DESCRIPTION

AGENT FOR PRODUCING RETINOIC ACID

Technical Field

The present invention relates to an agent for producing retinoic acid and an agent for enhancing immune function on the basis of the effect of the agent to promote retinoic acid production.

Background Art

When pathogenic bacteria, fungi, viruses, or the like invade and grow in the bodies of mammalian animals, including humans, symptoms such as diarrhea, gastrointestinal disease, or fever develop. Serious symptoms may occasionally result in death. Such infectious mammalian diseases have not yet been eradicated in the modern society, despite its advanced medicine. The defense against infectious diseases is a major issue of concern for humans seeking to lead healthy lives.

People of the ages with lower resistance, including with regard to the ability to defend against infection, such as elderly persons, infants, and children, than healthy adults, are more likely to be affected with such infectious diseases. Because of a decrease in self-defense function against infection associated with aging, for example, elderly persons are infected with indigenous bacteria with which healthy adults would not be infected (e.g., *Staphylococcus aureus* existing in the nasal mucous membrane). If the development of an infectious disease is observed in elderly persons, patients with the infectious disease are physically isolated and facilities in which patients who had developed the infectious diseases had stayed or visited are closed, so as to prevent infectious diseases from spreading. In addition, infants and/or children have immature immune systems and, accordingly, they are more likely to be affected with infectious diseases than healthy adults, because of their low ability of their own defense against infection.

In order to deal with an infectious disease, in general, the cause of such infectious

disease is first identified, and treatment by administration of a drug having antimicrobial activity is then carried out. When a drug having antimicrobial activity is administered to a patient, however, a drug having antimicrobial activity also attacks enteric bacteria such as so-called nonpathogenic bifidobacteria, that do not cause infectious diseases and are naturally colonized in the body, in addition to particular bacteria or viruses causing infectious diseases. Accordingly, side effects such as diarrhea may develop due to infection with other pathogens. As a result of repetitive administration or long-term administration of the same drug having antimicrobial activity, in addition, pathogenic bacteria, fungi, and viruses causing infectious diseases may gradually acquire tolerance to the drug, and antimicrobial effects may occasionally decline. If pathogens acquire drug tolerance, the treatment of infectious diseases becomes difficult. Accordingly, development of a substance that enables the prevention or treatment of infectious diseases based on a new mechanism has been awaited.

It has long been fundamental knowledge that organisms exert immune function to prevent invasion and colonization by pathogenic bacteria, fungi, and viruses causing infectious diseases. For example, retinoic acid, which is an indicator of immune function, is known to be involved in differentiation of regulatory T cells, cell migration, and induction of IgA production. In addition, retinoic acid has been used for treatment of various skin conditions, such as acne, wrinkles, psoriasis, age spots, and stains (Patent Literature 1).

Citation List

Patent Literature

Patent Literature 1: JP Patent Publication No. 10-036249 A (1998)

Summary of the Invention

Problem to Be Solved by the Invention

It is an object of the present invention to provide an agent that is capable of inducing body's own biological defense function in the body based on a new mechanism.

Means for Solving the Problem

The present inventors have conducted concentrated studies in light of the above

object. As a result, they have obtained the following findings. Specifically, the present inventors have found that ingestion and/or action of a certain type of lactic acid bacteria enhances the gene expression of retinal dehydrogenase that is an indicator of the immune function, and thereby promoting the production of retinoic acid that is also an indicator of the immune function, which can solve the above-mentioned problem. Lactic acid bacteria used as an active ingredient in the present invention have been eaten for a long period of time in the form of, e.g., fermented food. Therefore, the agent for producing retinoic acid and the agent for enhancing immune function are highly safe and have low risks of side effects.

The agent for producing retinoic acid or the agent for enhancing immune function of the present invention is ingested by and/or acts on a subject, thereby more strongly inducing retinoic acid from retinal with retinal dehydrogenase in the body, and thus immune function is enhanced. According to the present invention, for example, preventive and/or therapeutic effects on infectious diseases caused by opportunistic bacteria, infectious diseases caused by pathogenic bacteria or fungi, infection with *Helicobacter pylori*, infectious diseases caused by viruses, bacterial translocation, prolonged intestinal mucosal inflammation, changes in the intestinal flora balance (e.g., disruption of intestinal flora balance), diseases induced by the aforementioned inflammations, inflammatory bowel disease, such as Crohn's disease and ulcerative colitis, excessive inflammation, and allergic symptoms can be expected. In addition, promotion of retinoic acid production by lactic acid bacteria according to the present invention, can be expected to lead to a decrease of infection risk, suppression of inflammation, and anti-allergic effects in infants and children having immature immune function, elderly persons with lowered immune function, and patients with various diseases. According to the present invention, such effects can be expected, particularly for elderly persons or aged mammals.

Specifically, the present invention includes the followings.

[1] An agent for producing retinoic acid comprising, as an active ingredient, lactic acid bacteria.

[2] The agent for producing retinoic acid according to [1], wherein the lactic acid bacteria are the genus *Lactobacillus* bacteria.

[3] The agent for producing retinoic acid according to [1] or [2], wherein the lactic acid bacteria are *Lactobacillus delbrueckii* subsp. *bulgaricus*.

[4] The agent for producing retinoic acid according to any of [1] to [3], wherein the lactic acid bacteria are of *Lactobacillus delbrueckii* subsp. *bulgaricus* strain OLL1073R-1 (Accession Number FERM BP-10741).

[5] The agent for producing retinoic acid according to any of [1] to [4], wherein the lactic acid bacteria are present at100,000,000 or more cells per unit package.

[6] An agent for enhancing immune function comprising the agent for producing retinoic acid according to any of [1] to [5].

[7] An agent for enhancing immune function comprising, as an active ingredient, lactic acid bacteria.

[8] The agent for enhancing immune function according to [7], wherein the lactic acid bacteria are of the genus *Lactobacillus*.

[9] The agent for enhancing immune function according to [7] or [8], wherein the lactic acid bacteria are present at 100,000,000 or more cells per unit package.

[10] The agent for enhancing immune function according to any of [7] to [9], wherein the lactic acid bacteria are of *Lactobacillus delbrueckii* subsp. *bulgaricus* OLL1073R-1 (FERM BP-10741).

This description includes the disclosure in Japanese Patent Application No. 2014-051065 to which the present application claims priority.

Effects of the Invention

The present invention can provide an agent that is capable of strongly inducing body's own biological defense function by promoting retinoic acid production.

Embodiments for Carrying out the Invention

Hereafter, the present invention is described in greater detail, although the present invention is not limited to the embodiments described below.

In the present invention, the term "lactic acid bacteria" collectively refers to all organisms that are taxonomically recognized as lactic acid bacteria or also all bacteria closely related thereto, such as bifidobacteria, without limitation to any species or strains. In the present invention, any lactic acid bacteria having activity of enhancing retinoic acid production, such as activity of enhancing the expression of retinal dehydrogenase gene, can be used. While lactic acid bacteria can be classified as plant-derived or animal-derived lactic acid bacteria, either plant-derived or animal-derived lactic acid bacteria can be used in the present invention. In particular, lactic acid bacteria used in the present invention are preferably lactic acid bacteria of the genus *Lactobacillus* (*Lactobacillus* bacteria), and for example, one or more strains selected from among lactic acid bacteria of the genus *Lactobacillus* are preferred, since they have a history of being ingested in the form of fermented milk such as yogurt. Also, the lactic acid bacteria of the genus *Lactobacillus* used in the present invention are more preferably *Lactobacillus delbrueckii* subsp. *bulgaricus* (*Lactobacillus delbrueckii* ssp. *bulgaricus*), which is referred to as *Lactobacillus bulgaricus*, more preferably and further preferably *Lactobacillus delbrueckii* subsp. *bulgaricus* strain OLL1073R-1 (Accession Number FERM BP-10741). The *Lactobacillus delbrueckii* subsp. *bulgaricus* strain OLL1073R-1 is deposited internationally under the Budapest Treaty on February 22, 1999 (original deposition date) with the International Patent Organism Depositary, National Institute of Technology and Evaluation (NITE-IPOD) (Room #120, 2-5-8 Kazusakamatari, Kisarazu-shi, Chiba, Japan) under Accession Number FERM BP-10741. This strain was transferred from the domestic deposit to the international deposit on November 29, 2006.

The lactic acid bacteria used in the present invention may be viable or killed cells. Also, the lactic acid bacteria used in the present invention may be frozen or lyophilized cells. In addition, the lactic acid bacteria used in the present invention may be lactic acid bacterial cells alone, or they may be in a form comprising other components (e.g., cryoprotective agents, lyophilization protective agents or the like) in addition to lactic acid bacterial cells, or they may be dispersed in various media, such as water or starch, that have been used as pharmaceutical or food materials.

The lactic acid bacteria used in the present invention may include any substance derived from bacterial cells or metabolites thereof, which is obtainable by conventional techniques, such as disrupted cells, or cellular metabolites resulting from culture or fermentation, as well as bacterial cells (viable or killed cells) themselves. For example, lactic acid bacteria such as *Lactobacillus delbrueckii* subsp. *bulgaricus* strain OLL1073R-1 (Accession Number FERM BP-10741) produce extracellular polysaccharide (EPS) during the culturing. Use of the lactic acid bacteria or lactic acid bacterial cultures containing the extracellular polysaccharide produced is within the scope of the use of the lactic acid bacteria according to the present invention.

The present invention provides an agent for producing retinoic acid (or an agent for promoting retinoic acid production) comprising, as an active ingredient, the lactic acid bacteria as described above. In a preferred embodiment, the lactic acid bacteria used in the present invention (lactic acid bacteria having activity of enhancing retinoic acid production) and the agent for producing retinoic acid comprising, as an active ingredient, the lactic acid bacteria are capable of enhancing the expression of the retinal dehydrogenase gene in animal cells, thereby promoting a conversion of retinal into retinoic acid (production of retinoic acid) catalyzed by retinal dehydrogenase in animal cells. Whether or not the lactic acid bacteria used in the present invention have activity of enhancing the expression of the retinal dehydrogenase gene can be determined as described in Examples below. That is, animal cells (preferably mesenteric lymph node cells or dendritic cells derived from mesenteric lymph node cells) are cultured in the presence of the lactic acid bacteria for a given period of time (e.g., at 37°C for 24 hours), mRNAs are then extracted from the cells, mRNAs of the retinal dehydrogenase (e.g., RALDH2) gene in the animal cells are quantified via real-time RT-PCR, and whether or not the mRNA level is significantly increased relative to that of the control (the sample obtained by culturing animal cells in the absence of the lactic acid bacteria) is determined. With the use of the agent for producing retinoic acid according to the present invention, the mRNA levels of the retinal dehydrogenase gene can typically be increased by 20% or more, and preferably 40% or more, compared with the control.

The agent for producing retinoic acid according to the present invention is capable of

imparting various physiological activities to animals (preferably mammals) or cells thereof via promoting retinoic acid production. For example, it is known that retinoic acid imparts homing specificity to the small intestine tissue to T cells or B cells in the gut-associated lymphoid organs, thereby inducing allocation of the cells (lymphocytes) in the small intestine tissue, and it induces production of IgA, which is critical for defense against infectious diseases in gut-associated lymphoid organs. Thus, retinoic acid is regarded as an indicator of immune function of the gut immune system. It is also known that retinoic acid suppresses induction of inflammation-promoting T cell differentiation and promotes induction of inflammation-suppressing regulatory T cell differentiation, thereby suppressing inflammatory responses such as autoimmune responses. Accordingly, the agent for producing retinoic acid according to the present invention potentiates defense against infectious diseases to exert a preventive effect against infectious diseases, and it can exert effects of prevention, treatment, or amelioration of inflammatory diseases, such as autoimmune diseases, via suppression of inflammatory responses. Examples of the infectious diseases include those caused by opportunistic or pathogenic bacteria, fungi, or viruses. Examples of the inflammatory diseases, such as autoimmune diseases, include, but are not limited to, inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis, and allergic symptoms. The agent for producing retinoic acid according to the present invention can be used for improving various skin conditions, such as acne, wrinkle, psoriasis, age spots, and stains, by promoting retinoic acid production in the skin. Various other physiological activities of retinoic acid are also known. A subject to be administered with the agent for producing retinoic acid according to the present invention may be an animal, preferably a mammal, and particularly preferably an elderly person or aged mammal. The agent for producing retinoic acid according to the present invention may be used to treat cells or tissues of animals, such as mammals (e.g., the gut-associated lymphoid organs or dendritic cells thereof, or skin cells such as epidermal cells or dermal cells), for the purpose of promoting retinoic acid production in cells.

The lactic acid bacteria used in the present invention are capable of enhancing immune function on the basis of the increased amount of retinoic acid produced in gut-associated lymphoid organs, and, in particular, in dendritic cells thereof. The present

invention also provides an agent for enhancing immune function comprising the agent for producing retinoic acid according to the present invention. In addition, the present invention provides an agent for enhancing immune function comprising the lactic acid bacteria as described above.

The agent for enhancing immune function according to the present invention refers to an agent capable of inducing a gene or the like in the body by having the agent ingested by and/or act on organisms such as mammals, including humans, thereby producing an immune indicator derived from the gene. In particular, the agent for enhancing immune function according to the present invention is capable of enhancing the expression of the retinal dehydrogenase gene, thereby improving the immune function of organisms such as mammals. In the present invention, the term "ingest" or "ingestion" refers to introduction of an active ingredient into the gastrointestinal tract in the bodies of organisms, including humans. In the present invention, "administer" or "administration" encompasses ingestion and parenteral application (e.g., topical application to the skin and the like). The way of administration is not particularly limited, and includes any known way of administration, such as oral, tubal, enteral, transdermal (e.g., embrocating, patching, or spraying), and subcutaneous administration.

Examples of the improvement of immune function according to the present invention include prevention and/or treatment of infectious diseases caused by opportunistic bacteria, infectious diseases caused by pathogenic bacteria or fungi, infection with *Helicobacter pylori*, infectious diseases caused by viruses, bacterial translocation, prolonged intestinal mucosal inflammation, changes in the intestinal flora balance (e.g., disruption of intestinal flora balance), diseases induced by these inflammations, and inflammatory bowel disease, such as Crohn's disease and ulcerative colitis. The examples further include prevention and/or treatment of excessive inflammation and allergic symptoms via induction of regulatory T cell differentiation. In addition, examples of the improvement of immune function also include reduction of infection risk, suppression of inflammation, and anti-allergic effects in infants and children having immature immune function, elderly persons with lowered immune function, and patients with various diseases. In particular, a subject to be administered with

the agent for enhancing immune function according to the present invention is preferably an elderly person or aged mammal.

The effects of enhancing immune function according to the present invention can be evaluated by using, as an indicator, an increase in the production amount of any substance serving as an indicator for the immune function in the body (i.e., an immune indicator). For example, retinoic acid is involved in differentiation of regulatory T cell, cell migration, and induction of IgA production, and whether or not immune function is enhanced can be evaluated by using, as an indicator, responsiveness causing retinoic acid production in the body. The immune indicator may be retinoic acid, or it may be a so-called retinoic acid precursor, such as retinal, which is activated via hydrolysis at its particular site. The immune indicator may be a different substance that induces production of an immune indicator such as retinoic acid, for example, a protein that catalyzes the synthesis of an immune indicator such as retinoic acid, or mRNA of a gene encoding the protein. That is, induction of the production of an immune indicator in the present invention may be an increased expression of a gene encoding a protein being an immune indicator in the body, which thereby expresses the immune indicator, or the production of further immune indicators via the activity of the immune indicator. While a gene encoding an immune indicator is not particularly limited, an example of a preferred gene is a retinal dehydrogenase (e.g., RALDH2) gene.

The present invention also provides an agent for prevention and/or treatment of various diseases associated with a reduction or lack of retinoic acid. Moreover, the present invention provides a method for prevention and/or treatment of various diseases associated with a reduction or lack of retinoic acid with the use of the agent for producing retinoic acid. Examples of the various diseases include any diseases, symptoms, and syndromes associated with a reduction or lack of retinoic acid, including infectious diseases caused by opportunistic bacteria, infectious diseases caused by pathogenic bacteria or fungi, infection with *Helicobacter pylori*, infectious diseases caused by viruses, bacterial translocation, prolonged intestinal mucosal inflammation, changes in the intestinal flora balance (e.g., disruption of intestinal flora balance), diseases induced by these inflammations, inflammatory bowel disease, such as Crohn's disease and ulcerative colitis, excessive inflammation, and allergic

symptoms; and any conditions suspected of the diseases, symptoms, and syndromes, regardless of before or after the filing date and/or the priority date of the present invention. Further, the present invention provides a method of whitening comprising parenterally administering the agent for producing retinoic acid according to the present invention to the skin or the like on the basis of the whitening effect disclosed in Patent Literature 1, for example (a preventive, therapeutic, or cosmetic method). A subject to be administered with the agent for producing retinoic acid according to the present invention may be an animal, such as a mammal, and particularly preferably an elderly person or aged mammal.

The agent for producing retinoic acid and the agent for enhancing immune function according to the present invention may comprise a material fermented with the aforementioned lactic acid bacteria (lactic acid bacteria having activity of enhancing retinoic acid production). The material fermented with the aforementioned lactic acid bacteria is not particularly limited, provided that the material is prepared by fermentation of materials that have been ingested by mammals, including humans, with the lactic acid bacteria. Examples of the material particularly include materials that are prepared by fermentation of milk, soybeans, adzuki beans, cereals, vegetables, meat, fish, or any mixture thereof with the aforementioned lactic acid bacteria.

An example of the material prepared by fermentation of milk with the lactic acid bacteria is one obtained by combining milk as material, a dairy product, and water as material to have a milk fat content of 3.0% by weight and a solid-not-fat content of 9.2% by weight, optionally homogenizing it to adjust milk fat globules as needed, and then heat-sterilizing the resultant under sterile conditions equivalent to keeping at 65°C for 30 minutes, cooling to 40°C to 45°C, adding a mixed starter comprising *Lactobacillus delbrueckii* subsp. *bulgaricus* (e.g., *Lactobacillus delbrueckii* subsp. *bulgaricus* strain OLL1073R-1) and *Streptococcus salivarius* subsp. *thermophiles*, as *Lactobacillus* lactic acid bacteria, fermenting it at 40°C to 45°C for 2 to 12 hours, and adjusting the acidity of lactic acid to 0.6% to 1.2% by weight. In that case, strains of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophiles* are not particularly limited, and lactic acid bacteria other than the two bacterial species (e.g., *Lactobacillus*, *Bifidobacterium*, *Lactococcus*, or *Leuconostoc*) and

other microorganisms, materials, or additives useful for fermentation or health may be optionally added. The term "milk as material" used herein refers to, but is not limited to, so-called fresh milk or raw milk from a cow, goat, sheep, buffalo, or the like or reconstituted milk having the same composition as the fresh milk or raw milk, and also may be one prepared with the addition (or enrichment) of milk fat and/or a solid-not-fat (e.g., milk protein, lactose, or ash), low-fat milk or non-fat milk prepared by removal of milk fat, or lactose-removed milk or lactose-degraded milk for human individuals who are less tolerant to lactose. In addition, the milk as material may also be demineralizated milk prepared for patients who are restricted from ingesting minerals such as sodium and/or for moderation of saltiness sensed during ingestion, or calcium-enriched milk prepared for patients who are required to ingest minerals such as calcium. When such materials fermented with lactic acid bacteria are used, the agent for producing retinoic acid and the agent for enhancing immune function according to the present invention may comprise other lactic acid bacteria, such as *Streptococcus* bacteria, in addition to the aforementioned lactic acid bacteria having activity of enhancing retinoic acid production.

The number of the lactic acid bacteria contained in the agent for producing retinoic acid and the agent for enhancing immune function according to the present invention is not particularly limited, provided that the agents contain lactic acid bacteria. In the agents, the lactic acid bacteria are preferably present at 100,000,000 or more cells, more preferably 500,000,000 or more cells, and further preferably 1,000,000,000 or more cells, per unit package. While the effects of the present invention can be expected to grow stronger as the number of the lactic acid bacteria is increased, its typical upper limit is 10,000,000,000,000 cells from the viewpoint of the growth of general lactic acid bacteria.

The weight of the agent for producing retinoic acid and the agent for enhancing immune function according to the present invention per unit package is not particularly limited. When lactic acid bacteria are present at 100,000,000 or more cells per 1 g of the agent for producing retinoic acid and the agent for enhancing immune function, the weight of the agent for producing retinoic acid and the agent for enhancing immune function is preferably 10 g to 500 g, more preferably 25 g to 250 g, further preferably 50 g to 200 g, and

still further preferably 75 g to 150 g per unit package. The term "unit package" used in the present invention refers not only to a packed bag, box, or container itself, but also a single-dose unit pack form or one day-dose unit pack form in such a packed bag, box, or container.

When the agent for producing retinoic acid or the agent for enhancing immune function according to the present invention is to be administered orally, for example, the lactic acid bacteria or materials fermented with the lactic acid bacteria may be administered (or ingested) in unchanged form. Alternatively, the lactic acid bacteria or materials may be administered (or ingested) in the form of, for example, tablets, granules, powdered formulations, capsules, or powders. The agent for producing retinoic acid and the agent for enhancing immune function according to the present invention may be administered parenterally to the skin or the like in the form of a solution, emulsion, gel, powder, or cream (e.g., via transdermal application by embrocating, patching, or spraying).

The effect of enhancing immune function in the present invention is exerted in the gastrointestinal tract in a body after ingestion of the lactic acid bacteria of the present invention. Examples of gastrointestinal tracts include a set of organs, including oral cavity, throat, esophagus, stomach, duodenum, small intestine, large intestine, cecum, and up to anus. While representative parts of gastrointestinal tract are illustrated therein, for example, any parts of gastrointestinal tracts that are known and/or within the scope of the general technical knowledge in the art, such as colon (a lower part of small intestine), ileum, jejunum (an upper part of small intestine), epigastric gastrointestinal tract, hypogastric gastrointestinal tract, upper large intestine, and lower large intestine, also belong to the scope of the "gastrointestinal tract" according to the present invention.

The effects of enhancing immune function according to the present invention can be exerted at various sites inside and outside the body after making the lactic acid bacteria of the present invention act (e.g., via embrocating, spraying, or injection). Examples of sites inside and outside the body include skin, hair, eyeballs, nasal mucosa, external ear, middle ear, nails, and surfaces of various organs.

The agent for enhancing immune function according to the present invention exerts effects of enhancing immune function on various animals, and preferably mammals, including

humans. Accordingly, the present invention provides a method for treatment and/or prevention of various diseases comprising making an animal ingest the agent for enhancing immune function or administering the agent for enhancing immune function to an animal. In the present invention, mammals may be any known livestock animals, pet animals, ornamental animals, wild animals or the like that are classified as mammals. Examples thereof include humans, cows, pigs, sheep, dolphins, whales, tigers, lions, cheetahs, hippopotamuses, giraffes, camels, alpacas, dogs, cats, monkeys, foxes, raccoon dogs, bears, squirrels, sea bears, sea lions, giant pandas, wild boars, deer, horses, orangutans, and kangaroos. The agent for enhancing immune function of the present invention can also be used for enhancing the immune function of animals having gastrointestinal tracts other than mammals, such as insects, reptiles, birds, fish, amphibians, and mollusks. The various diseases include infectious diseases caused by opportunistic bacteria, infectious diseases caused by pathogenic bacteria or fungi, infection with *Helicobacter pylori*, bacterial translocation, prolonged intestinal mucosal inflammation, changes in the intestinal flora balance (e.g., disruption of intestinal flora balance), diseases induced by these inflammations, inflammatory bowel disease, such as Crohn's disease and ulcerative colitis, excessive inflammation, and allergic symptoms, although they are not particularly limited as long as the diseases can be treated, prevented, or ameliorated by inducing production of immune indicators such as retinoic acid.

Examples

Hereafter, the present invention is described in greater detail with reference to the examples, although the present invention is not limited to these examples.

12-month-old to 13-month-old BALB/c mice (aged mice) were used as subjects. Mesenteric lymph node cells and CD11c+ dendritic cells derived from mesenteric lymph nodes were removed from the small intestines of the mice.

The removed mesenteric lymph node cells or CD11c+ dendritic cells derived from mesenteric lymph nodes were separately co-cultured with *Lactobacillus delbrueckii* subsp. *bulgaricus* strain OLL1073R-1 (accession number FERM BP-10741) at 37°C in a 96-well

plate onto which killed cells of the strain OLL1073R-1 had been seeded at 100 μg of cell weight/ml. The mesenteric lymph node cells were seeded at 500,000 cells/well, and the CD11c+ dendritic cells derived from mesenteric lymph nodes were seeded at 100,000 cells/well. After the completion of co-culture for 24 hours, the mesenteric lymph node cells or CD11c+ dendritic cells were recovered from the resulting culture, mRNAs were extracted from the recovered cells in accordance with a conventional technique, and the expression level of mRNA of the retinal dehydrogenase RALDH2 (Aldh1a2) gene (GenBank Accession Number NM_009022) was measured by real-time RT-PCR method.

As a control, the culture and measurement of the gene expression level were carried out in the same manner as described above, except that *Lactobacillus delbrueckii* subsp. *bulgaricus* strain OLL1073R-1 (Accession Number FERM BP-10741) was not seeded.

On the basis of the expression levels of the RALDH2 gene obtained via co-culture with lactic acid bacteria, the gene expression level ratios of these cells were determined relative to the gene expression level of the control set as 1.0.

As a result, the expression level of mRNA of the RALDH2 gene in the mesenteric lymph node cells was found to have increased to 3.05 ± 0.56 when co-cultured with *Lactobacillus delbrueckii* subsp. *bulgaricus* strain OLL1073R-1 (Accession Number FERM BP-10741), in comparison with the control (1.00 ± 0.52; the value following the "±" in the calculated value indicates the standard deviation (S.D.)). Also, the expression level of mRNA of the RALDH2 gene in the CD11c+ dendritic cells derived from mesenteric lymph nodes was found to have increased to 7.91 ± 1.43 when co-cultured with *Lactobacillus delbrueckii* subsp. *bulgaricus* strain OLL1073R-1 (Accession Number FERM BP-10741), in comparison with the control (1.00 ± 0.62). It is to be noted that the value following the "±" in the calculated value indicates the standard deviation (S.D.).

The results demonstrate that *Lactobacillus delbrueckii* subsp. *bulgaricus* OLL1073R-1 (Accession Number FERM BP-10741) significantly enhanced the expression level of the retinal dehydrogenase RALDH2 gene in the cells. Retinoic acid is generated via oxidation of retinal, and retinal dehydrogenase RALDH2 catalyzes the retinal oxidation reaction. Accordingly, the enhancement of the expression of the retinal dehydrogenase gene can

promote retinoic acid production in the cells.

The results demonstrate that administration of lactic acid bacteria of *Lactobacillus delbrueckii* subsp. *bulgaricus* strain OLL1073R-1 causes an increase in the amount of retinoic acid in the body that can serve as an indicator of enhanced immune function.

Industrial Applicability

The agent for producing retinoic acid according to the present invention can be used for increasing the retinoic acid production in cells. The present invention can provide a novel agent for enhancing immune function that induces human's own biological defense function with greater safety and fewer side effects. Ingestion or action of the agent for producing retinoic acid and the agent for enhancing immune function according to the present invention is capable of inducing retinoic acid as an immune indicator in the body. Thus, the agent can be used for enhancement of the defenses against various infectious diseases and treatment, prevention, or amelioration of inflammation reactions.

All publications, patents, and patent applications cited herein are incorporated herein by reference in their entirety.

CLAIMS

1. An agent for producing retinoic acid comprising, as an active ingredient, lactic acid bacteria.

2. The agent for producing retinoic acid according to claim 1, wherein the lactic acid bacteria are *Lactobacillus* bacteria.

3. The agent for producing retinoic acid according to claim 1 or 2, wherein the lactic acid bacteria are *Lactobacillus delbrueckii* subsp. *bulgaricus*.

4. The agent for producing retinoic acid according to any one of claims 1 to 3, wherein the lactic acid bacteria are of *Lactobacillus delbrueckii* subsp. *bulgaricus* strain OLL1073R-1 (Accession Number FERM BP-10741).

5. The agent for producing retinoic acid according to any one of claims 1 to 4, wherein the lactic acid bacteria are present at 100,000,000 or more cells per unit package.

6. An agent for enhancing immune function comprising the agent for producing retinoic acid according to any one of claims 1 to 5.