

[DESCRIPTION]

[Title of Invention]

REGULATORY T CELL INDUCTION PROMOTER, THERAPEUTIC OR PROPHYLACTIC AGENT FOR AUTOIMMUNE DISEASE, THERAPEUTIC OR PROPHYLACTIC AGENT FOR ALLERGIC DISEASE, FOOD COMPOSITION FOR PREVENTING AUTOIMMUNE DISEASE, FOOD COMPOSITION FOR PREVENTING ALLERGIC DISEASE, FOOD COMPOSITION FOR IMPROVING ALLERGIC CONDITION, FOOD COMPOSITION FOR MAINTAINING HEALTHY IMMUNE FUNCTION, TREATMENT OR PREVENTION METHOD FOR AUTOIMMUNE DISEASE OR ALLERGIC DISEASE, AND SCREENING METHOD FOR SUBSTANCE PROMOTING INDUCTION OF REGULATORY T CELL

[Technical Field]

[0001]

The present disclosure of the present application relates to a regulatory T cell induction promoter, a therapeutic or prophylactic agent for an autoimmune disease, a therapeutic or prophylactic agent for an allergic disease, a food composition for preventing an autoimmune disease, a food composition for preventing an allergic disease, a food composition for improving an allergic condition, a food composition for maintaining a healthy immune function, a treatment or prevention method for an autoimmune disease or an allergic disease, and a screening method for substances promoting induction of regulatory T cells.

[Background Art]

[0002]

T cells are a type of cells called lymphocytes of white blood cells circulating in the blood and serve as an important cell population that can be called the command

center of mechanisms (immune responses) for protecting living bodies from foreign substances. Immune responses are important for eliminating harmful pathogens but involve inflammatory reactions and thus can attack one's own body when not coming to an end at an appropriate time. Thus, immune systems are provided with various mechanisms for suppressing and regulating immune responses.

[0003]

For example, when immune responses end, programmed cell death called apoptosis is induced for a major part of antigen-specific immune cells, or immune cells become active that suppress immune reactions called regulatory T cells, which may be also referred to as "Treg". Further, regulatory T cells also play an important role in maintaining immune cells that recognize body's own proteins (self-antigens) in an inactive state (immune tolerance). Differentiation and functions of T cells are regulated by intracellular energy metabolism. Among others, glycolytic pathways are important for fate selection of effector T cells or regulatory T cells.

[0004]

Monosaccharides, which are the smallest functional units of sugars, exist in nature in various forms such as sugar chains, glycoproteins, and nucleic acids and are responsible for the fundamental aspect of life activities. In contrast, rare sugars are defined as monosaccharides and their derivatives that exist in small quantities in nature, and while about 50 types of rare sugars exist, the ratio thereof relative to the sugars in nature is considered to be only around 0.1%. While having sweetness in common, these rare sugars have a property of being difficult to be utilized as energy and thus have been called "underperforming sugar".

[0005]

In recent years, however, it has been newly reported that monosaccharides

including rare sugars have some physiological effects. For example, it has been reported that D-mannose, which is a monosaccharide, suppresses autoimmune diseases (see Non-Patent Literature 2) via induction of regulatory T cells known to suppress immune responses by involving secretion of IL-10 or TGF- β , which is an inhibitory cytokine, or by involving suppression of dendritic cell maturation via intercellular interactions via CTLA-4 (see Non-Patent Literature 1).

[Citation List]

[0006]

Non-Patent Literature 1: Sakaguchi S. et al., "Regulatory T cells and immune tolerance", *Cell*.2008;133:775-787.

Non-Patent Literature 2: Zhang D. et al., "D-mannose induces regulatory T cells and suppresses immunopathology", *Nat Med*.2017;23:1036-1045.

[Summary of Invention]

[Technical Problem]

[0007]

As described above, it has been reported that D-mannose suppresses autoimmune diseases via induction of regulatory T cells. There are various mechanisms by which substances administered to living bodies affect cells or the like. Thus, search for substances other than D-mannose is also desired for substances that induce regulatory T cells.

[0008]

The disclosure in the present application has been made to solve the problem described above. The inventors have carried out intensive research and thereby found that D-sorbose and D-allose, which are rare sugars, promote induction of regulatory T cells.

[0009]

That is, the disclosure of the present application intends to provide a regulatory T cell induction promoter containing D-sorbose and/or D-allose as an active ingredient. Further, the disclosure of the present application intends to provide a therapeutic or prophylactic agent for an autoimmune disease, a therapeutic or prophylactic agent for an allergic disease, a food composition for preventing an autoimmune disease, a food composition for preventing an allergic disease, a food composition for improving an allergic condition, a food composition for maintaining a healthy immune function, a treatment or prevention method for an autoimmune disease or an allergic disease, and a screening method for substances promoting induction of regulatory T cells.

[Solution to Problem]

[0010]

- (1) A regulatory T cell induction promoter containing D-sorbose and/or D-allose as an active ingredient.
- (2) The regulatory T cell induction promoter according to (1) above including only D-sorbose as the active ingredient.
- (3) The regulatory T cell induction promoter according to (1) above including only D-allose as the active ingredient.
- (4) The regulatory T cell induction promoter according to (1) above,
wherein the D-sorbose is a D-sorbose derivative, and
wherein the D-allose is a D-allose derivative.
- (5) The regulatory T cell induction promoter according to any one of (1) to (4) above used for treatment or prevention against an autoimmune disease or an allergic disease via induction of a regulatory T cell.

- (6) The regulatory T cell induction promoter according to any one of (1) to (4) above, wherein a regulatory T cell is induced by inhibition of hexosamine pathway that is an intracellular sugar metabolism pathway.
- (7) The regulatory T cell induction promoter according to any one of (1) to (4) above used as a component of a pharmaceutical composition.
- (8) The regulatory T cell induction promoter according to any one of (1) to (4) above used as a component of a food composition.
- (9) A therapeutic or prophylactic agent for an autoimmune disease, the therapeutic or prophylactic agent containing at least one selected from a group consisting of D-sorbose, a D-sorbose derivative, D-allose, and a D-allose derivative as an active ingredient.
- (10) The therapeutic or prophylactic agent for an autoimmune disease according to (9) above, wherein the autoimmune disease is type 1 diabetes mellitus.
- (11) A therapeutic or prophylactic agent for an allergic disease, the therapeutic or prophylactic agent containing at least one selected from a group consisting of D-sorbose, a D-sorbose derivative, D-allose, and a D-allose derivative as an active ingredient.
- (12) A food composition for preventing an autoimmune disease, the food composition containing at least one selected from a group consisting of D-sorbose, a D-sorbose derivative, D-allose, and a D-allose derivative as an active ingredient.
- (13) A food composition for preventing an allergic disease, the food composition containing at least one selected from a group consisting of D-sorbose, a D-sorbose derivative, D-allose, and a D-allose derivative as an active ingredient.
- (14) A food composition for improving an allergic condition, the food composition containing at least one selected from a group consisting of D-sorbose, a D-sorbose

derivative, D-allose, and a D-allose derivative as an active ingredient.

(15) A food composition for maintaining a healthy immune function, the food composition containing at least one selected from a group consisting of D-sorbose, a D-sorbose derivative, D-allose, and a D-allose derivative as an active ingredient.

(16) A treatment or prevention method for an autoimmune disease or an allergic disease, the method comprising a step of:

administering an effective amount of at least one selected from a group consisting of D-sorbose, a D-sorbose derivative, D-allose, and a D-allose derivative to an animal (excluding human), the effective amount having an effect of promoting induction of regulatory T cells.

(17) A screening method for substances promoting induction of a regulatory T cell, the method comprising steps of:

(a) bringing a subject substance and a regulatory T cell into contact with each other;

(b) measuring an induction rate of the regulatory T cell brought into contact with the subject substance and comparing the measured induction rate with an induction rate of a regulatory T cell of a control group not brought into contact with the subject substance; and

(c) selecting a subject substance that promotes induction of a regulatory T cell based on a result of the comparison in the step (b).

[Advantageous Effects of Invention]

[0011]

Induction of regulatory T cells is promoted by the regulatory T cell induction promoter containing D-sorbose and/or D-allose as the active ingredient disclosed in the present application. The D-sorbose and/or D-allose can also be used for a

therapeutic or prophylactic agent for autoimmune diseases, a therapeutic or prophylactic agent for allergic diseases, a food composition for preventing autoimmune diseases, a food composition for preventing allergic diseases, a food composition for improving allergic conditions, a food composition for maintaining healthy immune functions, and a treatment or prevention method for autoimmune diseases or allergic diseases. Further, the use of the screening method for substances promoting induction of regulatory T cells disclosed in the present application is expected to lead to successful search for substances other than D-sorbose and/or D-allose that promote induction of regulatory T cells.

[Brief Description of Drawings]

[0012]

[FIG. 1]

FIG. 1 is a diagram illustrating results of evaluation ex vivo of the Treg induction ability of respective rare sugars.

[FIG. 2]

FIG. 2 is a diagram illustrating results of investigation into effects of long-term sustained oral administration of D-sorbose on living bodies.

[FIG. 3]

FIG. 3 is a diagram illustrating results of evaluation of Treg induction ability of D-sorbose using steady-state C57BL/6J mice.

[FIG. 4]

FIG. 4 is a diagram illustrating results obtained by harvesting mouse-derived splenic cells administered in advance with D-sorbose in order to emulate an immune activation state and stimulating spleen cells with PMA/ionomycin.

[FIG. 5]

FIG. 5 is a diagram illustrating evaluation of Treg induction ability and effects thereof within living bodies using NOD/ShiJcl mice representing a spontaneous onset model of autoimmune diabetes mellitus.

[FIG. 6]

FIG. 6 is a diagram illustrating that D-sorbose prevents onset of autoimmune diabetes mellitus.

[FIG. 7]

FIG. 7 is a diagram illustrating analysis of ratios of Treg in respective tissues for immunologically confirming preventive effects against diabetes mellitus onset due to D-sorbose.

[FIG. 8]

FIG. 8 is a diagram illustrating analysis of ratios of Treg in respective tissues for immunologically confirming preventive effects against diabetes mellitus onset due to D-sorbose.

[FIG. 9]

FIG. 9 is diagram illustrating results of evaluation of differences in sensitivity to D-sorbose between Treg and other T cells in terms of cell viability.

[FIG. 10]

FIG. 10 is a diagram illustrating that D-sorbose inhibits intracellular sugar metabolism and thereby induces Treg.

[FIG. 11]

FIG. 11 is a diagram illustrating results of evaluation of the Treg induction ability using inhibitors of enzymes catalyzing metabolism pathways branched from glucose-6-phosphate out of sugar metabolism.

[FIG. 12]

FIG. 12 is a diagram illustrating results of investigation into effects of long-term sustained oral administration of D-allose on living bodies.

[FIG. 13]

FIG. 13 is a diagram illustrating analysis of the ratio of Treg in mediastinal lymph nodes for confirming that D-sorbose prevents onset of OVA-induced allergic bronchitis.

[FIG. 14]

FIG. 14 is a diagram illustrating evaluation of lungs for confirming that D-sorbose prevents onset of OVA-induced allergic bronchitis.

[Description of Embodiments]

[0013]

A regulatory T cell induction promoter disclosed in the present application (hereafter, which may be also referred to as “induction promoter”) will be described below in detail. Note that, in the present specification, numerical values, numerical ranges, and qualitative expressions (for example, expressions such as “identical”, “same”, or the like) represent the numerical values, numerical ranges, and properties including errors generally accepted in this technical field.

[0014]

[Embodiment of the regulatory T cell induction promoter]

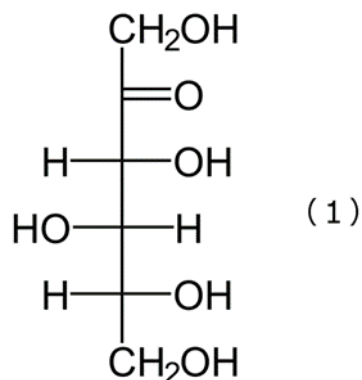
The induction promoter contains D-sorbose and/or D-allose as the active ingredient.

[0015]

D-sorbose is an epimer at the C4 position of D-fructose and, industrially, produced via D-tagatose by using D-tagatose isomerase from galactitol as a raw material resulted from natural monosaccharide D-galactose being reduced. The

structural formula of D-sorbose is expressed by Formula (1).

[Formula 1]



[0016]

Although there are few reports about physiological effects of D-sorbose, some effects have been reported that inhibit sucrase activity in small intestines and suppress blood glucose elevation and insulin secretion (Oku T. et al., "D-sorbose inhibits disaccharidase activity and demonstrates suppressive action on postprandial blood levels of glucose and insulin in the rat," *Nutr Res.* 2014; 34: 961-967.)

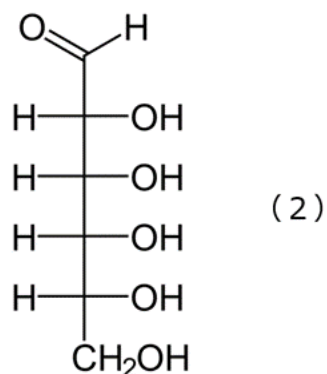
[0017]

D-sorbose has sweetness that is about 70 percent of the sweetness of sucrose (common sugar). It has been reported that, while 25% of orally taken D-sorbose is absorbed in the intestinal tract, 24% of the same is subjected to fermentation utilization by intestinal bacteria, and the remaining 51% of the same is excreted in feces, the 25% absorbed in the intestinal tract is also entirely excreted in urine (Machiko Kitagawa et al., "Evaluation of Absorption and Fermentative Utilization of D-Mannose, D-Sorbitol, and D-allose in Healthy Adults," *Luminacoids Research: Journal of the Japanese Society for Dietary Fiber.* 2018; 22: 75-82), and it is thus considered that such D-sorbose is not used as energy.

[0018]

D-allose is an epimer at the C3 position of glucose. The structural formula of D-allose is expressed in Formula (2).

[Formula 2]



[0019]

It is known that D-allose increases Thioredoxin interacting protein (TXNIP), negatively regulates expression of Glucose transporter 1 (GLUT1), and thereby suppresses enhanced sugar metabolism in tumor cells (Yamaguchi F. et al., "Rare sugar D-allose induces specific up-regulation of TXNIP and subsequent G1 cell cycle arrest in hepatocellular carcinoma cells by stabilization of p27kip1," *Int J Oncol.* 2008; 32: 377-385).

[0020]

Commercially available products can be used as D-sorbose and D-allose.

[0021]

D-sorbose and D-allose may be derivatives. Note that, in the present specification, "D-sorbose derivative" means those that may release "D-sorbose" by being metabolized within a living body. Further, "D-allose derivative" means those that may release "D-allose" by being metabolized within a living body. When metabolized within a living body, "D-sorbose derivative" and "D-allose derivative" will be changed to "D-sorbose" and "D-allose" within the living body, respectively.

Therefore, although “D-sorbose derivative” and “D-allose derivative” included in the induction promoter differ from “D-sorbose” and “D-allose” as substances, respectively, “D-sorbose derivative” and “D-allose derivative” achieve the similar effects as “D-sorbose” and “D-allose” after administered to a living body.

[0022]

The D-sorbose derivative is not particularly limited as long as it may release D-sorbose when metabolized within a living body. Further, the D-allose derivative is not particularly limited as long as it may release D-allose when metabolized within a living body.

[0023]

The D-sorbose derivative and the D-allose derivative may be, but not limited to, examples as follows.

(i) Polymers in which two or more units, preferably two to six units, and more preferably two to four units of D-sorbose are polymerized or polymers in which two or more units, preferably two to six units, and more preferably two to four units of D-allose are polymerized.

(ii) Sugar adducts in which one or more units, preferably one to six units, and more preferably one to four units of glucose are polymerized to D-sorbose or sugar adducts in which one or more units, preferably one to six units, and more preferably one to four units of glucose are polymerized to D-allose.

[0024]

While the polymers of (i) with two or more units of D-sorbose being polymerized or the polymers with two or more units of D-allose being polymerized may include other components as long as their constituent units include D-sorbose or D-allose as the main component, respectively, polymers having only D-sorbose or only

D-allose as their constituent units are more preferable.

[0025]

Specific examples of D-sorbose sugar adducts of (ii) may be, for example, monoglucosyl- α -D-sorbose, α -maltosyl- α -D-sorbose, α -maltotriosyl- α -D-sorbose, or α -maltotetraosyl- α -D-sorbose.

Specific examples of D-allose sugar adducts of (ii) may be, for example, monoglucosyl- α -D-allose, α -maltosyl- α -D-allose, α -maltotriosyl- α -D-allose, or α -maltotetraosyl- α -D-allose.

[0026]

Note that, in the following description except for the Examples, reference to "D-sorbose" means "D-sorbose" and "D-sorbose derivative" unless otherwise specified. Similarly, reference to "D-allose" means "D-allose" and "D-allose derivative" unless otherwise specified. The "D-sorbose" and "D-allose" in the Examples mean monosaccharide D-sorbose and monosaccharide D-allose, respectively.

[0027]

The dose form of the induction promoter according to the embodiment may be, for example, tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as solids or in liquid media), ointments, gelatin soft and hard capsules, suppositories, eye drops, nasal drops, patches, sterile injectable solutions, sterile powder fillings, and the like.

[0028]

Further, the induction promoter according to the embodiment may include a carrier, an excipient, and a diluent, which may be, for example, lactose, dextrose, sucrose, sorbitol, mannitol, starch, gum acacia, calcium phosphate, alginate, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose,

water, phosphate-buffered saline (PBS), syrup, methylcellulose, methyl and propyl oxybenzoates, talc, magnesium stearate, and mineral oil. Furthermore, a lubricant, a humectant, an emulsifier and a suspending agent, a preservative, a sweetener, or a flavoring agent may be added thereto. Further, when the induction promoter according to the embodiment is administered by injection, a known drug such as a water-soluble hyaluronic acid, a steroid, an anesthetic, an anti-inflammatory agent, or the like may be combined therewith in accordance with an injection site.

[0029]

The field of rare sugars has been less studied so far. The dose of the induction promoter according to the embodiment (the dose of D-sorbose and/or D-allose) can be set to an appropriate quantity through safety experiments using animals. For example, the lower limit in kg/day of D-sorbose may be, but not limited to, 1 mg or greater, 2 mg or greater, 3 mg or greater, 4 mg or greater, 5 mg or greater, 6 mg or greater, 7 mg or greater, 8 mg or greater, 9 mg or greater, 10 mg or greater, or the like. Further, the upper limit in kg/day of D-sorbose may be, for example, 50 mg or less, 45 mg or less, 40 mg or less, 35 mg or less, 30 mg or less, 25 mg or less, 20 mg or less, 15 mg or less, or the like. Further, the dose may be expressed by a predetermined range such as, for example, 1 mg to 50 mg/kg/day, 3 mg to 40 mg/kg/day, 5 mg to 30 mg/kg/day, 7 mg to 20 mg/kg/day, or the like by combining any numerical values indicated as examples.

[0030]

The lower limit in kg/day of D-allose may be, for example, 3 mg or greater, 4 mg or greater, 5 mg or greater, 6 mg or greater, 7 mg or greater, 8 mg or greater, 9 mg or greater, 10 mg or greater, 11 mg or greater, 12 mg or greater, or the like. The upper limit in kg/day of D-allose may be, for example, 50 mg or less, 45 mg or less, 40

mg or less, 35 mg or less, 30 mg or less, 25 mg or less, 20 mg or less, 15 mg or less, or the like. Further, the dose may be expressed by a predetermined range such as, for example, 3 mg to 50 mg/kg/day, 5 mg to 40 mg/kg/day, 7 mg to 30 mg/kg/day, 10 mg to 20 mg/kg/day, or the like by combining any numerical values indicated as examples.

[0031]

In the dose listed above, the dose of “D-sorbose derivative” or “D-allose derivative” means a quantity converted into the quantity of the D-sorbose or D-allose content in respective derivatives.

[0032]

As illustrated in the Examples described later, D-sorbose prevented onset of autoimmune diabetes via induction of differentiation into Treg. Furthermore, it was indicated that D-sorbose inhibited intracellular sugar metabolism and that, also when these metabolism pathways were inhibited by some existing inhibitors, differentiation into Treg was induced in the same manner as with D-sorbose.

[0033]

Autoimmune diseases and allergic diseases caused by excessive activation of immune systems in living bodies have been increasing year by year. To treat these diseases, however, symptomatic therapy using immunosuppressants and antihistamines are mainly employed, and there are many instances where resistance to these treatments are exhibited. Thus, establishment of a novel treatment method has been desired. Accordingly, since the induction promoter disclosed in the present application induces Treg that is an immune tolerance mechanism inherently provided to living bodies, the induction promoter can be used for a novel treatment method for these hyperactive immune disorders.

[0034]

The autoimmune diseases that can be treated or prevented by using the induction promoter disclosed in the present application may be rheumatoid arthritis (anti-CCP antibody, MMP-3, rheumatoid factor), systemic lupus erythematosus (anti-ds-DNA antibody, anti-Sm antibody, anti-nuclear antibody, others), systemic scleroderma (anti-centromere antibody, anti-Scl-70/topoisomerase I antibody, anti-RNA polymerase III antibody, anti-nuclear antibody, others), polymyositis/dermatomyositis (anti-Jo-1 antibody, anti-ARS antibody, others), mixed connective tissue disease (anti-U1-RNP antibody), Sjögren's syndrome (anti-SS-A antibody, anti-SS-B antibody, anti-nuclear antibody, others.), antiphospholipid antibody syndrome (lupus anticoagulant, anticardiolipin antibody, others), microscopic polyangiitis (MPO-ANCA), granulomatosis with polyangiitis (PR3-ANCA), eosinophilic granulomatosis with polyangiitis (MPO-ANCA), or crescentic glomerulonephritis (MPO-ANCA).

[0035]

Further, organ-specific autoimmune diseases may be Graves' disease (anti-thyroid receptor antibodies, others), Hashimoto's thyroiditis (anti-thyroglobulin antibodies, anti-peroxidase antibodies, anti-thyroid microsomal antibodies, others), autoimmune hepatitis (anti-smooth muscle antibodies, anti-nuclear antibodies, others), primary biliary cholangitis (anti-mitochondrial antibodies, anti-nuclear antibodies, elevated IgM levels, others), autoimmune pancreatitis (anti-nuclear antibodies, elevated IgG levels, others), Type 1 diabetes mellitus (anti-islet of Langerhans cell antibodies, others), myasthenia gravis (anti-acetylcholine receptor antibodies), Guillain-Barré syndrome (anti-ganglioside antibodies), multiple sclerosis (anti-aquaporin-4 antibody, others), Goodpasture syndrome (anti-GBM antibody), autoimmune hemolytic anemia (anti-erythrocyte antibody, others), megaloblastic

anemia (anti-intrinsic factor antibody, others), idiopathic thrombocytopenic purpura (anti-platelet antibody), or the like.

[0036]

In addition to the above, primary sclerosing cholangitis, polyarteritis nodosa, Takayasu's arteritis, giant cell arteritis, polymyalgia rheumatica, adult-onset Still's disease, Behçet's disease, sarcoidosis, ulcerative colitis, Crohn's disease, or the like are also considered as some types of autoimmune diseases though no autoantibody specific thereto has been discovered.

[0037]

The finding that Treg is involved in autoimmune diseases in general is described in detail by Tara Fiyouzi et. al., "Enhancing Regulatory T Cells to Treat Inflammatory and Autoimmune diseases", *Int. J. Mol. Sci.* 2023, 24 (9), 7797; <https://doi.org/10.3390/ijms24097797>. Therefore, D-sorbose and/or D-allose that promotes induction of Treg is useful for autoimmune diseases in general.

[0038]

Further, allergic diseases that can be treated or prevented by using the induction promoter disclosed in the present application may be bronchial asthma, atopic dermatitis, allergic rhinitis, hay fever, food allergies, allergic conjunctivitis, or the like.

[0039]

The finding that Treg is involved in asthma and airway allergies is described by Jing Zhang et. al., "Regulatory T Cells, a Viable Target Against Airway Allergic Inflammatory Responses in Asthma", *Front. Immunol.*, 10 June 2022, Sec. T Cell Biology, Volume 13-2022 |<https://doi.org/10.3389/fimmu.2022.902318>.

[0040]

The finding that Treg is involved in food allergies is described by Guirong Liu, et. al., “The Role of Regulatory T Cells in Epicutaneous Immunotherapy for Food Allergy”, *Front. Immunol.*, 08 July 2021, Sec. T Cell Biology, Volume 12-2021 |<https://doi.org/10.3389/fimmu.2021.660974>.

[0041]

The finding that Treg is involved in atopic dermatitis is described by Masutaka Furue, et. al., “Aryl Hydrocarbon Receptor in Atopic Dermatitis and Psoriasis”, *Int. J. Mol. Sci.* 2019, 20 (21), 5424; <https://doi.org/10.3390/ijms20215424>.

[0042]

The finding that Treg is involved in hay fever is described by Iris Bellinghausen, et. al., “Current Strategies to Modulate Regulatory T Cell Activity in Allergic Inflammation”, *Front. Immunol.*, 26 May 2022, Sec. Vaccines and Molecular Therapeutics, Volume 13-2022 |<https://doi.org/10.3389/fimmu.2022.912529>.

[0043]

Therefore, D-sorbose and/or D-allose that promotes induction of Treg is useful for allergic diseases in general.

[0044]

The induction promoter disclosed in the present application can induce regulatory T cells in a topical site in an immunologically activated state (for example, a topical site being inflamed), for example. The topical site described above may be, for example, a nose, an eye, a throat, an oral cavity, a pancreas, a respiratory system (a trachea, a lung, or the like), skin, a digestive tract (an esophagus, a stomach, a duodenum, a small intestine, a large intestine), a brain, a liver, a gallbladder, a spleen, a kidneys, a thymus, a thyroid, an adrenal gland, a muscle, a lymphatic vessel, a blood vessel, a heart, a testis, a uterus, a ovary, a prostate, or the like.

[0045]

The induction promoter according to the embodiment can be used as a component of pharmaceutical compositions or a component of food compositions.

[0046]

First, cases where the induction promoter according to the embodiment is used as a component of food compositions will be described. As long as the induction promoter according to the embodiment is included as a component of a food composition, other components of the food composition are not particularly limited.

[0047]

The food composition can be of any form of a solid, a gel, or a liquid, which may be, for example, fermented dairy products such as lactic acid bacteria beverages, various processed food and beverage products, dried powders, tablets, capsules, granules, or the like, and furthermore, can be various beverages, yogurt, liquid foods, jellies, candies, retort pouch foods, tablet candies, cookies, castella cakes, breads, biscuits, chocolates, or the like. Further, the food composition may be improved in nutritional balance, flavor, or the like by adding an additive such as a sugar, a protein, a fat, a vitamin, a mineral, a flavoring, carbohydrate, a sweetener, a flavor enhancer, a colorant, a texture modifier, or the like or a mixture thereof. Note that the food composition represents the concept including drinking water.

[0048]

Although the intake period of the food composition is not particularly limited, intake for a long term is preferable in terms of prevention or suppression of progression. The intake period may be, for example, but not limited to, one week or longer, two weeks or longer, three weeks or longer, one month or longer, three months or longer, six months or longer, one year or longer, or the like. The lower limit of D-

sorbose (mass %) included in the food composition may be, for example, 1% or greater, 2% or greater, 3% or greater, 4% or greater, 5% or greater, 6% or greater, 7% or greater, 8% or greater, 9% or greater, 10% or greater, or the like. On the other hand, the upper limit of D-sorbose (mass %) included in the food composition may be, for example, 30% or less, 29% or less, 28% or less, 27% or less, 26% or less, 25% or less, 24% or less, 23% or less, 22% or less, 21% or less, 20% or less, or the like. Further, the content thereof may be expressed by a predetermined range such as, for example, 1% to 30%, 3% to 25%, 5% to 20%, or the like by combining any numerical values indicated as examples.

[0049]

The lower limit of D-allose (mass %) included in the food composition may be, for example, 1% or greater, 2% or greater, 3% or greater, 4% or greater, 5% or greater, 6% or greater, 7% or greater, 8% or greater, 9% or greater, 10% or greater, or the like. On the other hand, the upper limit of D-allose (mass %) included in the food composition may be, for example, 20% or less, 19% or less, 18% or less, 17% or less, 16% or less, 15% or less, 14% or less, 13% or less, 12% or less, 11% or less, 10% or less, or the like. Further, the content thereof may be expressed by a predetermined range such as, for example, 1% to 20%, 3% to 15%, 5% to 10%, or the like by combining any numerical values indicated as examples.

[0050]

Next, cases where the induction promoter according to the embodiment is used as a component of pharmaceutical compositions will be described. As long as the induction promoter according to the embodiment is included as a component of a pharmaceutical composition, other components of the pharmaceutical composition are not particularly limited. The pharmaceutical composition including the

induction promoter according to the embodiment can also be provided as pharmaceutical compositions having efficacy of treatment or prevention for autoimmune diseases or allergic diseases via induction of regulatory T cells or as pharmaceutical compositions to which such efficacy is added.

[0051]

The pharmaceutical composition can be topically administered or systemically administered. The administration form is not particularly limited and may be any of oral administration and parenteral administration (for example, ophthalmic or nasal administering, or the like). Further, in the pharmaceutical composition, a pharmaceutically acceptable carrier can be included in accordance with an administration form in addition to lactoferrin that is the active ingredient. The carrier may be, for example, an excipient, a disintegrant or disintegration aid, a binder, a lubricant, a coating agent, a colorant, a diluent, a base, a solvent or a solubilizing agent, a tonicity agent, a pH regulator, a stabilizer, a propellant, an adhesive, and the like.

[0052]

The component included in the pharmaceutical composition other than the induction promoter according to the embodiment may be, for example, an immunosuppressant, an immune checkpoint inhibitor, an allergy medication, an antibody formulation, or the like. Examples of the immunosuppressant may be, but not limited to, cyclosporine, mizoribine, cyclophosphamide, azathioprine, tacrolimus, mycophenolate mofetil, or the like. Further, the immune checkpoint inhibitor may be, but not limited to, nivolumab, pembrolizumab, durvalumab, avelumab, atezolizumab, cemiplimab, ipilimumab, tremelimumab, or the like.

[0053]

The allergy medication may be, but not limited to, Alesion Epinastine 1994, Ebastel Ebastine 1996, Zyrtec Cetirizine 1998, Talion Bepotastine 2000, Allegra Fexofenadine 2001, Allelock Olopatadine 2001, Claritin Loratadine 2002, Xyzal Levocetirizine 2010, Bilanoa Bilastine 2016, Desalex Desloratadine 2016, Rupafin Rupatadine 2017, or the like.

[0054]

The antibody formulation may be, but not limited to, omalizumab, which is an anti-IgE antibody, mepolizumab, which is an anti-IL-5 antibody, benralizumab, which is an anti-IL-5 receptor antibody, dupilumab, which is an anti-IL-4/13 receptor antibody, nemolizumab, which is an IL-31 receptor antibody, tezepelumab, which is an anti-TSLP antibody, or the like.

[0055]

The dose of the pharmaceutical composition varies in accordance with the body weight, the age, or the like of a patient. The dose can be set as appropriate by a physician. For example, the dose can be set, but not limited to, such that the quantity of the induction promoter included in the pharmaceutical composition is the same as the dose of the induction promoter described above (the dose of D-sorbose and/or D-allose).

[0056]

[Embodiment of the therapeutic or prophylactic agent for autoimmune diseases and Embodiment of the therapeutic or prophylactic agent for allergic diseases]

As described above, the induction promoter disclosed in the present application can be used for treatment or prevention of autoimmune diseases or allergic diseases via induction of regulatory T cells. In other words, D-sorbose and/or D-allose can be used as a therapeutic or prophylactic agent for autoimmune

diseases or allergic diseases via induction of regulatory T cells. Note that, in the present specification, the term “treatment/therapeutic” refers to reversing, reducing, inhibiting, or preventing progression of autoimmune diseases and allergic diseases, disorders or symptoms due to such diseases, or one or more signs of such disorders or symptoms. Further, alleviation of symptoms or improvement of QOL are also included. The term “prevention/prophylactic” means suppressing onset of symptoms.

[0057]

With respect to the therapeutic or prophylactic agent for autoimmune diseases according to the embodiment, “regulatory T cell induction promoter” described above in “Embodiment of the regulatory T cell induction promoter” can be simply replaced with “therapeutic or prophylactic agent for autoimmune diseases”. Similarly, with respect to the therapeutic or prophylactic agent for allergic diseases according to the embodiment, “regulatory T cell induction promoter” described above in “Embodiment of the regulatory T cell induction promoter” can be simply replaced with “therapeutic or prophylactic agent for allergic diseases”. Therefore, to avoid duplicated description, detailed description for the embodiment of the therapeutic or prophylactic agent for autoimmune diseases and the embodiment of the therapeutic or prophylactic agent for allergic diseases will be omitted.

[0058]

[Embodiment of the food composition for preventing autoimmune diseases, Embodiment of the food composition for preventing allergic diseases, Embodiment of the food composition for improving allergic conditions, and Embodiment of the food composition for maintaining a healthy immune function]

As described above, the induction promoter disclosed in the present

application can be used for prevention of autoimmune diseases or allergic diseases via induction of regulatory T cells. In other words, when added to foods, D-sorbose and/or D-allose can be used as a food composition for preventing autoimmune diseases or a food composition for preventing allergic diseases. Note that the meaning of prevention is the same as that described above in “Embodiment of the therapeutic or prophylactic agent for autoimmune diseases and Embodiment of the therapeutic or prophylactic agent for allergic diseases”.

[0059]

The food composition for preventing autoimmune diseases and the food composition for preventing allergic diseases according to the embodiment are the same as the part: “cases where the induction promoter according to the embodiment is used as a component of food compositions will be described” described above in “Embodiment of the regulatory T cell induction promoter”. That is, the part describing that the induction promoter is included as an ingredient of food compositions can be simply replaced with the food composition for preventing autoimmune diseases or the food composition for preventing allergic diseases containing D-sorbose and/or D-allose as the active ingredient. Therefore, detailed description for the embodiment of the food composition for preventing autoimmune diseases and the embodiment of the food composition for preventing allergic diseases will be omitted.

[0060]

The food composition for improving allergic conditions according to the embodiment and the food composition for maintaining a healthy immune function according to the embodiment are the same as the part: “cases where the induction promoter according to the embodiment is used as ingredients of food compositions

will be described” described above in “Embodiment of the regulatory T cell induction promoter”. That is, the part describing that the induction promoter is included as an ingredient of a food composition can be simply replaced with the food composition for improving allergic conditions or the food composition for maintaining healthy immune functions containing D-sorbose and/or D-allose as the active ingredient. Therefore, detailed description for the embodiment of the food composition for improving allergic conditions and the embodiment of the food composition for maintaining a healthy immune function will be omitted.

[0061]

Note that, in the present specification, “allergic conditions” are not particularly limited as long as including a physical discomfort caused by allergies and mean inclusion of conditions that do not correspond to any diseases (for example, a condition before developing a disease). More specific examples of allergic conditions may be discomfort of a topical site (for example, a nose, an eye, a throat, skin, a respiratory tract, or a digestive tract), itching or irritation, nasal congestion, sneezing, watery eye, or increased redness of an eye due to the allergy.

[0062]

Further, the food composition according to the embodiment can be used for maintaining a healthy immune function. In the present specification, “maintaining a healthy immune function” means suppression of an excessive immune response (for example, a condition with the immune being activated at a topical site where inflammation occurs) to allow the condition to be closer to the healthy condition.

[0063]

[Embodiment of the treatment or prevention method for autoimmune diseases or allergic diseases]

Next, a treatment or prevention method for autoimmune diseases or allergic diseases according to the embodiment (hereafter, which may be simply referred to as “treatment/prevention method”) will be described.

[0064]

The treatment/prevention method includes a step of administering an effective amount of D-sorbose and/or D-allose having an effect of promoting induction of regulatory T cells to an animal (however, excluding human). The effective amount is not particularly limited as long as it is an amount achieving the effect of treatment or prevention and, for example, can be set to be the same as the dose of the induction promoter (the dose of D-sorbose and/or D-allose) described above in “Embodiment of the regulatory T cell induction promoter”.

[0065]

The animals include human (however, human is excluded in countries where medical treatment on human is excluded from the patent protection) or other mammals. The mammals may be mouse, rat, rabbit, hamster, sheep, monkey, pig, cat, dog, cow, horse, or the like. Further, a subject organism that is a target where a subject substance is brought into contact, is taken, or is administered may include individuals, tissues, cells (including cultured cells), or the like of human or animals. The cells are not limited and may be cell lines derived from mammals such as human, mouse, and rat or cell lines or cell lines derived from these cell lines, primary cells derived from various tissues, stem cells, ES cells, or iPS cells.

[0066]

[Screening method for substance promoting induction of regulatory T cells]

While it was confirmed in the Examples described later that D-sorbose and D-allose promoted induction of regulatory T cells, the use of this experimental system

can also screen substances promoting induction of regulatory T cells. The screening method for substances promoting induction of regulatory T cells according to the embodiment includes steps of:

(a) bringing a subject substance and regulatory T cells into contact with each other;

(b) measuring the induction rate of the regulatory T cells brought into contact with the subject substance and comparing the measured induction rate with the inductivity of regulatory T cells of a control group not brought into contact with the subject substance; and

(c) selecting a subject substance that promotes induction of regulatory T cells based on a result of the comparison in the step (b).

[0067]

The subject substance is not particularly limited as long as it is a substance that may promote induction of regulatory T cells. The subject substance may be, but not limited to, a nucleic acid, an antibody, a peptide, a protein, a non-peptide compound, a synthetic compound, a fermentation product, a cell extract, a cell culture supernatant, a plant extract, a mammalian tissue extract, plasma, or the like. The subject substance may be a novel substance or may be a known substance. These subject substances may form salts, and salts with physiologically acceptable acids or bases may be used as the salts of the subject substances.

[0068]

In selection of the subject substance, a threshold to the induction rate of the control group can be determined if necessary, and a substance exhibiting induction rate above the threshold can be selected.

[0069]

While the Examples will be presented below and the embodiments disclosed in the present application will be specifically described, these examples are only for the purpose of illustration of the embodiments. The Examples are neither to limit nor to express restriction on the scope of the invention disclosed in the present application

[EXAMPLES]

[0070]

<Example 1>

Materials and experiment methods used in Example 1 of the present application will be described below.

(1) Laboratory animal

C57BL/6J or C57BL/6N wild-type male mice were purchased from Charles River Japan, and NOD/ShiJcl female mice were purchased from CLEA Japan, Inc. All the mice were acclimated under a housing environment for at least one week and then used for the experiments at 6 to 11 weeks of age. The mice were housed in an environment without any specific pathogen being present and allowed to freely move in dedicated breeding cages (three mice/cage) and freely take feed and drinking water. The breeding room was maintained at a temperature of 23 ± 3 °C and a humidity of $50 \pm 10\%$, and the mice were kept under 12-hour light-dark cycles (lighting on at 8:00 AM).

[0071]

(2) Reagent

Roswell Park Memorial Institute medium (RPMI-1640 medium) was purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan), and 10% FBS (HyClone; Cytiva, Tokyo, Japan), 100 U/mL of penicillin, and 100 mg/mL of

streptomycin (Sigma-Aldrich, St. Louis, MO) were added thereto to obtain a complete RPMI medium.

[0072]

Among the monosaccharides listed in Table 1 below, D-glucose was purchased from Nacalai tesque (Kyoto, Japan), and D-mannose, D-allose, D-psicose, D-tagatose, and D-sorbose were provided by Matsutani Chemical Industry Co., Ltd. (Hyogo, Japan).

[0073]

[Table 1]

Monosaccharide	Category	M.W.
D-Glucose	Aldohexose	180.16
D-Mannose	Aldohexose	180.16
D-Allose	Aldohexose	180.16
D-Psicose	Ketohexose	180.16
D-Tagatose	Ketohexose	180.16
D-Sorbose	Ketohexose	180.16

[0074]

D-glucose, D-mannose, D-allose, D-psicose, D-tagatose, and D-sorbose were dissolved in the complete RPMI medium and adjusted to 500 mM. D-sorbose for oral administration to mouse was dissolved in distilled water to be 1.1 M or 0.28 M.

[0075]

As sugar metabolites, monosodium glucose-6-phosphate, sodium ribose-5-phosphate, and lactic acid were purchased from Nacalai tesque, DL-malic acid and monopotassium phosphoenolpyruvate were purchased from FUJIFILM Wako Pure Chemical Corporation, DL-glyceraldehyde-3-phosphate was purchased from Cayman Chemical (Ann Arbor, MI), and UDP-N-Acetyl-D-glucosamine, Disodium salt was

purchased from Calbiotech (El Cajon, CA). All the metabolites were dissolved in distilled water and used as a reference solution for intracellular metabolite measurement.

[0076]

As glucose metabolism inhibitor, KAN0438757 (KAN) was purchased from Selleck Biotech (Tokyo, Japan), 6-Aminonicotinamide (6-AN) was purchased from Cayman Chemical, and 6-Diazo-5-oxo-L-norleucine (DON) was purchased from FUJIFILM Wako Pure Chemical Corporation.

[0077]

The inhibitors were dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich), and then diluted with the complete RPMI medium for use. The final concentration of DMSO in the cell culture was adjusted to be 0.033% or less.

[0078]

(3) Cell separation from mouse tissue

Tissues collected from the mice were treated as follows, and lymphocytes were separated therefrom. After spleens were mashed, a pH 7.3 hemolysis buffer (NH₄Cl:8.26g, NaHCO₃: 1.19g, EDTA-2Na: 37.8mg in 100mL milli-Q water) was added thereto, and the mixture was allowed to react in ice for 3 minutes to be subjected to hemolysis treatment. The resulted solution was subjected to filtration with a 70 mm filter to obtain a suspension of spleen cells. Peripheral blood was collected in a tube containing ethylenediamine-N,N,N',N'-tetraacetic acid-2Na (EDTA-2Na; 0.5 mg, Dojindo Laboratories, Kumamoto, Japan) and subjected to hemolysis treatment with a pH 7.3 hemolysis buffer in the same manner as with the spleens. After mashed, lymph nodes were subjected to filtration with a 70 mm filter.

[0079]

(4) Differentiation culture of mouse Treg

CD3⁺ T cells were isolated from spleen cells of 8-week-old male C57BL/6J mice by using Magnetic cell sorting (MACS; Pan T Cell Isolation Kit II mouse, Miltenyi Biotec, Tokyo, Japan). The isolated CD3⁺ T cells were seeded on a 96-well plate flat bottom (2 x 10⁵ cells/well) and stimulated with anti-CD3/CD28 (beads: cells = 1:5, Dynabeads^R Mouse T-Activator CD3/CD28, Thermo Fisher Scientific, Waltham, MA) and rIL-2 (30IU/mL, Recombinant Human IL-2, Bio-techne, Minneapolis, MN) in the presence of 25 mM various monosaccharides, and the cells were cultured under conditions of 5% CO₂ at 37 °C. The complete RPMI medium instead of monosaccharides was added to the control group. The cells were restimulated on the third day after the culture, and the cells harvested on the sixth day after the culture were analyzed by fluorescence-activated cell sorting (FACS).

[0080]

(5) Flow cytometry analysis (FACS)

Foxp3 intranuclear staining was performed according to the manufacturer's instruction by using a fixation/permeabilization buffer solution (Foxp3/Transcription Factor Staining Buffer Set, eBioscience; Affymetrix, Santa Clara, CA). To explain briefly, the cells were subjected to blocking of FC receptor by anti-CD16/CD32 antibody (Clone: 93, BioLegend, San Diego, CA), subjected to dead cell staining with Ethidium Bromide (Sigma-Aldrich) and cell surface staining with anti-CD3 antibody (PE, Clone: 17A2, BioLegend) and anti-CD4 antibody (FITC, Clone: GK1.5, BioLegend), then subjected to fixation/permeabilization, and subjected to intranuclear staining with anti-Foxp3 biotin antibody (Clone: FJL-16s, BioLegend) and streptavidin (APC, BioLegend). Further, CD8 cell surface staining was similarly performed. To explain briefly, the cells were subjected to blocking of FC receptor by anti-CD16/CD32

antibody, subjected to cell surface staining with anti-CD8a antibody (FITC, Clone: 53-6.7, BioLegend), and then subjected to dead cell staining with 7-AAD Viability Staining Solution (BioLegend). The stained cells were analyzed by FACS-Calibur (BD, Franklin Lakes, NJ) or Galios (Beckman Coulter, Brea, CA). Data were analyzed by FlowJo ver. 9.9.5 or Kaluza ver. 1.1.

[0081]

(6a) Safety test of D-sorbose

Instead of drinking water, 1.1 M and 0.28 M D-sorbose solutions were administered to 6-week-old male C57BL/6J mice with free access to water. Distilled water was administered to the control group. During the administration period, the body weight, the water intake amount, and the feed intake amount were measured every two to three days, and spleens and peripheral blood were collected in 4 weeks after the administration. The peripheral blood was centrifuged (1400 x g, 24 °C, 10 min), and the supernatant was collected to obtain serum. The serum was stored at -80 °C until biochemical analysis was performed.

[0082]

(6b) Safety test of D-allose

Instead of drinking water, 0.28 M and 0.56 M D-allose solutions were administered to 6-week-old male C57BL/6J mice with free access to water. Distilled water was administered to the control group. During the administration period, the body weight, the water intake amount, and the feed intake amount were measured every two to three days.

[0083]

(7) Biochemical analysis of mouse serum components

Total protein (TP), alanine aminotransferase (ALT), creatinine (Cre), and

glucose (Glu) in the serum was measured by using a biochemical automatic analyzer (JCA-BM9130, JEOL, Tokyo, Japan).

[0084]

(8) Evaluation of IL-10 production capacity

Stimulation of spleen cells was performed in accordance with the following procedure. Spleen cells separated from spleens of C57BL/6J mice administered with 0.28 M D-sorbose solution with free access to water for 6 weeks from 6 weeks of age were seeded onto a 96-well plate flat bottom (5×10^5 cells/well), stimulated by Phorbol 12-myristate 13-acetate (PMA; 50ng/mL, Sigma-Aldrich) and ionomycin (500ng/mL, Sigma-Aldrich), and cultured under conditions of 5% CO₂ at 37 °C. For the control group, spleen cells from mice administered with distilled water were used. After 72 hours from the stimulation, the cell proliferation morphology was photographed, and the culture supernatant was collected. The IL-10 production quantity in the culture supernatant was measured by Mouse IL-10 ELISA kit (Life Technologies; Thermo Fisher Scientific).

[0085]

(9) Non-obese autoimmune diabetic (NOD) model

NOD/ShiJcl is an autoimmune spontaneous diabetes onset model that causes T-cell-dependent insulinitis and is characterized in hyperglycemia and high urine sugar due to various causes such as a negative selection disorder in the thymus due to MHC-II mutations. Since the onset thereof starts from about 18 weeks of age and cumulative onset of 60% is found by 35 weeks of age (provided by CLEA Japan, Inc), 17 weeks of age corresponding to the early stage of onset and 30 weeks of age corresponding to the terminal stage of disease are set as experiment end points. From 7.5 weeks of age, 1.1 M D-sorbose solution was administered to the mice of the

model with free access to water, and distilled water was administered to the control group. During the administration period, the blood was collected, the body weight, the water intake amount, and the feed intake amount were measured every 3 days. Serum was separated from the peripheral blood in accordance with the procedure described above in (6), and blood glucose levels were measured by a microplate assay using a glucose kit (GLU Reagent L, Sysmex, Hyogo, Japan). Persistent hyperglycemia (a case where the blood glucose level above 200 mg/dL was recorded for consecutive 6 days or longer) was evaluated as the onset of autoimmune diabetes mellitus. Peripheral blood was collected in EDTA-2Na-containing tube at 15 weeks of age, peripheral blood, spleens, pancreases, and pancreatic lymph nodes were collected at 17 weeks of age and 30 weeks of age.

[0086]

(10) Preparation of pathological tissue specimens

A mouse pancreas is classified into the gastric lobe, the splenic lobe, and the duodenal lobe, and the pancreases were collected for each of these sites. Among such collection, duodenal lobes where pancreatic islets were most densely distributed were fixed in 10% neutral buffered formalin, wrapped in 100 μ m mesh, and infiltrated and embedded in paraffin.

[0087]

(11) Histological evaluation

A plurality of 3 mm-thick sections were prepared at intervals of 50 μ m from duodenal lobes of mouse pancreases. The prepared sections were subjected to hematoxylin and eosin (HE) staining, and lymphocyte infiltration into pancreatic islets of Langerhans was scored as follows.

0: No lymphocyte is observed.

- 1: Lymphocytes surround pancreatic islets but not infiltrate.
- 2: Infiltration is observed in less than 25% of pancreatic islets.
- 3: Infiltration is observed in greater than or equal to 25% and less than 50% of pancreatic islets.
- 4: Infiltration is observed in greater than or equal to 50% of pancreatic islets.
- 5: Infiltration is observed in 100% of pancreatic islets, or pancreatic islets disappear leaving only debris.

For each individual, 30 pancreatic islets were scored, and the ratio occupied by each grade was analyzed.

[0088]

(12) Immunohistochemical staining

From duodenal lobes of mouse pancreases, 1 μ m-thick paraffin sections were prepared and subjected to Foxp3 immunohistochemical staining. As primary antibody, rabbit anti-Foxp3 antibody (ab215206, Abcam, Cambridge, England) was used. After deparaffinization and dehydration, the sections were subjected to heat treatment at 121°C for 20 minutes using Histofine antigen retrieval solution (pH 9.0) (Nichirei Bioscience, Tokyo, Japan). To eliminate endogenous peroxidase activity, the sections were immersed in a methanol solution containing 0.3% hydrogen peroxide for 30 minutes. After nonspecific binding was blocked with 1% BSA, the sections were incubated overnight at 4°C together with the primary antibody. The secondary antibody conjugated to peroxidase polymer (ImmPRESS Reagent anti-rabbit IgG, Vector Laboratories, Newark, CA) was incubated at room temperature for 30 minutes and then reacted with the substrate solution of 3,3'-diaminobenzidine tetrahydrochloride (DAB; Dako, Santa Clara, CA). The sections were then counterstained with Meyer's hematoxylin.

[0089]

(13) Measurement of cell viability

Treg (CD4⁺CD25⁺) and non-Treg (CD4⁺CD25⁻) were isolated from spleens of an 11-week-old male C57BL/6N mouse by using MACS (Pan T Cell Isolation Kit II mouse, CD4⁺CD25⁺ Regulatory T Cell Isolation Kit mouse, Miltenyi Biotec). Respective cells were seeded onto a 96-well plate flat bottom (1 x 10⁵ cells/well), stimulated with anti-CD3/CD28 (beads : cells = 1:5) and rIL-2 (30 I U/mL) in the presence of 25 mM monosaccharides, and cultured under conditions of 5% CO₂ at 37 °C. The cells were harvested after 24 hours and 72 hours from the stimulation, dead cells were stained with a trypan blue staining solution (Fujifilm Wako Pure Chemical Corporation), and the cell viability was measured.

[0090]

(14) Measurement of intracellular sugar metabolite

The mouse T lymph tumor cells (EL-4 cells) were purchased from JCRB cell bank (NIBIOHN; National Institutes of Biomedical Innovation, Health and Nutrition) and cultured in suspension under conditions of 37°C and 5% CO₂ by using complete RPMI medium. In cell passaging, cells were harvested together with the old medium, the number of cells was calculated by using the trypan blue staining solution, 1 x 10⁶ cells were then reseeded, and a fresh medium was added thereto. The cells were used for the experiment after the proliferation rate thereof was stabilized. Extraction of metabolites from the cells was performed as follows. The EL-4 cells were seeded onto 96-well plate flat bottom (4 x 10⁵ cells/well) and cultured under conditions of 5% CO₂ at 37 °C. To stabilize the metabolism, D-sorbose was added to obtain the final concentration of 25 mM and cultured again under conditions of 5% CO₂ at 37 °C after preculture for 24 hours. Instead of D-sorbose, the complete RPMI

medium was added to the control group. The cells were harvested in 6 hours after the addition, and the medium was completely removed by washing the cells 3 times with PBS (800 x g, 4 °C, 5 min). Distilled water was added to cell pellets and well blended, and the cells were crushed by an ultrasonic homogenizer (VP-050N, TAITEC, Aichi, Japan). Methanol and distilled water were added in equal volume, and the supernatant was collected after centrifugation (20000 x g, 4°C, 10 min). The supernatant was stored at -80 °C until analyzed. After 10 µM 2-morpholinoethanesulfonic acid of 30 µL and the supernatant of 200 µL dissolved on ice were mixed and the mixture was concentrated to dryness under a reduced pressure and redissolved with water of 30 µL, the solution was analyzed by liquid chromatograph-mass spectrometry (LC-MS/MS).

[0091]

(15) High-performance liquid chromatography tandem mass spectrometry (LC-MS/MS)

To comprehensively investigate the dynamics of glucose metabolism, seven types in total of metabolites (see (2) above) that account for various branched metabolism pathways were measured. In measurement, LCMS-8060 mass spectrometer (Shimadzu Corporation, Kyoto, Japan) was used along with the Nexcera HPLC system. In the HPLC, Inert Sustain PFP column (150 mm x 2.1 mm, 1.9 µm, GL Science, Tokyo, Japan) was used at 40°C, and 0.1% formic acid aqueous solution was used as the initial mobile phase. The cell extract (1 µL) was injected thereto, and the metabolites were separated at a 3-minute mobile phase gradient for increasing 0.1% formic acid-containing acetonitrile (Fujifilm Wako Pure Chemical) to 25% at a flow rate of 300 µL/min. The total analysis time was 6 minutes. The data was acquired by LabSolutions software ver. 2.2, and the peak area of the metabolites was

determined. The conversion efficiency was calculated from the quantified value of the metabolites and utilized as metabolic enzyme activity.

[0092]

(16) Induction of Treg differentiation by inhibition of sugar metabolism

To investigate the effect of intracellular metabolism on Treg differentiation, Treg differentiation and culture were performed by using sugar metabolism inhibitors. The CD3⁺ T cells were isolated by MACS from spleens of 9-week-old male C57BL/6N mice. The isolated CD3⁺ T cells were seeded onto 96-well plate flat bottom (2 x 10⁵ cells/well), stimulated with anti-CD3/CD28 (beads : cells = 1:2) and rIL-2 (30 IU/mL) in the presence of various inhibitors, and cultured under conditions of 5% CO₂ at 37 °C. The final concentration of the inhibitors was set to about 10 times the IC₅₀ of respective inhibitors, and KAN (2 μM), 6-AT (5 μM), and DON (2 μM) were used, respectively. For the group without inhibitor addition, the complete RPMI medium including DMSO at the same concentration as the inhibitor solvent was added. The cells were restimulated on the third day from the culture, and cells harvested on the sixth day from the culture were analyzed by FACS.

[0093]

(17) Statistical processing

GraphPad Prism software ver. 6 was used for statistical analysis. All the data were expressed as mean ± standard deviation, the unpaired Student-t test was used for testing significant differences between two groups, and One-way ANOVA or Two-way ANOVA and Saidak's-multiple comparison test or Dunnett's-multiple comparison test were used for testing significance differences between three or more groups. P < 0.05 was determined to be statistically significant.

[0094]

The experiment results of Example 1 performed by the above experiment method will be described below.

[0095]

(1) Ex vivo evaluation of Treg induction ability of various rare sugar

FIG. 1 illustrates the results of analysis by a flow cytometry method. T cells represent a type of lymphocytes, which are precursor cells produced in the bone marrow and then differentiated and matured through selection in the thymus. T cells ($CD3^+$ cells) are classified into multiple types in accordance with proteins such as CD4, CD8, or the like as an index that are specifically expressed on cell surfaces. For example, $CD4^+$ T cells serve to assist immune reactions and are also called helper T cells. $CD8^+$ T cells have the ability to kill cells infected with viruses or the like and tumor cells and are called killer T cells. In the present application, $CD3^+CD4^+Foxp3^+$ cells are defined as Treg (see Hori S. et al., "Control of regulatory T cell development by the transcription factor Foxp3", Science, 2003; 299: 1057-1061).

[0096]

As is apparent from FIGS. 1A and 1B, compared to the D-glucose group, D-sorbose and D-allose groups, which are rare sugars, exhibited an increasing trend in the ratio of $Foxp3^+$ Treg relative to $CD4^+$ cells. Treg is known to have Foxp3, which is a transcription factor, as the master regulator. That is, this means that the larger the ratio of $Foxp3^+$ Treg relative to $CD4^+$ cells is, the more the differentiation to Treg is induced. The results illustrated in FIGS. 1A and 1B are the same as with the D-mannose group already known to have Treg induction ability. This result indicates that the rare sugars D-sorbose and D-allose directly acted on T cells and induced differentiation into Treg in the same manner as with D-mannose.

[0097]

On the other hand, as is apparent from FIGS. 1C and 1D, while CD8⁺ T cells remarkably decreased in the D-mannose group compared to the D-glucose group, no significant change was observed in the D-sorbose and D-allose groups. CD8⁺ T cells (killer T cells) play an important role in antiviral responses and antitumor activity and are primary cells involved in excessive immune responses to self-antigens. Thus, while increase in Treg caused by D-mannose and depletion of CD8⁺ T cells are expected to provide synergetic suppression activity against excessive immune responses such as autoimmune diseases, long-term administration may cause susceptibility to viral infections and tumor proliferation. That is, while having the same effect as D-mannose in terms of inducing differentiation into Treg, D-sorbose and D-allose have a different effect from D-mannose in terms of not causing depletion of CD8⁺ T cells. Note that screening of substances that promote induction of regulatory T cells can be implemented by using the systems used in the present Example to administer the subject substance instead of D-sorbose and D-allose.

[0098]

(2) Effect of long-term sustained oral administration of D-sorbose on living bodies

The safety of D-sorbose that induced differentiation into Treg *ex vivo* was evaluated *in vivo*. FIG. 2 illustrates the results. As illustrated in FIG. 2A, in the administration groups of 1.1 M D-sorbose solution, body weights of mice sharply decreased in several days after start of the administration, but then gradually recovered, and in two weeks after the start of the administration, recovered to the same level as with the group with administration of distilled water (the control group). During the administration period, no physical finding such as diarrhea was observed in the D-sorbose group (not illustrated), and although the water intake amount and the feed intake amount continuously decreased, no direct factor for the reduction in

the body weight was found. These reductions in the body weight, the water intake amount, and the feed intake amount were mitigated by reducing the administration concentration of D-sorbitose to 0.28 M.

[0099]

Further, as illustrated in FIG. 2B, while the D-sorbitose group did not exhibit any pathological and significant change in the values of the total protein (TP), alanine aminotransferase (ALT), creatinine (Cre), and glucose (Glu) in serum compared to the control group, an increasing trend was found in the serum glucose value. It has been confirmed from the above result that, while causing initial decrease in body weights, the D-sorbitose administration ensures basic safety in the liver function, the kidney function, and the nutritional condition.

[0100]

(3) Induction of Treg by D-sorbitose requires immune activated state

The Treg induction ability of D-sorbitose was evaluated by using steady-state C57BL/6J mice. FIG. 3 illustrates the results. As is apparent from FIG. 3, no significant change was observed in the proportion of Tregs within spleens in the D-sorbitose group compared to the control group. This result is contrary to the effect of D-sorbitose ex vivo and implies that the Treg induction by D-sorbitose in the steady state is extremely less sensitive.

[0101]

Next, to emulate the immune activated state, spleen cells derived from mice administered in advance with D-sorbitose were harvested and stimulated with PMA/ionomycin. FIG. 4 illustrates the results. As is apparent from FIG. 4A (scale bar is 100 μ m), formation of small and numerous cell colonies was observed after 72 hours from the stimulation in the D-sorbitose group compared to the control group.

Furthermore, as illustrated in FIG. 4B, an increasing trend was found in the IL-10 production quantity in the D-sorbose group compared to the control group.

[0102]

From the above results, the induction of Treg by D-sorbose is less sensitive in the steady state but would have some effect in an immune activated state (inflammatory background). In particular, the inflammatory cytokine IL-2 is important for Treg differentiation, maturation, or the like and is more important for induced Treg (iTreg) than for natural Treg (nTreg). Therefore, it is implied that IL-2 supplied by the inflammatory background is required for Treg induction by D-sorbose, and it can be said that D-sorbose promotes induction of differentiation into iTreg. Further, since IL-2 is produced in a large quantity when immune system runaway is likely to occur, IL-2 functions only when an anomaly occurs even with daily intake as a health functional food (a food for specified health uses, a food with functional claims, or the like) and thus is expected to have less side effects.

[0103]

(4) D-sorbose prevents onset of autoimmune diabetes mellitus

Since the inflammatory background is important for Treg induction by D-sorbose was implied, the Treg induction ability and the effect thereof within living bodies were evaluated by using NOD/ShiJcl mice representing a spontaneous onset model of autoimmune diabetes mellitus. First, as illustrated in FIG. 5, because the body weight (FIG. 5A), the feeding status (FIGS. 5B and 5C), and the values of the total protein in serum, ALT, and creatinine (FIG. 5D) indicated the same results as with the change in the steady state C57BL/6J during the administration period, it has been confirmed that D-sorbose can be safely administered also in NOD/ShiJcl.

[0104]

Further, as illustrated in FIG. 6A, while the control group exhibited onset of diabetic hyperglycemia in some of the individuals, the D-sorbose group maintained the blood glucose level at a normal level until the terminal stage of disease. As if to support this, as illustrated in FIGS. 6B and 6C, compared to the control group, the D-sorbose group exhibited a reduction in lymphocytic infiltration to pancreatic islets in the terminal stage of disease (the black arrow parts in FIG. 6C, the scale bar of FIG. 6C is 100 μm), and many normal pancreatic islets remained.

[0105]

Next, to immunologically confirm the preventive effect against diabetes mellitus onset due to D-sorbose, the ratio of Treg in each tissue was analyzed. Compared to the control group, D-sorbose group exhibited a decreasing trend in CD3⁺ T cells within the pancreatic lymph nodes in the early stage of the onset (17 weeks of age, 10 weeks after the administration of D-sorbose) as illustrated in FIGS. 7A and 7B, and exhibited a significant increase in the ratio of Treg of the peripheral blood (FIGS. 7C and 7D) and the ratio of Treg within the pancreatic lymph nodes (FIGS. 7E, 7F) as illustrated in FIGS. 7C to 7F. In contrast, as illustrated in FIGS. 8A and 8B, no significant change in the ratio of Treg within spleens was observed.

[0106]

Further, as illustrated in FIG. 8C (scale bar is 100 μm), in the D-sorbose group, local infiltration of Foxp3⁺ Treg was observed in the pancreatic islet infiltrating lymphocyte population in the early stage of onset (17 weeks of age, 10 weeks after administration of D-sorbose). These results imply that D-sorbose induces Treg differentiation within local lymph nodes, which are places of T cell differentiation, and functionally exerts a suppression effect at the local inflammation site.

[0107]

(5) D-sorbose does not affect the viability between subsets of T cells

D-sorbose exhibited an increasing trend in the ratio of Treg at local inflammation sites as illustrated in FIGS. 7E and 7F, however, exhibited a decreasing trend in CD3⁺ T cells as illustrated in FIGS. 7A and 7B. This result may be caused by the fact that the ratio of Treg merely, relatively increased not due to the induction of Treg by D-sorbose but due to the reduction in T cells except for Treg. Accordingly, the difference in sensitivity to D-sorbose between Treg and other T cells was evaluated by the cell viability. FIG. 9 illustrates the results. The D-sorbose group exhibited a cell viability similar to that of the D-glucose group regardless of the culture time and T cell population. These results imply that the increase in Treg due to D-sorbose is not a relative increase but an increase caused by induction of differentiation from naive T cells.

[0108]

(6) D-sorbose inhibits intracellular sugar metabolism to induce Treg

To reveal the effect of D-sorbose on intracellular sugar metabolism, intracellular metabolite was measured. As illustrated in FIGS. 10A and 10B, compared to the group without monosaccharide addition (the control group), the D-sorbose group exhibited a remarkable increase in hexose-6-phosphate at the initial stage of the glycolytic pathway, while exhibiting a significant reduction in the metabolic enzyme activity for glyceraldehyde-3-phosphate of the glycolytic pathway branched therefrom, ribose-5-phosphate in the pentose phosphate pathway (PPP), and UDP-GluNAc in the hexosamine pathway (HP).

[0109]

Next, as illustrated in FIG. 10B, the Treg induction ability was evaluated by using an inhibitor of enzyme that catalyzes the metabolism pathway branched from

glucose-6-phosphate of sugar metabolism. As illustrated in FIGS. 11A and 11B, compared to the group without inhibitor addition (Vehicle group), the inhibition of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3) in the glycolytic pathway by KAN and the inhibition of glucose-6-phosphate dehydrogenase (G6PD) in the PPP by 6-AN did not change the ratio of Treg, but the inhibition of glutamine-fructose-6-phosphate aminotransferase (GFAT) in the HP by DON significantly increased the ratio of Treg. These results imply that the glycolytic pathway, the pentose phosphate pathway, and the hexosamine pathway, which are the intracellular sugar metabolism pathways among various metabolism pathways considered to be inhibited by D-sorbose, are inhibited, and in particular, the inhibition of the hexosamine synthetic pathway is involved with the Treg induction.

[0110]

Further, it was indicated that, also when the metabolism pathway inhibited by D-sorbose was inhibited by an existing inhibitor, differentiation into Treg was induced in the same manner as with D-sorbose. Therefore, D-sorbose can be used as an inhibitor for the glycolytic pathway, the pentose phosphate pathway, and the hexosamine pathway, in particular, an inhibitor for the hexosamine pathway and can be used as a pharmaceutical composition for diseases that can be treated or prevented by inhibition of such pathways. Specific diseases may be the autoimmune diseases and the allergic diseases described in “Embodiment of the regulatory T cell induction promoter”.

[0111]

(7) Effect of long-term sustained oral administration of D-allose on living bodies

The safety of D-allose was evaluated in vivo in the same manner as with D-sorbose. FIG. 12 illustrates the results. As illustrated in FIG. 12A to FIG. 12C, as the

concentration of D-allose increased, the water intake amount and the feed intake amount decreased, and a reduction in the body weight was found. During the administration period, however, no physical finding such as diarrhea was observed (not illustrated), and no particular health problem was observed in the D-allose group.

[0112]

<Example 2>

Next, an experiment to confirm whether or not D-sorbose is also useful for allergic diseases was performed. The experiment was performed in accordance with the same procedure as in Example 1 except that the following procedure was changed from the materials and experiment methods described above in (1) to (17) for Example 1. Note that, since Example 2 represents the experiment to confirm the usefulness of D-sorbose for allergic diseases, some of the materials and the experiment methods described in (1) to (17) for Example 1 were not performed.

(1) Laboratory animal

Balb/c wild-type female mice (purchased from Charles River Japan) were used as the mice and used in the experiment at 5 weeks of age.

[0113]

(2) Reagent

Only D-sorbose was used as the monosaccharide and dissolved in distilled water to be 1.1 M. Further, Ovalbumin (OVA; A5503; Sigma-Aldrich, St. Louis, MO, USA) was used as the inhibitor and dissolved with pH 7.4 PBS. As the adjuvant, aluminum hydroxide (Alhydrogel adjuvant 2%; invivoGen, San Diego, CA, USA) was used. OVA and aluminum hydroxide were mixed to be 25 μ g, 2 mg/200 μ L, respectively.

[0114]

(9) Non-obese autoimmune diabetic (NOD) model

Instead of “Non-obese autoimmune diabetic (NOD) model” of Example 1, OVA-induced allergy (OVA) model mice were prepared in accordance with the following procedure.

Balb/c mice were divided into two groups: a group administered with 1.1 M D-sorbose solution with free access to water starting from 5 weeks of age after birth and a control group administered with distilled water, and the administration was continued until 10 weeks of age. At 6, 7, and 8 weeks of age after birth, a mixed solution of OVA (25 µg) and aluminum hydroxide (2mg) was administered intraperitoneally. Then, OVA (100 µg/20 µL) was administered intranasally everyday for 1 week from 9 weeks of age after birth. During the administration period, blood was collected, and the body weight, the water intake amount, and the feed intake amount were measured every 3 days. Peripheral blood, lungs, mediastinal lymph nodes were collected at 10 weeks of age.

[0115]

(10) Preparation of pathological tissue specimens

Pathological tissue specimens were prepared in accordance with the same procedure as in Example 1 except that lungs were used instead of the pancreases of Example 1.

[0116]

(11) Histological evaluation

Instead of periodic histological evaluation of insulitis of Example 1, 1 µm-thick paraffin sections were prepared from the lungs of OVA model mice and subjected to HE staining, and the following findings were evaluated.

- Inflammatory cell infiltration

- Bronchial epithelial hyperplasia
- Goblet cell hyperplasia
- Bronchial smooth muscle hypertrophy
- Perivascular and peribronchial mucosal edema

[0117]

Experiment results of Example 2 performed in accordance with the above experiment method will be described below.

(1) D-sorbose prevents onset of OVA-induced allergic bronchitis

As described above in “(4) D-sorbose prevents onset of autoimmune diabetes” in Example 1, administration of D-sorbose significantly increased Treg induction ability in the living body.

As illustrated in FIGS. 13A and 13B, also in the experiment using the OVA-induced allergic bronchitis model, the ratio of Treg within mediastinal lymph nodes significantly increased in the D-sorbose group compared to the control group. Further, as illustrated in FIG. 13C (scale bar is 50 μm), in the D-sorbose group, local infiltration of Foxp3⁺ Tregs was observed within the pulmonary infiltrating lymphocyte population. These results imply that D-sorbose induces Treg differentiation within the local lymph nodes, which are places of T cell differentiation, and functionally exerts an allergy suppression effect at a local inflammation site.

[0118]

Furthermore, as if to support the above, as illustrated in FIG. 14A, the inflammatory cell infiltration into the lungs (scale bar is 100 μm), the bronchial epithelial hyperplasia, the goblet cell hyperplasia, the bronchial smooth muscle hypertrophy, the vascular and bronchial perimucosal edema, and the like decreased in the D-sorbose group compared to the control group. As being consistent with these

findings, as illustrated in FIG. 14B, the lung/body weight ratio significantly decreased in the D-sorbose group compared to the control group.

[0119]

It is known that allergic diseases cause onset when Th2 cells are induced. The OVA model mouse prepared in Example 2 is the Th2 cell-induced model mouse. It has been therefore confirmed from the result of Example 2 that D-sorbose is useful for allergic diseases.

[Industrial Applicability]

[0120]

The regulatory T cell induction promoter disclosed in the present application can be used for treatment or prevention of autoimmune diseases such as autoimmune diabetes mellitus, allergic diseases, or the like. Therefore, the regulatory T cell induction promoter disclosed in the present application is useful for medical institutions, pharmaceutical companies, and the health food industry.

[CLAIMS]

[Claim 1]

A regulatory T cell induction promoter containing D-sorbose and/or D-allose as an active ingredient.

[Claim 2]

The regulatory T cell induction promoter according to claim 1 including only D-sorbose as the active ingredient.

[Claim 3]

The regulatory T cell induction promoter according to claim 1 including only D-allose as the active ingredient.

[Claim 4]

The regulatory T cell induction promoter according to claim 1,
wherein the D-sorbose is a D-sorbose derivative, and
wherein the D-allose is a D-allose derivative.

[Claim 5]

The regulatory T cell induction promoter according to any one of claims 1 to 4 used for treatment or prevention against an autoimmune disease or an allergic disease via induction of a regulatory T cell.

[Claim 6]

The regulatory T cell induction promoter according to any one of claims 1 to 4, wherein a regulatory T cell is induced by inhibition of hexosamine pathway that is an intracellular sugar metabolism pathway.

[Claim 7]

The regulatory T cell induction promoter according to any one of claims 1 to 4 used as a component of a pharmaceutical composition.

[Claim 8]

The regulatory T cell induction promoter according to any one of claims 1 to 4 used as a component of a food composition.

[Claim 9]

A therapeutic or prophylactic agent for an autoimmune disease, the therapeutic or prophylactic agent containing at least one selected from a group consisting of D-sorbose, a D-sorbose derivative, D-allose, and a D-allose derivative as an active ingredient.

[Claim 10]

The therapeutic or prophylactic agent for an autoimmune disease according to claim 9, wherein the autoimmune disease is type 1 diabetes mellitus.

[Claim 11]

A therapeutic or prophylactic agent for an allergic disease, the therapeutic or prophylactic agent containing at least one selected from a group consisting of D-sorbose, a D-sorbose derivative, D-allose, and a D-allose derivative as an active ingredient.

[Claim 12]

A food composition for preventing an autoimmune disease, the food composition containing at least one selected from a group consisting of D-sorbose, a D-sorbose derivative, D-allose, and a D-allose derivative as an active ingredient.

[Claim 13]

A food composition for preventing an allergic disease, the food composition containing at least one selected from a group consisting of D-sorbose, a D-sorbose derivative, D-allose, and a D-allose derivative as an active ingredient.

[Claim 14]

A food composition for improving an allergic condition, the food composition containing at least one selected from a group consisting of D-sorbose, a D-sorbose derivative, D-allose, and a D-allose derivative as an active ingredient.

[Claim 15]

A food composition for maintaining a healthy immune function, the food composition containing at least one selected from a group consisting of D-sorbose, a D-sorbose derivative, D-allose, and a D-allose derivative as an active ingredient.

[Claim 16]

A treatment or prevention method for an autoimmune disease or an allergic disease, the method comprising a step of:

administering an effective amount of at least one selected from a group consisting of D-sorbose, a D-sorbose derivative, D-allose, and a D-allose derivative to an animal (excluding human), the effective amount having an effect of promoting induction of regulatory T cells.

[Claim 17]

A screening method for substances promoting induction of a regulatory T cell, the method comprising steps of:

(a) bringing a subject substance and a regulatory T cell into contact with each other;

(b) measuring an induction rate of the regulatory T cell brought into contact with the subject substance and comparing the measured induction rate with an induction rate of a regulatory T cell of a control group not brought into contact with the subject substance; and

(c) selecting a subject substance that promotes induction of a regulatory T cell based on a result of the comparison in the step (b).