% Soln | 0.13 |
| Magnesium chloride | 1.2 | Taurine | 0.12 |
| Sucrose | 11.9 | Water sol. vit. premix | 0.11 |
| Fructooligosaccharide | 5.9 | Ascorbyl palmitate | 0.03 |
| Medium chain triglycerides | 2.6 | Choline chloride | 0.25 |
| Canola oil | 1.5 | L-carnitine | 0.0681 |
| Soy oil | 0.87 | Flavor # 1 | 1.6 |
| 57% Vitamin A palmitate | 0.007 | Flavor # 2 | 0.27 |

**Liquid Nutritional #2 (Low Glycemic Index Formula)**

| Ingredient | Amt per 1,000 kg | Ingredient | Amt per 1,000 kg |
|---|---|---|---|
| Water | QS | Vitamin C | 584 gm |
| Maltodextrin | 56 kg | Potassium chloride | 530 gm |
| Acid casein | 41.09 kg | Choline chloride | 472.1 gm |
| Fructose | 28 kg | 45% KOH soln. | 402.5 gm |
| High oleic safflower oil | 27.2 kg | UTM/TM premix | 369.3 gm |
| Maltitol syrup | 16 kg | K phosphate | 333 gm |
| Maltitol | 12.63 kg | Carnitine | 230.5 gm |
| Fibersol 2E | 8.421 kg | Gellan gum | 125 gm |
| Caseinate | 6.043 kg | Taurine | 100.1 gm |
| FOS | 4.607 kg | Vitamin E | 99 gm |
| Soy polysaccharide | 4.3 kg | Lutein Esters (5%) | 92 gm |
| Canola oil | 3.2 kg | WSV premix | 75.4 gm |
| Tricalcium phosphate | 2.8 kg | Vit. DEK premix | 65.34 gm |
| Mg chloride | 2.4 kg | 30% Beta carotene | 8.9 gm |
| Lecithin | 1.6 kg | Vitamin A | 8.04 gm |
| Sodium citrate | 1.18 kg | Pyridoxine HCl | 3.7 gm |
| Potassium citrate | 1.146 kg | Chromium chloride | 1.22 gm |
| Sodium hydroxide | 1.134 kg | Folic acid | 0.64 gm |
| Mg phosphate | 1.028 kg | Potassium iodide | 0.20 gm |
| Calcium HMB monohydrate | 5.7 kg | Cyanocobalamin | 0.013 gm |
| m-inositol | 914gm | Vitamin C | 584 gm |

**Liquid Nutritional # 3 (Pediatric Formula)**

| Ingredient | per 771kg | Ingredient | per 771 kg |
|---|---|---|---|
| **Stock PIF Slurry** | | **Final Blend** | |
| High oleic safflower oil | 40.7 kg | PIW slurry | 251 kg |
| Soy oil | 24.4 kg | PIF slurry | 53 kg |
| MCT oil | 16.3 kg | MIN slurry | 12.6 kg |
| Lecithin | 840.2 g | Sodium chloride | 127.4 g |
| Monoglycerides | 840.2 g | Sucrose | 77.6 kg |
| Carrageenan | 508.9 g | Tricalcium phosphate | 2.5 kg |
| Caseinate | 32.8 kg | Water | 167 kg |
| **Stock OSV Blend** | | **Stock WSF Soln** | |
| DEK premix | 83.3 g | Water | 31.7 kg |
| Vitamin A | 7.1 g | Potassium citrate | 3.74 g |
| Lutein esters (5%) | 92 g | UTM/TM premix | 172.2 g |
| **Stock PIW Slurry** | | WSV premix | 134.1 g |
| Water | 530 kg | m-inositol | 176.7 g |
| Caseinate | 11.3 kg | Taurine | 145.5 g |
| Whey protein | 11.9 kg | L-carnitine | 34.92 g |
| **Stock MIN Slurry** | | Choline chloride | 638.7 g |
| Water | 18 kg | **Stock ascorbic acid soln.** | |
| Cellulose gum | 1696 g | Water | 18.6 kg |
| Calcium HMB monohydrate | 4.4kg | Ascorbic acid | 550.0 g |
| Magnesium chloride | 2.7 kg | 45% KOH | 341 g |
| Potassium chloride | 1.0 kg | **Stock vanilla soln.** | |
| Potassium citrate | 2.7 kg | Water | 38.5 kg |
| Potassium iodide | 0.25g | Vanilla flavor | 4.3 kg |
| Dipotassium phosphate | 1.45 kg | | |

**Nutritional Liquid #4 (Nutritional Supplement)**

| Ingredient | per 1,000kg | Ingredient | per 1,000kg |
|---|---|---|---|
| Water | QS | Magnesium chloride | 558 gm |
| Corn Syrup | 33 kg | Vanilla Flavor | 544 gm |
| Maltodextrin | 28 kg | Sodium Chloride | 272 gm |
| Sucrose | 19.4 kg | Carrageenan | 227 gm |
| Caseinate | 8.7 kg | Choline chloride | 218 gm |
| Calcium HMB monohydrate | 5.7 kg | UTM/TM Premix | 165 gm |
| High Oleic Safflower Oil | 4.1 kg | Potassium Chloride | 146 gm |
| Canola Oil | 4.1 kg | Ascorbic Acid | 145 gm |
| Soy Protein | 3.7 kg | Sodium Citrate | 119 gm |
| Whey Protein | 3.2 kg | Potassium Hydroxide | 104 gm |
| Caseinate | 2.9 kg | Lutein (5%) | 46 gm |
| Corn Oil | 2.0 kg | WSV Premix | 33 gm |
| Tricalcium Phosphate | 1.4 kg | Vit DEK Premix | 29 gm |
| Potassium Citrate | 1.3 kg | Vitamin A | 3.7 gm |
| Magnesium Phosphate | 952 gm | Potassium Iodide | 86 mcg |
| Lecithin | 658 gm | | |

**Liquid Nutritional #5 (Asthma and Allergy formula)**

| Ingredient | kg per 1000kg | Ingredient | kg per 1000 kg |
|---|---|---|---|
| Ingredient water | Q.S. | Natural Vitamin E | 0.645 |
| Borage oil | 61.1 | Micronized tri calcium phosphate | 0.631 |
| Marine oil | 53.4 | Tocopherol-2 antioxidant | 0.600 |
| Milk protein isolate | 30.4 | Taurine | 0.456 |
| Sucrose | 11.7 | Vanilla | 0.400 |
| Whey protein conc. | 8.41 | Sucralose 25% sol | 0.375 |
| Gum arabic | 8.00 | Zinc Sulfate | 0.251 |
| Calcium HMB Monohydrate | 5.7 | Ascorbyl palmitate | 0.143 |
| Soy lecithin | 4.77 | Sodium chloride | 0.143 |
| Cellulose gum | 4.00 | Acesulfame K | 0.0750 |
| Potassium citrate | 2.64 | Cupric sulfate | 0.0177 |
| Orange Cream Flavor | 2.50 | FD&C Red #3 | 0.0150 |
| Ascorbic acid | 1.13 | B carotene 30% | 0.00992 |
| Turmeric powder | 1.00 | Vit. A palmitate | 0.00315 |
| Sodium citrate | 0.901 | Sodium molybdate | 0.000529 |
| KOH 45% solution | 0.799 | Sodium selenate | 0.000441 |
| Orange Oil | 0.750 | | |

**Powder Nutritional #6 (Exercise Formula)**

| Ingredient Name | Amount per 1000 kg | Ingredient Name | Amoun 1000 k |
|---|---|---|---|
| Whey Protein Concentrate | 282.051 kg | Potassium Chloride | 5.128 k |
| Calcium Caseinate | 192.308 kg | Salt | 3.205 k |
| Maltodextrin | 165.416 kg | Xanthan Gum | 3.205 k |
| Milk Protein Isolate | 138.782 kg | Choline Bitartrate 41% choline | 2.782 k |
| Dutch Cocoa 10/12 | 76.932 kg | Acesulfame K | 2.718 k |
| Sunflower Oil Creamer | 21.474 kg | Vanilla | 1.923 k |
| Myoplex Oil PreBlend | 19.231 kg | Disodium Phosphate Anhydrous | 1.667 k |
| Chocolate Cream | 15.256 kg | MicroChill WPI | 1.282 k |
| Calcium HMB monohydrate | 13.157 kg | Beta Carotene 1% CWS | 1.128 k |
| Oat Fiber | 10.897 kg | Sucralose | 692.3 g |
| Tricalcium Phosphate | 8.526 kg | Potassium Citrate 38% K | 641.0 g |
| Vitamin Mineral Preblend | 8.462 kg | Alpha Ketoglutaric Acid | 321.0 g |
| Dipotassium Phosphate | 8.333 kg | Egg Albumin Powder | 321.0 g |
| Rich Dark Chocolate | 7.051 kg | L-Glutamine | 321.0 g |
| Carrageenan CSM 2 | 6.474 kg | Taurine | 321.0 g |

**Working Example I**

**[0074]** A 28-year old individual who suffers from seasonal allergies in the spring is given 0.25-1g of HMB (Nutritional Liquid #5) four times a week for a year. The symptoms of seasonal allergies are reduced the following spring.

**Working Example II**

**[0075]** A 30-year old white male who normally has four exacerbations of asthma per year is administered 1-10 g of HMB (Nutritional Liquid #5) four times a week for a year. Exacerbation frequency decreases to once a year.

**Working Example III**

**[0076]** A 45-year old female who has undergone chemotherapy for ovarian cancer is administered 2-10 g of HMB (Nutritional Liquid #1) four times a week for a year. One year later the ovarian cancer has not returned.

**Working Example IV**

**[0077]** A 50-year old male diagnosed with and has been treated for kidney tumors is administered 750 mg of HMB (capsules) four times a week for a year. Six months later the tumor has not spread to other parts of the individual's body.

**Working Example V**

**[0078]** A 42-year old female diagnosed with and treated for melanoma is administered 1 g of HMB 4 times ((Nutritional Liquid #1) a week for a year. Six months later the melanoma has not spread to other parts of the individual's body.

**Working Example VI**

**[0079]** A 37-year old male suffering from severe symptoms of psychological stress as a result of a burn injury is administered 500 mg (Nutritional Liquid #1) of HMB 4 times a week for a year. One year later the symptoms of psychological stress are reduced.

**Working Example VII**

**[0080]** A 29-year old female suffering from symptoms of surgical stress is administered 200 mg of HMB (Nutritional Liquid #2) 7 times a week for 2 months. Two months later the symptoms of surgical stress are reduced.

## Working Example VIII

**[0081]** A 25-year old male suffering from symptoms of pre-surgical stress is administered 200 mg of HMB (capsules) once a day for 3 weeks preceding the individual's scheduled surgery. At the end of the 3 weeks, symptoms of pre-surgical stress are reduced.

## Working Example IX

**[0082]** A 24-year old male suffering from moderate persistent asthma is tested to determine the individual's $FEV_1$ percentage value and the value is recorded. The individual is then administered 5-10 g of HMB 4 times ((Nutritional Liquid #5) a week for a year. One year later the individual's $FEV_1$ has not decreased and symptoms of asthma are reduced.

## Working Example X

**[0083]** A 33-year old male suffering from mild intermittent asthma is tested to determine the individual's $FEV_1$ percentage value and a value of 83% is recorded. The individual is then administered 1.5 -6 g of HMB 4 times (Nutritional Liquid #5) a week for a year. One year later the individual's $FEV_1$ remains above 80% and symptoms of asthma are reduced.

## Working Example XI

**[0084]** A 14-year old female with a family history of seasonal allergies shows no signs of suffering from seasonal allergies. The individual is administered 0.1-1.5 g of HMB (Nutritional Liquid #3) once a day for 6 months. Six months later the individual still shows no signs of suffering from seasonal allergies.

## Working Example XII

**[0085]** A 16-year old male with a family history of asthma shows no signs of suffering from the symptoms of asthma. The individual is administered 250 mg of HMB (Nutritional Liquid #4) once a day for 6 months. Six months later the individual still shows no signs of suffering from symptoms of asthma.

## Working Example XIII

**[0086]** A 72 year old male, after conventional treatment for and recovery from pneumonia, is administered 250 mg of HMB (Nutritional Liquid #1) once a day for 6 months. During those six months, the individual remains free of respiratory tract infections, including any recurrence of pneumonia.

## Working Example XIV

**[0087]** A 24-year old male is training for the New York Marathon. During his training period and for 3 months following the event he takes two servings per day of Nutritional formula #6 (containing 1 gram HMB per serving). Contrary to his previous year's experience, he does not experience any respiratory infections during this intense training regimen (such infections reflect the immune suppression that is known to be associated with extreme physical training programs).

**WHAT IS CLAIMED IS:**

1. A method of treating an individual having a condition characterized by ~~induced~~ an imbalance in type 1 and type 2 cytokine levels, comprising administering to the individual an effective amount of β-hydroxy-β-methylbutyrate to modulate the imbalance in type 1 and type 2 cytokine production.

2. The method of claim 1 wherein the ratio of type 1 to type 2 cytokine levels is increased by the administration of β-hydroxy-β-methylbutyrate.

3. The method of claim 2 wherein the level of type 1 cytokines is increased by the administration of β-hydroxy-β-methylbutyrate without an increase in type 2 cytokines.

4. The method of claim 2 wherein the effective amount of β-hydroxy-β-methylbutyrate ranges from 0.5 g to 10 g per day.

5. The method of claim 2 wherein the β-hydroxy-β-methylbutyrate is administered as a nutritional product further comprising one or more of fat, protein, and carbohydrate.

6. The method of claim 1 wherein the condition is an allergy.

7. The method of claim 6 wherein the amount of β-hydroxy-β-methylbutyrate is effective to ameliorate allergic symptoms.

8. The method of claim 1 wherein the condition is asthma.

9. The method of claim 8 wherein the amount of β-hydroxy-β-methylbutyrate is effective to ameliorate asthmatic symptoms.

10. The method of claim 8 wherein the amount of β-hydroxy-β-methylbutyrate is effective to prevent a decrease in $FEV_1$.

11. The method of claim 8 wherein the amount of β-hydroxy-β-methylbutyrate is sufficient to maintain basal $FEV_1$ above 80%.

12. The method of claim 1 wherein the condition is cancer.

13. The method of claim 1 wherein the type 1 cytokine is interferon-γ or interleuken 2, and the type 2 cytokine is interleukin 4.

14. The method of claim 2 wherein the type 1 cytokine is interferon-γ, interleukin 2, or interleukin-12, and the type 2 cytokine is interleukin 4, interleukin 5, interleukin 13, or interleukin 10.

15. A method of treating allergies in an individual having or at risk for developing allergies, said method comprising the administration to the individual an effective amount of β-hydroxy-β-methylbutyrate.

16. The method of claim 15 wherein the β-hydroxy-β-methylbutyrate is administered as a nutritional product further comprising one or more of fat, protein, and carbohydrate.

17. The method of claim 16 wherein an effective amount of β-hydroxy-β-methylbutyrate ranges from 0.5 g to 10 g per day.

18. A method of treating asthma in an individual having or at risk for developing asthma, said method comprising the administration to the individual an effective amount of β-hydroxy-β-methylbutyrate.

19. The method of claim 18 wherein the β-hydroxy-β-methylbutyrate is administered as a nutritional product further comprising one or more of fat, protein, and carbohydrate.

20. The method of claim 19 wherein an effective amount of β-hydroxy-β-methylbutyrate ranges from 0.5 g to 10 g per day.

21. A Method of reducing the risk of infection in an elderly individual at risk for developing infection, said method comprising the administration to the individual an effective amount of β-hydroxy-β-methylbutyrate.

22. The method of claim 21 wherein the β-hydroxy-β-methylbutyrate is administered as a nutritional product further comprising one or more of fat, protein, and carbohydrate.

23. The method of claim 22 wherein an effective amount of β-hydroxy-β-methylbutyrate ranges from 0.5 g to 10 g per day.