DESCRIPTION

Title of Invention

# COMPOSITION COMPRISING PROBIOTICS AND POLYPEPTIDE HAVING BINDING AFFINITY FOR IGE AND USE THEREOF

## Technical Field

The present invention relates to a composition for treating or preventing an allergic disease.

## Background Art

Food allergy is a disease caused by decreased immunological resistance against non-pathogenic food antigens (allergens). The disease may lead to deteriorated quality of life due to dietary restrictions and may be life-threatening in a case where acute and chronic anaphylaxis develops. Allergic diseases such as allergic rhinitis and atopic dermatitis as well as such food allergy are spreading at a high rate in industrialized and westernized modern societies. In addition, development of anaphylaxis, a severe allergic reaction, is also increasing. These immune diseases severely impair quality of life and socioeconomic costs are soaring accordingly. Thus, there is a desperate need for measures to overcome such diseases.

Although food allergic diseases may develop through an IgE-mediated or non-IgE-mediated immune response, IgE-mediated food allergy is the most common. In the IgE-mediated food allergy, allergens bind to IgE, and allergen-bound IgE crosslinks FcεRI, a high-affinity IgE Fc receptor on effector cells such as mast cells and basophils, thereby inducing activation of the effector cells. In a case where the effector cells are activated, modulators are released, thereby causing immediate hypersensitivity. In addition to food allergic diseases, most allergic diseases are caused by an excessive immune response due to immunoglobulin E (IgE). IgE is an antibody that is normally present in serum at a very low concentration. IgE is also produced by innocuous antigens. In a case where the number of IgE is increased without any particular stimulus, an allergic disease may be caused. The

abnormally increased number of IgE can bind to high-affinity IgE Fc receptors (FcεRIs) which are expressed on the surface of mast cells, basophils, and the like.

Such binding between IgE and the IgE Fc receptor causes mast cells or basophils to release chemical mediators such as histamine, leukotriene, prostaglandin, bradykinin, and platelet-activating factors. Release of these chemical mediators by the mast cells or basophils results in allergic symptoms. In particular, worsened allergic symptoms may be exhibited in a case where IgE and FcεRI are bound to each other. FcεRI-expressing cells are known to increase in allergic patients.

Various methods, such as allergen avoidance, administration of anti-allergic drugs, modulation of IgE synthesis in the body, and development of anti-IgE antibodies, have been proposed to treat allergic diseases. However, such methods have many drawbacks, such as inability to cure an underlying cause of allergy, insufficient drug efficacy, and occurrence of serious side effects.

Meanwhile, a method of using microorganisms such as lactic acid bacteria has been studied for the purpose of treating or ameliorating allergic diseases. Such healthy microorganisms are called probiotics. However, techniques to discover and evaluate probiotics for immune control such as allergy inhibition have not yet been established. In particular, studies on the underlying action mechanism of probiotics are inadequate, and most of the studies are conducted *in vitro*. In other words, although probiotics are orally ingested, most of the studies so far have focused on *in vitro* experiments using cell lines, and these experimental methods have a major drawback that it is not possible to provide a substitute for studies on functions that may be exhibited in a case where a human ingests probiotics.

In addition, immunoglobulin compositions have been studied to treat allergic diseases. Such compositions have been reported to be useful for treating IgE-mediated disorders including allergy and asthma (KR10-1783272B1). In particular, omalizumab (trade name: Xolair), which targets an Fc portion of an IgE antibody, has been developed and used as a therapeutic agent for intractable severe asthma and intractable urticaria. However, a high-dose administration of omalizumab is required in order to maintain effects. Thus, it has been reported that omalizumab has a high cost burden, and side effects such as angioedema and anaphylactic reaction (*The Journal of Clinical Investigation*, Volume 99, Number 5, March

1997, 915-925).

Although the underlying mechanism by which omalizumab causes side effects has not yet been identified, it can be expected that omalizumab is an IgG1 antibody. A study which targets a mouse model showed that a large number of antigen-specific IgG1 antibodies can induce passive systemic anaphylaxis (PSA) through FcγRIII, a low-affinity IgG receptor, and platelet-activating factors in an antigen-rich environment. In addition, such IgG-mediated anaphylaxis occurs exaggeratedly due to lack of FcεRI signaling. Therefore, passive systemic anaphylaxis may be caused in a case where a considerable amount of omalizumab is injected into a patient with an allergic disease who exhibits a high level of IgE. Recently, it has been reported that FcγRIIA, a low-affinity IgG receptor expressed in a human, is also associated with IgG-mediated anaphylaxis. In addition, post-marketing studies have reported abnormal reactions such as allergic granulomatous vasculitis and idiopathic severe thrombocytopenia.

## Technical Problem

Although many studies have been conducted on allergic diseases, a method for dramatically ameliorating allergic diseases has not been developed so far. An object of the present invention is to provide a composition for treating or preventing such allergic diseases.

## Solution to Problem

According to an aspect of the present invention, there is provided a composition comprising, as active ingredients, probiotics and a polypeptide with a binding ability to IgE.

In another aspect, there is provided a pharmaceutical composition for treating or preventing an allergic disease, comprising the composition as an active ingredient. In yet another aspect, there is provided a health functional food composition for ameliorating or alleviating an allergic symptom, comprising the composition as an active ingredient.

In still yet another aspect, there is provided a kit for treating or preventing an allergic disease, comprising a first composition that contains probiotics and a second composition that contains a polypeptide with a binding ability to IgE.

## Advantageous Effects of Invention

The composition comprising, as active ingredients, probiotics and a polypeptide with a binding ability to IgE, according to the present invention, exhibits an excellent effect of ameliorating allergy *in vivo*. Thus, the composition can be used as a pharmaceutical composition for treating or preventing a severe allergic disease. Furthermore, from the viewpoint that the composition of the present invention can be applied to oral immunotherapy, the composition may not only be more effective for food allergy while decreasing side effects, but also ideal for treating children suffering from IgE-mediated allergies. Therefore, the composition can be used as a health functional food for ameliorating or alleviating an allergic symptom.

Brief Description of Drawings

FIG. 1 illustrates a schematic diagram of the constitution of a monomer forming an embodiment ($IgE_{TRAP}$) of the polypeptide dimer of the present invention. An embodiment of $IgE_{TRAP}$ can consist of 425 amino acids from human FcεRIα (region from 26$^{th}$ amino acid to 205$^{th}$ amino acid in FcεRIα extracellular domain, 180 aa) to human IgD/IgG4 hybrid Fc (245 amino acids). The IgD/IgG4 hybrid Fc has an FcRn-binding site (right hatched line) but lacks binding sites for FcγR and C1q (left hatched line). Here, IgD may be a region (38 aa) from 133$^{rd}$ amino acid to 170$^{th}$ amino acid, and IgG4 may be a region (207 aa) from 121$^{st}$ amino acid to 327$^{th}$ amino acid.

FIG. 2 illustrates a three-dimensional structural model of an $IgE_{TRAP}$ homodimer. The structure shows an FcεRIα extracellular domain (blue), an IgD hinge (yellow), and an IgG4 Fc (green).

FIG. 3 illustrates SDS-PAGE results for polypeptides with a binding ability to IgE produced in each cell line (FIG. 3A). Here, it can be seen that a truncated form is not generated at both reducing and non-reducing conditions (FIGS. 3A and 3B).

FIG. 4 illustrates results of isoelectric focusing (GEL-IEF) experiments performed to identify an increase in sialic acid content of polypeptides with a binding ability to IgE which have been produced in each cell line. A content of proteins with lowered major isoelectric point (pI) is increased due to an increase in content of negatively charged sialic acid caused by introduction of a sialic acid transferase gene. From this, it can be seen that a content of

acidic proteins is increased through addition of sialic acid transferase.

FIG. 5 illustrates SDS-PAGE results for non-reduced and reduced forms of a polypeptide dimeric protein ($IgE_{TRAP}$) according to an embodiment of the present invention. In particular, it can be seen that the polypeptide dimer has high purity even in culture supernatant which corresponds to Input.

FIG. 6 illustrates results obtained by performing SDS-PAGE analysis of $IgE_{TRAP}$ under non-reduced and reduced conditions.

FIG. 7 illustrates a graph showing a binding ability of omalizumab to IgE. The graph shows results obtained by immobilizing omalizumab and analyzing a binding ability thereof depending on IgE concentrations treated. Interaction between human IgE and omalizumab was analyzed using surface plasmon resonance (SPR), and a binding affinity of each molecule was calculated.

FIG. 8 illustrates a graph showing a binding ability, to IgE, of the polypeptide dimeric protein ($IgE_{TRAP}$) according to an embodiment of the present invention. The graph shows results obtained by immobilizing the $IgE_{TRAP}$ and analyzing a binding ability thereof depending on IgE concentrations treated. Interaction between human IgE and the $IgE_{TRAP}$ was analyzed using surface plasmon resonance (SPR), and a binding affinity of each molecule was calculated.

FIGS. 9 to 13 illustrate results obtained by identifying interactions of the dimeric protein ($IgE_{TRAP}$), an embodiment of the present invention, and omalizumab with IgG receptors FcγRI (FIG. 9), FcγRIIA (FIG. 10), FcγRIIB (FIG. 11), FcγRIIIA (FIG. 12), and FcγRIIIB (FIG. 13) using bio-layer interferometry (BLI) assay.

FIG. 14 illustrates a graph obtained by quantifying a binding capacity between $IgE_{TRAP}$ and IgG receptors, and between omalizumab and IgG receptors.

FIG. 15A illustrates a graph showing an inhibitory ability, on activity of mouse-derived mast cells, of the polypeptide dimeric protein ($IgE_{TRAP}$) according to an embodiment of the present invention depending on concentrations thereof.

FIG. 15B illustrates a graph showing a comparison between inhibitory abilities, on activity of human FcεRI-expressing mouse-derived mast cells, of the polypeptide dimeric protein ($IgE_{TRAP}$) according to an embodiment of the present invention and Xolair

(omalizumab) depending on concentrations thereof.

FIG. 16 illustrates potencies of a polypeptide dimeric protein according to an embodiment of the present invention in a food allergy-induced disease model.

FIG. 17 illustrates an experimental schedule for food allergy induction, and $IgE_{TRAP}$, *B. longum*, and combination therapy. i.p., intraperitoneal; i.g., intragastric

FIG. 18 illustrates potencies of $IgE_{TRAP}$, *B. longum*, and combination therapy to inhibit allergy-induced diarrhea symptoms. n = 16 to 18 mice per group, OVA vs OVA + $IgE_{TRAP}$: $*P < .05$, OVA vs OVA + *B. longum* + $IgE_{TRAP}$ , and OVA vs PBS: $***P < .0001$

FIG. 19A illustrates an experimental plan to identify that *B. longum* improves therapeutic effects of $IgE_{TRAP}$. Specifically, an experimental plan for food allergy induction, and single or combined administration of $IgE_{TRAP}$ and *B. longum* is shown. i.p., intraperitoneal; i.g., intragastric

FIG. 19B illustrates a graph obtained by identifying effects of combined administration of probiotics and the polypeptide dimeric protein ($IgE_{TRAP}$) with a binding ability to IgE depending on increased doses in a food allergy-induced disease model. Effects of $IgE_{TRAP}$, *B. longum*, and a combination thereof to inhibit food allergic diarrhea are shown. n = 14 mice per group, OVA vs OVA + *B. longum* + $IgE_{TRAP}$ (20 μg), OVA vs OVA + *B. longum* + $IgE_{TRAP}$ (200 μg), and OVA vs PBS: $**P < .001$

FIG. 20 illustrates results obtained by analyzing, with ELISA, mast cell protease-1 (MCPT-1) levels in sera obtained from respective experimental groups, at the time of administration of $IgE_{TRAP}$, *B. longum*, and a combination thereof, in a food allergy-induced disease model.

FIG. 21 illustrates results obtained by measuring, with ELISA, total IgE (free IgE and IgE-$IgE_{TRAP}$ complex) levels in sera obtained from respective experimental groups, at the time of administration of $IgE_{TRAP}$, *B. longum*, and a combination thereof, in a food allergy-induced disease model. n = 16 to 18 mice per group, OVA vs OVA + $IgE_{TRAP}$, OVA vs OVA + *B. longum* + $IgE_{TRAP}$, and OVA vs PBS: $***P < .0001$

FIG. 22 illustrates results obtained by measuring, with ELISA, free IgE levels in sera obtained from respective experimental groups, at the time of administration of $IgE_{TRAP}$, *B. longum*, and a combination thereof, in a food allergy-induced disease model. n = 16 to 18

mice per group, OVA vs OVA + $IgE_{TRAP}$, OVA vs OVA + *B. longum* + $IgE_{TRAP}$, and OVA vs PBS: ***$P < .0001$

FIG. 23 illustrates results obtained by identifying inhibitory effects on mast cell proliferation and goblet cell proliferation in respective experimental groups, at the time of administration of $IgE_{TRAP}$, *B. longum*, and a combination thereof, in a food allergy-induced disease model. Results obtained by staining mast cells (red) in representative paraffin sections of the jejunum in the respective experimental groups are shown (magnification 400x). Enlargement of the jejunum clearly shows the mast cells (red).

FIG. 24 illustrates results obtained by enlarging the mast cells 400 times in FIG. 23 and identifying the mast cells. n = 10 to 12 mice per group, OVA vs *B. longum*, and OVA vs $IgE_{TRAP}$: **$P < .001$, OVA vs OVA + *B. longum* + $IgE_{TRAP}$, and OVA vs PBS: ***$P < .0001$

FIG. 25 illustrates results obtained by identifying inhibitory effects on mast cell proliferation and goblet cell proliferation in respective experimental groups, at the time of administration of $IgE_{TRAP}$, *B. longum*, and a combination thereof, in a food allergy-induced disease model. Results obtained by staining goblet cells for identification in representative paraffin sections of the jejunum in the respective experimental groups are shown (purple, magnification 400x).

FIG. 26 illustrates results obtained by randomly selecting globet cells from villus-crypt units (VCUs) in Figure 25 and counting 10 VCUs. n = 5 to 6 mice per group, OVA vs *B. longum*, and OVA vs $IgE_{TRAP}$: *$P < .05$, OVA vs OVA + *B. longum* + $IgE_{TRAP}$, and OVA vs PBS: ***$P < .0001$

FIG. 27 illustrates a schematic diagram of mechanism of food allergy inhibition caused by combined therapy with *B. longum* and $IgE_{TRAP}$. Food allergens ingested can induce activation of effector cells (mast cells and basophils) by binding of IgE to a high-affinity IgE Fc receptor (FcεRI) on the effector cells. Activated effector cells release modulators, thereby causing an immediate hypersensitivity reaction. *B. longum* induces apoptosis of mast cells through secretion of extracellular vesicle (EV) which decreases the number of mast cells. In the meantime, $IgE_{TRAP}$ can block IgE binding to FcεRI on effector cells, and thus inhibit activation and proliferation of the effector cells. Combined administration of *B. longum* and $IgE_{TRAP}$ made it possible to effectively alleviate food allergic symptoms and goblet cell

hyperplasia.

FIG. 28 illustrates a graph obtained by identifying changes in IL-33 expression in intestinal tissue after administration of *B. longum* and $IgE_{TRAP}$. Administration of *B. longum* and $IgE_{TRAP}$ decreased expression of IL-33 mRNA in the jejunum of food allergy model mice. n = 16 to 18 mice per group, OVA vs OVA + *B. longum* + $IgE_{TRAP}$: $*P < .05$

FIG. 29 illustrates a graph obtained by identifying diarrhea frequency after intraperitoneal injection of $IgE_{TRAP}$ and *L. casei*. n = 7 to 10 mice per group

FIG. 30 illustrates a graph obtained by identifying diarrhea frequency after intraperitoneal injection of $IgE_{TRAP}$ and *Lc. lactis*. n = 5 mice per group

FIG. 31 illustrates a graph obtained by identifying diarrhea frequency after intraperitoneal injection of $IgE_{TRAP}$ and *S. thermophilus*. n = 6 to 10 mice per group

FIG. 32 illustrates a graph obtained by identifying diarrhea frequency after intraperitoneal injection of $IgE_{TRAP}$ and *L. rhamnosus*. n = 5 to 10 mice per group

FIG. 33 illustrates a graph obtained by identifying diarrhea frequency after intraperitoneal injection of $IgE_{TRAP}$ and *L. reuteri*. n = 5 to 10 mice per group

FIG. 34 illustrates a graph obtained by identifying diarrhea frequency after intraperitoneal injection of $IgE_{TRAP}$ and *L. fermentum*. n = 7 to 10 mice per group

FIG. 35 illustrates a graph obtained by orally administering $IgE_{TRAP}$ to normal mice and then identifying $IgE_{TRAP}$ adsorbed in serum of the mice.

Detailed Description of Invention

In an aspect of the present invention, there is provided a composition comprising, as active ingredients, probiotics and a polypeptide with a binding ability to IgE.

As used herein, the term "probiotics" collectively refers to microorganisms that are favorable to the human body in a case of being ingested in an appropriate amount, indicating bacteria beneficial to the human body. The probiotics may be lactic acid bacteria or Bifidobacterium. The lactic acid bacteria collectively refer to bacteria that ferment sugar to produce lactic acid. Most intestinal beneficial bacteria are classified as lactic acid bacteria, and the lactic acid bacteria can degrade sugars, of which 50% or more thereof is produced as lactic acid.

The lactic acid bacteria may be any one selected from the group consisting of *Lactobacillus*, *Lactococcus*, *Enterococcus*, and *Streptococcus*. Specifically, the *Lactobacillus* may be any one selected from the group consisting of *L. acidophilus*, *L. casei*, *L. gasseri*, *L. delbrueckii ssp. bulgaricus*, *L. helveticus*, *L. fermentum*, *L. paracasei*, *L. plantarum*, *L. reuteri*, *L. rhamnosus*, *L. pentosus*, and *L. salivarius*. In addition, the *Lactococcus* may be *Lc. lactis*, and the *Streptococcus* may be *S. themophilus*. In addition, the Bifidobacterium may be any one selected from the group consisting of *B.bifidum*, *B.breve*, *B. longum*, and *B.animalis ssp. lactis*.

Preferably, the probiotics may be *Lactobacillus casei* or *Bifidobacterium longum*. In particular, *Bifidobacterium longum* may be accession no. KACC 91563 (KR10-1778734B1). In particular, the KACC 91563 strain targets mast cells which are important cells in allergic reactions, and thus can be utilized as lactic acid bacteria that treat allergy. Usually, the probiotics can be used in the form of live bacteria, in which the live bacteria can be used in a lyophilized form. In addition, the probiotics may be used in the form of dead bacteria.

As used herein, the term "polypeptide with a binding ability to IgE" means a polypeptide capable of binding to IgE. As used herein, the term "IgE" means an antibody protein known as immunoglobulin E. IgE has an affinity to mast cells, blood basophils, or the like. In addition, reaction between an IgE antibody and an antigen (allergen) corresponding thereto causes an inflammatory reaction. In addition, IgE is known to be an antibody that causes anaphylaxis.

Specifically, the polypeptide with a binding ability to IgE may be any one of recombinant proteins including an anti-IgE antibody, an IgE Fc receptor, an extracellular domain of an alpha subunit of the IgE Fc receptor, a fragment of the extracellular domain fragment of the alpha subunit of the IgE Fc receptor, and an extracellular domain of an alpha subunit of the IgE Fc receptor or a fragment thereof.

Here, the anti-IgE antibody means an antibody capable of recognizing IgE as an antigen and binding IgE. Here, a fragment of the anti-IgE antibody may be any one selected from the group consisting of Fab, scFv, $F(ab)^2$, and Fv, as long as the fragment can bind to IgE. Antibody fragments mean antigen binding domains excluding a crystallizable region (Fc region) that performs a function (effector function) to transfer, to a cell or a complement,

stimulus due to binding with an antigen. An embodiment of the anti-IgE antibody may be omalizumab.

As used herein, the term "IgE Fc receptor" is also referred to as Fcε receptor and binds to an Fc portion of IgE. There are two types for the receptor. The receptor having high affinity to IgE Fc is called Fcε receptor I (FcεRI). The receptor having low affinity to IgE Fc is called Fcε receptor II (FcεRII). FcεRI is expressed in mast cells and basophils. In a case where IgE antibodies bound to FcεRI are cross-linked by polyvalent antigens, degranulation occurs in mast cells or basophils, thereby releasing various chemical transmitter substances including histamine. This release leads to an immediate allergic reaction.

The FcεRI is a membrane protein composed of one α chain, one β chain, and two γ chains linked by a disulfide bond. Among these chains, a portion to which IgE binds is the α chain (FcεRIα), and FcεRIα has a size of about 60 kDa. FcεRIα is composed of a hydrophobic domain existing inside the cell membrane and a hydrophilic domain existing outside the cell membrane. In particular, IgE binds to an extracellular domain of the α chain.

Specifically, the alpha subunit of the IgE Fc receptor may have the amino acid sequence set forth in NP_001992.1. In addition, the extracellular domain (FcεRIaECD) of the alpha subunit of the IgE Fc receptor may have the amino acid sequence of SEQ ID NO: 1. In the present specification, the extracellular domain of the alpha subunit of the IgE Fc receptor may be a fragment or variant of the extracellular domain of the alpha subunit of the IgE Fc receptor, as long as the fragment or variant is capable of binding to IgE.

Production of the variant can be achieved through a method of substituting, deleting, or adding one or more proteins in the wild-type FcεRIaECD (extracellular domain), as long as the method does not alter a function of the α chain of FcεRI. Such various proteins or peptides may be 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 1. In addition, the FcεRIaECD of SEQ ID NO: 1 may be encoded by a polynucleotide having the sequence of SEQ ID NO: 5.

Therefore, the extracellular domain itself of the alpha subunit of the IgE Fc receptor or a fragment of the extracellular domain of the alpha subunit of the IgE Fc receptor can be used as the polypeptide with a binding ability to IgE. An embodiment of the fragment of the extracellular domain may be in a form in which some of the amino acids at the N-terminus of

the extracellular domain of the alpha subunit of the IgE Fc receptor are deleted. In an embodiment, the fragment of the extracellular domain may be one in which 1 to 30 amino acids at the N-terminus are deleted. In addition, the fragment of the extracellular domain may be one in which 5 to 25 amino acids at the N-terminus are deleted. In addition, the fragment of the extracellular domain may be in a form in which 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids at the N-terminus are deleted. In addition, an embodiment of the fragment of the extracellular domain may be in a form in which some of the amino acids at the C-terminus of the extracellular domain of the alpha subunit of the IgE Fc receptor are deleted. In an embodiment, the fragment of the extracellular domain may be one in which 1 to 30 amino acids at the C-terminus are deleted. In addition, the fragment of the extracellular domain may be one in which 5 to 25 amino acids at the C-terminus are deleted. In addition, the fragment of the extracellular domain may be in a form in which 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids at the C-terminus are deleted. In addition, an embodiment of the fragment of the extracellular domain may be in a form in which some of the amino acids at the N-terminus and C-terminus of the extracellular domain of the alpha subunit of the IgE Fc receptor are deleted. In an embodiment, the fragment of the extracellular domain may be in a form in which 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids, respectively, at the N-terminus and C-terminus are deleted.

However, the extracellular domain of the alpha subunit of the wild type IgE receptor, or a fragment thereof, is poorly persistent in the body. In order to improve this, the extracellular domain of the alpha subunit of the IgE receptor, or a fragment thereof, can be modified through various methods. As an embodiment of the modification method, polyethylene glycol (PEG) may be bound thereto. As another embodiment of the modification method, an Fc region of an immunoglobulin may be bound thereto. Here, in addition to a native form of immunoglobulin Fc, a modified Fc region may be used.

In addition, as used herein, the term "modified Fc region" means a region in which a part of an Fc portion of an antibody has been modified. Here, the term "Fc region" refers to a protein which contains heavy chain constant region 2 (CH2) and heavy chain constant region 3 (CH3) of an immunoglobulin, and does not contain variable regions of heavy and light chains and light chain constant region 1 (CH1) of an immunoglobulin. In particular, the

modified Fc region means a region obtained by substituting some amino acids in the Fc region or by combining different types of Fc regions. Specifically, the modified Fc region may have the amino acid sequence of SEQ ID NO: 2. In addition, the modified Fc region of SEQ ID NO: 2 may be encoded by a polynucleotide having the sequence of SEQ ID NO: 6.

In addition, the modified Fc region of the present invention may be in the form of having sugar chains in a native form, increased sugar chains relative to a native form, or decreased sugar chains relative to a native form, or may be in the form of being sugar chain-removed. Immunoglobulin Fc sugar chains may be modified by conventional methods such as chemical methods, enzymatic methods, and genetic engineering methods using microorganisms.

Here, the modified Fc region of the present invention may be a region that lacks antibody dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC) functions due to having no binding site for FcγR or C1q.

In addition, the FcεRIα-ECD or a fragment thereof may be linked to a wild-type Fc or modified Fc region via a linker. The linker may be composed of 20 to 60 consecutive amino acids, 25 to 50 consecutive amino acids, or 30 to 40 amino acids. In an embodiment, the linker may be composed of 30 or 49 amino acids as shown below. Also, the linker may contain at least one cysteine. Specifically, the linker may contain one, two, or three cysteines. Preferably, the linker contains one cysteine. In an embodiment, the linker may be a hinge region derived from an IgD antibody. In addition, the linker may be a hinge variant obtained by modifying the hinge region of the IgD antibody. The hinge variant may be obtained by modifying some in a hinge sequence of the IgD antibody in order to minimize generation of truncated forms during a protein production process.

In an embodiment, the hinge may contain the following sequence:

Arg Asn Thr Gly Arg Gly Gly Glu Glu Lys Lys Xaa1 Xaa2 Lys Glu Lys Glu Glu Gln Glu Glu Arg Glu Thr Lys Thr Pro Glu Cys Pro (SEQ ID NO: 17), where Xaa1 may be Lys or Gly, and Xaa2 may be Glu, Gly, or Ser. Specifically, the linker may have the amino acid sequence of SEQ ID NO: 3 and SEQ ID NO: 19, thereby minimizing generation of truncated forms during a protein production process.

In another embodiment, the hinge may contain the following sequence:

Ala Gln Pro Gln Ala Glu Gly Ser Leu Ala Lys Ala Thr Thr Ala Pro Ala Thr Thr Arg Asn Thr Gly Arg Gly Gly Glu Glu Lys Lys Xaa3 Xaa4 Lys Glu Lys Glu Glu Gln Glu Glu Arg Glu Thr Lys Thr Pro Glu Cys Pro (SEQ ID NO: 18), where Xaa3 may be Lys or Gly, and Xaa4 may be Glu, Gly, or Ser. Specifically, the linker may have the amino acid sequence of SEQ ID NO: 4, thereby minimizing generation of truncated forms during a protein production process.

In particular, in the linker having the amino acid sequence of SEQ ID NO: 4, at least one of Thr's may be glycosylated. Specifically, among the amino acids of SEQ ID NO: 18, the $13^{th}$, $14^{th}$, $18^{th}$, and $19^{th}$ Thr's may be glycosylated. Preferably, all four amino acids may be glycosylated. Here, the glycosylation may be O-glycosylation.

$IgE_{TRAP}$, which is an embodiment of the polypeptide with a binding ability to IgE of the present invention, means an Fc-fusion protein of an FcεRIα extracellular domain and an IgD/IgG4 hybrid Fc domain. IgE Fc receptor FcεR consists of α-chain, β-chain, and two identical disulfide-linked γ-chains. FcεRIβ and FcεRIγ have no extracellular domain. However, FcεRIα has two extracellular immunoglobulin-related domains and is involved in IgE binding. Thus, in order to produce a more safe and effective IgE inhibitor, a human FcεRIα extracellular domain was linked to a human IgD/IgG4 hybrid Fc domain (FIGS. 1 and 2) to produce $IgE_{TRAP}$. Unlike omalizumab, $IgE_{TRAP}$ does not bind to IgG receptors and is likely to decrease risk of IgG-mediated anaphylaxis (FIGS. 9 to 13). In addition, $IgE_{TRAP}$ has an affinity to IgE which is 69-fold higher than omalizumab. Therefore, $IgE_{TRAP}$ would be more safe and effective than omalizumab as a therapeutic agent for food allergy.

The polypeptide with a binding ability to IgE serves to block binding between FcεRIα on effector cells and IgE. The human IgD/IgG4 hybrid Fc contains the upper CH2 domain of IgD and the last CH2 and CH3 domains of IgG4, which do not have a binding site for FcγR or C1q (FIG. 1). However, this hybrid Fc may have a binding site for a neonatal Fc receptor (FIG. 1). In addition, a theoretical molecular weight of $IgE_{TRAP}$ in homodimeric form is about 97.6 kDa. However, an actual molecular weight thereof is about 150 kDa due to glycosylation (FIG. 6).

The polypeptide dimeric protein with a binding ability to IgE according to an embodiment of the present invention not only has excellent safety and persistence in the body

as compared with conventionally used anti-IgE antibodies, but also binds to IgE very strongly due to having a binding capacity to IgE which is about 70-fold higher than the conventionally used anti-IgE antibody, omalizumab, which allows an extended administration cycle. In addition, the polypeptide dimeric protein according to the present invention is a substance obtained by applying a modified Fc, which has IgE alone as a single target and does not bind to an Fc gamma receptor, and thus lacks antibody dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC) functions. Therefore, unlike conventional anti-IgE antibodies containing an IgG1 Fc region, the polypeptide dimeric protein does not bind to an Fc gamma receptor, and thus can inhibit release of mediators caused by being bound to the Fc gamma receptor on the surface of mast cells. Therefore, the polypeptide with a binding ability to IgE of the present invention can minimize severe side effects such as occurrence of anaphylaxis which can be caused by binding between IgG1 and Fc gamma receptor III on mast cells. Accordingly, the polypeptide dimeric protein according to the present invention can be utilized as a new pharmaceutical composition which can replace therapeutic agents containing a conventional anti-IgE antibody.

In addition, an embodiment of the polypeptide with a binding ability to IgE which is provided by the present invention may be in the form of a monomer. In particular, in a case where there is no cysteine in the linker used, the polypeptide may be in a monomeric form.

In addition, an embodiment of the polypeptide with a binding ability to IgE, which is provided by the present invention, may be a polypeptide dimer. Here, as described above, the polypeptide dimer may be in a form in which two monomers are bound to each other and each monomer is obtained by binding between an extracellular domain of an alpha subunit of an IgE Fc receptor and a modified Fc region. The polypeptide dimer may be in a form in which the same two monomers are bound to each other by cysteine located at a linker site. In addition, the polypeptide dimer may be in a form in which two different monomers are bound to each other. For example, in a case where the two monomers are different from each other, the polypeptide dimer may be in a form in which one monomer contains the extracellular domain of the alpha subunit of the IgE Fc receptor, and the other monomer contains a fragment of the extracellular domain of the alpha subunit of the IgE Fc receptor. Here, an embodiment of the monomer may have the amino acid sequence of SEQ ID NO: 20, SEQ ID

NO: 21, or SEQ ID NO: 22.

In another aspect of the present invention, there is provided a pharmaceutical composition for treating or preventing an allergic disease, comprising a composition that contains, as active ingredients, probiotics and a polypeptide with a binding ability to IgE.

The probiotics and the polypeptide with a binding ability to IgE are as described above. A mixing amount of the probiotics and the polypeptide with a binding ability to IgE in the composition can be appropriately determined. In an embodiment, the probiotics in the composition may be contained in an amount of $1 \times 10^5$ cfu to $1 \times 10^{12}$ cfu. Alternatively, the probiotics in the composition may be contained in an amount of $1 \times 10^6$ cfu to $1 \times 10^{11}$ cfu, $1 \times 10^7$ cfu to $1 \times 10^{10}$ cfu, or $1 \times 10^9$ cfu to $5 \times 10^9$ cfu. In addition, the polypeptide with a binding ability to IgE may be contained in an amount of, but is not limited to, 0.1 ug to 5 mg, 0.5 ug to 1 mg, 1 ug to 500 ug, 10 ug to 400 ug, or 200 ug to 300 ug. In addition, a mixing ratio of the probiotics and the polypeptide with a binding ability to IgE in the composition can be appropriately altered.

In the present specification, "allergic disease" means a pathological symptom caused by an allergic reaction mediated by mast cell activation such as mast cell degranulation. Such allergic diseases include food allergy, atopic dermatitis, asthma, allergic rhinitis, allergic conjunctivitis, allergic dermatitis, allergic contact dermatitis, anaphylaxis, urticaria, pruritus, insect allergy, chronic idiopathic urticaria, drug allergy, and the like. In particular, the allergic diseases may be IgE-mediated.

In an embodiment of the present invention, a polypeptide with a binding ability to IgE which contains an FcεRIα extracellular domain blocks binding of IgE to FcεRI on effector cells through its binding with IgE, and thus can be referred to as $IgE_{TRAP}$. In addition, it was identified that *B. longum* can improve a therapeutic effect of $IgE_{TRAP}$ and remarkably decrease a dose of $IgE_{TRAP}$ required for treatment.

In the composition for treating or preventing an allergic disease of the present invention, an active ingredient may be contained in any amount (effective amount) depending on use, formulation, blending purpose, and the like, as long as the active ingredient can exhibit anti-allergic activity. A typical effective amount of the active ingredient may be determined within a range of 0.001% by weight to 20.0% by weight based on a total weight

of the composition. Here, "effective amount" refers to an amount of an active ingredient which is capable of inducing an anti-allergic effect. Such an effective amount can be determined experimentally within the ordinary skill of those skilled in the art.

Here, the pharmaceutical composition may further contain a pharmaceutically acceptable carrier. For the pharmaceutically acceptable carrier, any carrier can be used as long as the carrier is a non-toxic substance suitable for delivery to a patient. Distilled water, alcohol, fat, wax, and an inert solid may be contained as carriers. Pharmacologically acceptable adjuvants (buffers and dispersants) may also be contained in the pharmaceutical composition. In particular, any pharmaceutically acceptable formulation can be used as long as the probiotics and the polypeptide with a binding ability to IgE can maintain their stability in the formulation.

Specifically, the pharmaceutical composition of the present invention contains, in addition to active ingredients, a pharmaceutically acceptable carrier, and may be made into an oral or parenteral formulation depending on a route of administration by a conventional method known in the art. Here, the term "pharmaceutically acceptable" means not having more toxicity than a subject to be applied (prescribed) can accommodate without inhibiting activity of the active ingredient.

In a case where the pharmaceutical composition of the present invention is made into an oral formulation, the pharmaceutical composition may be made into formulations such as powders, granules, tablets, pills, sugar coating tablets, capsules, liquids, gels, syrups, suspensions, and wafers, together with suitable carriers, in accordance with methods known in the art. Here, examples of suitable pharmaceutically acceptable carriers can include sugars such as lactose, glucose, sucrose, dextrose, sorbitol, mannitol, and xylitol, starches such as corn starch, potato starch, and wheat starch, celluloses such as cellulose, methylcellulose, ethylcellulose, sodium carboxymethyl cellulose, and hydroxypropylmethyl cellulose, polyvinyl pyrrolidone, water, methylhydroxybenzoate, propylhydroxybenzoate, magnesium stearate, mineral oil, malt, gelatin, talc, polyol, vegetable oil, and the like. In a case of being made into preparations, the preparations can be carried out, as necessary, by including diluents and/or excipients such as a filler, an extender, a binder, a wetting agent, a disintegrant, and a surfactant.

In a case where the pharmaceutical composition of the present invention is made into a parenteral formulation, the pharmaceutical composition may be made into preparations in the form of injections, transdermal drugs, nasal inhalers, and suppositories, together with suitable carriers, in accordance with methods known in the art. In a case of being prepared into injections, sterilized water, ethanol, polyol such as glycerol and propylene glycol, or a mixture thereof may be used as a suitable carrier. For the carrier, isotonic solutions such as Ringer's solution, phosphate buffered saline (PBS) containing triethanolamine, sterile water for injection, and 5% dextrose, and the like may be preferably used.

Preparation of the pharmaceutical composition is known in the art, and specifically, reference can be made to Remington's Pharmaceutical Sciences (19th ed., 1995) and the like. The document is considered part of the present specification.

A preferable daily dosage of the pharmaceutical composition of the present invention is ranged from 0.01 ug/kg to 10 g/kg, and preferably from 0.01 mg/kg to 1 g/kg, depending on the patient’s condition, body weight, sex, age, disease severity, or route of administration. Administration may be carried out once or several times a day. Such a dosage should in no way be interpreted as limiting the scope of the present invention.

The subject to which the composition of the present invention can be applied (prescribed) is a mammal and a human, with a human being particularly preferred. The composition for anti-allergy of the present invention may further comprise, in addition to the active ingredient, any compound or natural extract, on which safety has already been verified and which is known to have anti-allergic activity, for the purpose of raising and reinforcing the anti-allergic activity. Here, the pharmaceutical composition may further comprise extracellular endoplasmic reticulum isolated from *Bifidobacterium longum* KACC 91563.

In yet another aspect of the present invention, there is provided a health functional food composition for ameliorating or alleviating an allergic symptom, comprising a composition that contains, as active ingredients, probiotics and a polypeptide with a binding ability to IgE. Here, the food composition may further comprise, as an active ingredient, extracellular endoplasmic reticulum isolated from *Bifidobacterium longum* KACC 91563.

Meanwhile, the food composition of the present invention may further comprise a sitologically acceptable carrier. In addition, the food composition may be used together with

another food or food ingredient, and may be suitably used according to conventional methods. A mixing amount of the active ingredient can be suitably determined according to its intended use (prevention, health, or therapeutic treatment).

There is no particular limitation on a type of the food. Examples of the food include meats, sausages, bread, chocolates, candies, snacks, confections, dairy products including ice cream, various soups, beverages, tea, drinks, alcoholic beverages, and vitamin complexes. All functional health foods in a conventional sense are included. Foods to which the above substances may be added contain ingredients that are typically added during manufacture, and examples of the ingredients include proteins, carbohydrates, fat, nutrients, flavoring agents, and seasonings. The above-mentioned carbohydrates are typical sugars, for example, a monosaccharide such as glucose and fructose, a disaccharide such as maltose and sucrose, and a polysaccharide such as dextrin and cyclodextrin, and sugar alcohol such as xylitol, sorbitol, and erythritol. As the flavoring agent, a natural flavoring agent such as thaumatin and a stevia extract, a synthetic flavoring agent such as saccharin and aspartame, or the like may be used.

For example, in a case where the food composition of the present invention is prepared as a drink, citric acid, liquid fructose, sugar, glucose, acetic acid, malic acid, juice, extract, or the like may be further contained in addition to the composition of the present invention.

In addition to the above, the composition of the present invention may contain various nutrients, vitamins, electrolytes, flavors, colorants, pectic acid and salts thereof, alginic acid and salts thereof, organic acids, protective colloid thickeners, pH adjusting agents, stabilizers, preservatives, glycerin, alcohol, carbonating agent used in carbonated beverages, and the like. In addition, the composition of the present invention may contain flesh for the production of fruit juice beverages and vegetable beverages. These ingredients may be used independently or in admixture.

In addition, the food composition of the present invention may fall within any product category in legal or functional classification as long as the food composition complies with the enforcement regulations at the time of being manufactured and distributed. For example, the food composition may be a health functional food according to the Health

Functional Foods Act, or may fall within confectioneries, beans, teas, beverages, special-purpose foods, or the like according to each food type in the Food Code of Food Sanitation Act (standards and specifications for food, notified by Food and Drug Administration). With regard to other food additives that may be contained in the food composition of the present invention, reference can be made to the Food Code or the Food Additive Code according to the Food Sanitation Act.

In still yet another aspect of the present invention, there is provided a kit for treating or preventing an allergic disease, comprising a first composition that contains probiotics; and a second composition that contains a polypeptide with a binding ability to IgE. Here, the second composition may be a composition for subcutaneous or intravenous administration.

In still yet another aspect of the invention, there is provided a method for treating or preventing an allergic disease, comprising a step of administering probiotics; and administering a polypeptide with a binding ability to IgE.

The probiotics are as described above and may be orally administered. Here, the polypeptide with a binding ability to IgE may be orally administered, and may be parenterally administered. Here, parenteral administration can be carried out by a method such as subcutaneous administration, intravenous administration, mucosal administration, or the like.

In a mouse food allergic model, it has been shown that $IgE_{TRAP}$ not only lowers a free IgE level but also decreases the number of mast cells, thereby alleviating food allergic symptoms (FIGS. 21 to 26). A decreased number of mast cells caused by $IgE_{TRAP}$ is expected to be due to the fact that IgE increases the number of mast cells by increasing a survival rate of the mast cells. In addition, over-proliferation of goblet cells in the small intestine was significantly inhibited by administration of $IgE_{TRAP}$ and *B. longum* alone, and was even further inhibited at the time of combined administration (FIGS. 23 to 26). Since Th2 cytokines such as IL-13 are known to induce over-proliferation of goblet cells, it is expected that $IgE_{TRAP}$, *B. longum*, and a combination thereof mitigate an environment for Th2 cytokines in the small intestine. As support for this, $IgE_{TRAP}$ and *B. longum* showed a tendency to decrease mRNA expression of IL-33, which is involved in promoting IL-13 secretion, in the small intestine, by activating type 2 innate lymphoid cells (ILC2s); and a combination thereof significantly decreased the expression of IL-33 in a more effective

manner (FIG. 28). In addition, IL-33 is not only secreted in IgE-activated mast cells but also involved in promoting degranulation of mast cells. Thus, IL-33 has a close correlation with severity of food allergies. This suggests that $IgE_{TRAP}$ and *B. longum* can ameliorate food allergic symptoms by various mechanisms.

Previously, *B. longum* has been reported to induce apoptosis of mast cells and to improve food allergic symptoms, which is consistent with the results of the present inventors. However, daily administration of *B. longum* for the treatment of food allergy was less effective than administration of $IgE_{TRAP}$ alone. However, *B. longum* remarkably improved a therapeutic effect of $IgE_{TRAP}$ (FIG. 18), and $IgE_{TRAP}$ used in combination with *B. longum* exhibited a therapeutic effect which is similar to that obtained by administration of $IgE_{TRAP}$ alone at a 10-fold higher dose (FIG. 19B). In addition, it has been reported that some intestinal bacteria can ameliorate an allergic disease by increasing Treg cells or decreasing levels of IgE and Th2 cytokines. Thus, other probiotics in addition to *B. longum* are expected to be capable of improving a therapeutic effect of $IgE_{TRAP}$. Indeed, it was identified that an elevated therapeutic effect is exhibited at the time of combined administration of various types of probiotics and $IgE_{TRAP}$ (FIGS. 29 to 34).

In still yet another aspect of the present invention, there is provided a method for treating or preventing an allergic disease, comprising a step of administering, to an individual, the polypeptide with a binding ability to IgE and the probiotics in combination. The individual may be a mammal, preferably a human. Here, administration may be performed orally or parenterally. Here, the polypeptide with a binding ability to IgE and the probiotics can be prepared into suitable formulations for oral administration. In addition, parenteral administration may be performed by methods such as subcutaneous administration, intravenous administration, mucosal administration, and muscular administration.

## DETAILED DESCRIPTION OF THE INVENTION

Hereinafter, the present invention will be described in more detail with reference to the following examples. However, the following examples are intended to merely illustrate the present invention, and the scope of the present invention is not limited only thereto.

## Materials and methods

### Cell line construction for IgETRAP and purification thereof

A nucleotide sequence of $IgE_{TRAP}$ was constructed by linking the C-terminus ($26^{th}$ to $205^{th}$) of the FcεRIα extracellular domain to the N-terminus of the IgD/IgG4 hybrid Fc domain (IgD, $133^{rd}$ to $170^{th}$; IgG4, $121^{st}$ to $327^{th}$). The protein was expressed in dihydrofolate reductase-deficient Chinese hamster ovary DG44 cells. $IgE_{TRAP}$ was purified using the HiTrap rProtein A FF column (GE Healthcare), and its purity was identified by SDS-PAGE under reducing and non-reducing conditions.

### 3D structure modeling

A structural model of $IgE_{TRAP}$ was designed using WinCoot and was built with the PyMOL software on the basis of information on FcεRIα (PDB accession 1F6A) and IgD/IgG4 Fc (PDB accession 1ADQ) of the Protein Data Bank.

### Surface plasmon resonance (SPR) assay

SPR assay was conducted using the ProteOn XPR36 (Bio-Rad) apparatus. A degree of binding of omalizumab and $IgE_{TRAP}$ to human IgE (Calbiochem) was identified using kinetic analysis. 850 response units (RUs) of omalizumab in acetate buffer (pH 5.5) and 500 RUs of $IgE_{TRAP}$ in acetate buffer (pH 4.0) were immobilized on the ProteOn™ GLC sensor chip (Bio-Rad). PBS containing Tween-20 was used as a running buffer and a flow rate was set at 30 ul/min. A graph of each data set was analyzed using the ProteOn Manager software (Bio-Rad).

### Biolayer interferometry (BLI) assay

A degree of binding of $IgE_{TRAP}$ and omalizumab to IgG receptors was identified using the Octet RED384 system (Pall ForteBio, CA, USA). FcγRI, FcγRIIA, FcγRIIB, FcγRIIIA, and FcγRIIIB recombinant proteins (R & D Systems Inc., 5 μg/ml) which had been diluted in 300 mM acetate buffer (pH 5) were immobilized on the Amine Reactive 2 Generation (AR2G) biosensor activated by a combination of 400 mM EDC and 10 mM sulfo-NHS. Then, association with and dissociation from $IgE_{TRAP}$ and omalizumab at various concentrations were measured, respectively, for 300 seconds. Here, the kinetic buffer used was PBS containing 0.1% Tween-20 and 1% bovine serum, and all experiments were carried out at 30°C with a sample plate shaker at a rate of 1,000 rpm.

**β-Hexosaminidase release assay**

Bone marrow-derived mast cells (BMMCs) were cultured at 37°C in RPMI containing 10% heat-inactivated FBS, 10 ng/mL mouse IL-3 (PeproTech, Inc.), and 50 ng/mL mouse SCF (PeproTech, Inc.). Before analysis, 1 ug/mL of anti-dinitrophenyl IgE (Sigma-Aldrich) and various concentrations of $IgE_{TRAP}$ were incubated at room temperature for 30 minutes. The bone marrow-derived mast cells were incubated in a mixture of anti-dinitrophenyl IgE (Sigma-Aldrich) and $IgE_{TRAP}$ at 37°C for 30 minutes, and 0.1 μg/ml of anti-dinitrophenyl IgE was added thereto. The resultant was incubated again at 37°C for 30 minutes. Culture supernatant was collected and incubated with 3 mM p-nitrophenyl-N-acetyl-β-D-glucosaminide at 37°C for 20 minutes. 0.1 M sodium carbonate buffer (pH 10) was added to stop the reaction, and absorbance at 405 nm was measured. A ratio of released β-hexosaminidase was calculated by comparison with a total intracellular content of BMMCs dissolved with 0.1% Triton X-100.

**Food allergy induction and administration of *B. longum***

On days 0 and 14, 50 ug of OVA (Grade V; Sigma-Aldrich) and 1 mg of aluminum potassium sulfate adjuvant (Sigma-Aldrich) were administered intraperitoneally into mice. After 14 days, the mice were orally administered 50 mg of OVA (Grade III; Sigma-Aldrich) 5 times at 2-day intervals. The mice were fasted for about 4 to 5 hours prior to oral administration of OVA. Diarrhea occurrence was evaluated by monitoring the mice for up to 1 hour after OVA inoculation. *B. longum* was lyophilized and mixed with powdered mouse feed at $3 \times 10^9$ cfu/g. The mice were allowed *ad libitum* access to the feed. In order to maintain freshness, the mouse feed mixed with *B. longum* was replaced every 2 to 3 days.

**Histology**

The jejunum of the small intestine was fixed with 4% paraformaldehyde and embedded in paraffin to make a block. Then, a paraffin section slide was produced. The slide was deparaffinized and mast cells were stained with a naphthol AS-D chloroacetate esterase kit (Sigma-Aldrich). For goblet cells, the slide was stained with a periodic acid-Schiff stain kit (ScyTek Laboratories, Inc.). An image of the stained slide was taken using Pannoramic MIDI (3D HISTECH Ltd.).

**ELISA**

Mouse Total IgE ELISA kit (BioLegend) and MCPT-1 ELISA kit (Invitrogen) were used according to the manufacturers' protocol to measure total concentrations of IgE and MCPT-1 in mouse serum. In order to measure free IgE, the plate was coated with 1 mg/mL of $IgE_{TRAP}$ and allowed to react overnight at 4°C. The rest of the analysis was conducted according to the manufacturer's protocol for the Mouse Total IgE ELISA kit.

**Statistical analysis**

Statistical analysis for all data was performed using the GraphPad Prism 5 software (GraphPad Software Inc.). Kaplan-Meier survival curve analysis with log-rank (Mantel-Cox) assay was used to calculate diarrhea occurrence. One-way ANOVA with Newman-Keuls multiple comparison test was used to identify meaningful differences in the test.

**I. Preparation and characterization of IgETRAP**

**Example 1. Preparation of polypeptide containing FcεRIα-ECD and modified Fc region**

A C-terminal modified polypeptide of the extracellular domain (FcεRIα-ECD) of the alpha subunit of the IgE Fc receptor was prepared according to the method disclosed in U.S. Patent No. 7,867,491.

First, a fusion protein that contains the extracellular domain of the α-chain of FcεRI having the amino acid sequence of SEQ ID NO: 1 and the modified immunoglobulin Fc of SEQ ID NO: 2 was prepared. Specifically, in order to express a protein (FcεRIαECD-Fc1), a protein (FcεRIαECD-Fc2), and a protein (FcεR1αECD-Fc3), which were linked, respectively, via a hinge of SEQ ID NO: 19, a hinge of SEQ ID NO: 3, and a hinge of SEQ ID NO: 4, cassettes obtained by linking the gene encoding each protein were cloned into the pAD15 vectors (Genexin, Inc.) to construct FcεRIαECD-Fc protein expression vectors. Then, each of the expression vectors was transduced into CHO DG44 cells (from Dr. Chasin, Columbia University, USA).

Here, at the time of being transduced into the cell line, an expression vector obtained by cloning an α-2,6-sialic acid transferase gene into the pCI Hygro vector (Invitrogen) was simultaneously transduced to separately prepare cell lines which were capable of expressing FcεRIαECD-Fc2ST and FcεRIα ECD-Fc3ST proteins to which sialic acid was added.

As a primary screening procedure, HT selection was carried out using 5-

hydroxytryptamine (HT)-free 10% dFBS medium (Gibco, USA, 30067-334), MEMα medium (Gibco, 12561, USA, Cat No. 12561-049), and HT+ medium (Gibco, USA, 11067-030). Then, methotrexate (MTX) amplification was performed using HT-selected clones to amplify productivity using the dihydrofolate reductase (DHFR)-system.

After completion of the MTX amplification, subculture was carried out about 1 to 5 times for cell stabilization for the purpose of evaluation of productivity. Thereafter, unit productivity evaluation of the MTX-amplified cells was performed. The results are shown in Table 1 below.

[Table 1]

| Version | Media | MTX concentration | Productivity | | |
|---|---|---|---|---|---|
| | | | 3-day culture | | Batch culture (mg/ml) |
| | | | ug/mL | ug/$10^6$cells | |
| FcεRIαECD-Fc2 | Ex-cell DHFR | 500nM | 37.23 | 20.9 | 225 |
| FcεRIαECD-Fc2 + a2,6-ST | | 100nM | 45.4 | 25.1 | 338.2 |
| FcεRIαECD-Fc3 | | 2uM | 27.0 | 16.9 | 180.4 |
| FcεRIαECD-Fc3 + a2,6-ST | | 1uM | 17.5 | 10.2 | 101.7 |

As shown in Table 1, the FcεRIαECD-Fc3 cell line exhibited productivity of 16.9 ug/$10^6$ cells after the methotrexate amplification at 2 uM. On the other hand, the FcεRIαECD-Fc3 cell line (FcεRIαECD-Fc3ST) co-transduced with 2,6-sialic acid transferase exhibited productivity of 17.5 ug/$10^6$ cells after the methotrexate amplification at 1 uM. In addition, the FcεRIαECD-Fc2 cell line exhibited productivity of 20.9 ug/$10^6$ cells under the methotrexate amplification condition at 0.5 uM. In addition, the FcεRIαECD-Fc2 cell line (FcεRIαECD-Fc2ST) co-transduced with 2,6-sialic acid transferase exhibited productivity of 25.1 ug/$10^6$ cells after the methotrexate amplification at 0.1 uM. That is, it was identified that the FcεRIαECD-Fc2 cell line co-transfected with 2,6-sialic acid transferase, which had been selected under the methotrexate amplification condition at 0.1 uM, exhibited the most excellent productivity.

**Example 2. Purification of FcεRIα ECD fusion protein and identification of purity thereof**

Among the cell lines selected in Example 1 above, i) FcεRIαECD-Fc3, ii) FcεRIαECD-Fc3ST, and iii) FcεRIαECD-Fc2ST were cultured at a 60 ml scale by a batch culture method. The resulting cultures were purified using a Protein-A affinity column, and

then purified proteins were subjected to SDS-PAGE and size-exclusion HPLC (SE-HPLC) to identify purity of the proteins.

As shown in FIGS. 3A and 3B, it was identified that all respective proteins purified by the SE-HPLC method have purity of 93% or higher. In addition, as a result of SDS-PAGE analysis, it was identified that proteins having sizes of about 150 kDa and about 75 kDa were detected, respectively, in the non-reducing and reducing conditions (FIG. 3A, Lanes 1 to 6). From this, it was found that the Fc-bound FcεRIαECD forms a dimer. In addition, no impurities such as a truncated form were observed in the SDS-PAGE results. In particular, even after the process of thawing/freezing (FIG. 3A, Lanes 7 to 9), it was identified that all proteins have purity of 93% or higher, and has no impurities. Here, Gel-IEF was performed under the following test conditions to identify a degree of sialic acid content in the proteins following introduction of the sialic acid transferase. From this, it was identified that a content of acidic proteins was increased due to increased sialic acid content.

[Table 2]

| Test conditions | |
|---|---|
| Gel | pH3-10 IEF gel 1.0mm |
| Sample buffer | IEF sample buffer (2X) |
| Loading condition | 100 V 1hr, 200 V 1hr, 500 V 2hr |

In order to identify reproducibility of purification yield, the FcεRIαECD-Fc2ST cell line was batch-cultured in a 1 L flask at a 250 ml scale and purified using a Protein-A affinity column. Subsequently, the culture supernatant and the purified product were subjected to running on a 4% to 15% TGX$^{TM}$ gel (Bio-Rad Laboratories, Inc.) for 30 minutes at a condition of Tris-Glycine SDS (TGS) buffer and 200 V, and then subjected to SDS PAGE analysis. As a result, it was identified that proteins with very high purity (98% or higher) were purified even by only the first step purification and proteins were expressed with very high purity even in the culture supernatant. This indicates that process development steps can be simplified in developing the FcεRIαECD-Fc protein, which was expressed in the cell line in question, into a medical product, and as a result, it is highly likely for the development cost of the medical product to be remarkably decreased.

**Experimental Example 1. Identification of binding ability of FcεRIα ECD fusion protein to IgE**

A binding ability to IgE was comparatively measured for the four proteins, i) FcεRIαECD-Fc2, ii) FcεRIαECD-Fc2ST, iii) FcεRIαECD-Fc3, and iv) FcεRIαECD-Fc3ST which had been purified through the method of Example 2 above, and the commercially available anti-IgE antibody, omalizumab (trade name: Xolair). Specifically, the binding ability to IgE was measured by coating IgE on the channel of the Protein GLC sensor chip (Bio-Rad Laboratories, Inc.), and causing omalizumab or each FceR1αECD-Fc protein at various concentrations to flow at a rate of 30 μl per minute.

The experiments were conducted by identifying zero base using 25 mM NaOH as a regeneration buffer, and then repeating the above steps. Thereafter, a binding curve was identified using a protein binding analyzer (ProteOn XPR36, Bio-Rad Laboratories, Inc., USA). The results are shown in Table 3, and FIGS. 7 and 8. $IgE_{TRAP}$ in FIG. 8 means FceR1αECD-Fc2ST, which is an embodiment of the polypeptide with a binding ability to IgE of the present invention.

[Table 3]

| Samples / items | | FcεRIa ECD-Fc | | Omalizumab | Remarks |
|---|---|---|---|---|---|
| Drug type | | Fc fusion protein | | Anti-IgE Ab | |
| Binding affinity | ka (association rate) | Fc2 | $2.14 \times 10^{5}$ | $4.05 \times 10^{5}$ | 1.9-fold weaker than omalizumab |
| | | Fc2ST | $2.64 \times 10^{5}$ | | 1.5-fold weaker than omalizumab |
| | | Fc3 | $1.98 \times 10^{5}$ | | 2.0-fold weaker than omalizumab |
| | | Fc3ST | $2.40 \times 10^{5}$ | | 1.7-fold weaker than omalizumab |
| | kd (dissociation rate) | Fc2 | $8.29 \times 10^{-5}$ | $6.02 \times 10^{-3}$ | 73-fold better than omalizumab |
| | | Fc2ST | $5.69 \times 10^{-5}$ | | 106-fold better than omalizumab |
| | | Fc3 | $1.33 \times 10^{-4}$ | | 45-fold better than omalizumab |
| | | Fc3ST | $1.49 \times 10^{-4}$ | | 40-fold better than omalizumab |
| | KD (kd/ka) | Fc2 | $3.88 \times 10^{-10}$ | $1.49 \times 10^{-8}$ | 38-fold better than omalizumab |
| | | Fc2ST | $2.16 \times 10^{-10}$ | | 69-fold better than omalizumab |
| | | Fc3 | $6.72 \times 10^{-10}$ | | 22-fold better than omalizumab |
| | | Fc3ST | $6.21 \times 10^{-10}$ | | 24-fold better than omalizumab |

As shown in Table 3, the association rate (ka) value of the polypeptide dimer according to an embodiment of the present invention was measured to be 1.5- to 2.0-fold lower than that of omalizumab. That is, it was found that a binding ability thereof to substances other than IgE was 1.5- to 2.0-fold lower than that of omalizumab. In addition, the dissociation rate (kd) value of the polypeptide dimer according to an embodiment of the present invention was measured to be 40- to 106-fold higher than that of omalizumab. In addition, as shown in FIGS. 7 and 8, it was able to identify that omalizumab lost its binding to IgE in a case where a certain period of time has passed after the binding, whereas once the

polypeptide dimer of the FcεRIαECD fusion protein of the present invention binds to IgE, the polypeptide dimer was not separated from IgE. That is, it can be seen that the polypeptide dimer of the present invention is not easily separated from IgE, and has a much better ability to maintain its bound state than omalizumab. As a result, it was found that the polypeptide dimer according to an embodiment of the present invention has an equilibrium dissociation constant (KD <kd/ka>) value which was 22- to 69-fold higher than omalizumab.

From this, it can be seen that the FcεRIαECD fusion protein of the present invention has a remarkably increased binding ability to IgE as compared with omalizumab. In particular, it was identified that the FcεRIαECD-Fc2 (FcεRIαECD-Fc2ST) to which sialic acid was added exhibits the highest IgE-binding capacity which was 69-fold higher than omalizumab. In particular, for FceR1αECD-Fc2ST, the association rate (Ka) and dissociation rate (Kd) of $IgE_{TRAP}$ were about 1.5-fold and 94.5-fold lower than omalizumab, respectively (FIGS. 7 and 8, and Table 4). It has previously been reported that IgE was dissociated very slowly from FcεRIα. It was identified that $IgE_{TRAP}$ was also dissociated very slowly (FIG. 8). As a result, a binding ability of $IgE_{TRAP}$ to human IgE was 69-fold higher than omalizumab (FIG. 8 and Table 4).

[Table 4]

| | Ka ($M^{-1}S^{-1}$) | Kd ($S^{-1}$) | KD (M) |
|---|---|---|---|
| $IgE_{TRAP}$ | 2.64 X $10^{5}$ | 5.69 X $10^{-5}$ | 2.15 X $10^{-10}$ |
| Omalizumab | 4.05 X $10^{5}$ | 6.02 X $10^{-3}$ | 1.49 X $10^{-8}$ |
| Comparison of KD [Omalizumab/$IgE_{TRAP}$] | | | About 69-fold |

**Experimental Example 2. Identification of binding ability of IgETRAP to IgG-mediated IgG receptors**

$IgE_{TRAP}$ does not bind to low-affinity IgG receptors associated with IgG-mediated anaphylaxis. Since anaphylaxis, a major side effect of omalizumab, was expected to be caused by the possibility of binding to a low-affinity IgG receptor, BLI assay was used to check a binding ability of $IgE_{TRAP}$ to IgG receptors while using omalizumab as a control. Specifically, a degree of binding of $IgE_{TRAP}$ and omalizumab to IgG receptors was identified using the Octet RED384 system (Pall ForteBio, CA, USA).

FcγRI, FcγRIIA, FcγRIIB, FcγRIIIA, and FcγRIIIB recombinant proteins (R & D Systems Inc., 5 μg/ml) which had been diluted in 300 mM acetate buffer (pH 5) were

immobilized on the Amine Reactive 2 Generation (AR2G) biosensor activated by a combination of 400 mM EDC and 10 mM sulfo-NHS. Then, association with and dissociation from $IgE_{TRAP}$ and omalizumab at various concentrations were measured, respectively, for 300 seconds. Here, the kinetic buffer used was PBS containing 0.1% Tween-20 and 1% bovine serum, and all experiments were carried out at 30°C with a sample plate shaker at a rate of 1,000 rpm.

As expected, omalizumab showed a significant binding ability to FcγRI, a high-affinity IgG receptor, as well as to low-affinity IgG receptors such as FcγRIIA, FcγRIIB, FcγRIIIA, and FcγRIIIB (FIGS. 9 to 14). This means that, unlike omalizumab, $IgE_{TRAP}$ cannot bind to IgG receptors such as FcγRIIA and FcγRIII, and thus has a very low risk of inducing IgG-mediated anaphylaxis (FIGS. 9 to 14). Binding abilities of omalizumab and $IgE_{TRAP}$ to the IgG receptors were quantified and shown in FIG. 14.

**Experimental Example 3. Identification of activity of FcεRIα ECD fusion protein through beta-hexosaminidase assay in mouse bone marrow-derived mast cells**

Beta-hexosaminidase assay was performed for *in vitro* activity analysis of the FcεRIαECD fusion protein of the present invention. Specifically, the FcεRIαECD-Fc2 protein according to an embodiment of the present invention was mixed, at each concentration, with mouse IgE (1 ug/mL), and incubated at room temperature (20°C) for 30 minutes to prepare samples. Mouse bone marrow-derived mast cells in culture for mast cell activation were washed with Hank's balanced salt solution (HBSS) buffer to remove the medium, and the number of cells was measured. Then, an adjustment was made so that $5 \times 10^5$ cells were injected into 40 μL of HBSS buffer.

Then, 50 uL of the sample solution prepared through the pre-incubation was added to the activated mast cells. Then, the resultant was incubated in a 5% $CO_2$ incubator at 37°C for 30 minutes. Subsequently, after the addition of each 10 μL of DNP (2,4-dinitrophenol, 100 ng/mL), which is a foreign antigen, incubation was performed again at 37°C for 30 minutes in 5% $CO_2$, and then 30 μL of the supernatant was separated. 30 uL of the separated supernatant and 30 uL of the substrate (4-nitrophenyl N-acetyl-β-D-glucosaminide, 5.84 mM) were mixed well, and then incubated at 37°C for 20 minutes in 5% $CO_2$. Then, 140 μL of 0.1 M sodium carbonate buffer (pH 10) as a stop solution was added to terminate the reaction.

Thereafter, absorbance at 405 nm was measured to identify a secretion amount of β-hexosaminidase secreted by the foreign antigen in the activated mast cells. The results are shown in FIG. 15A.

$IgE_{TRAP}$ inhibited degranulation of mast cells in a dose-dependent manner with 0.45 μg/ml of $IC_{50}$ (concentration of drug necessary to show inhibitory effect of 50%) in the presence of 1 μg/ml of mouse IgE (FIG. 15A). $IgE_{TRAP}$ completely inhibited degranulation of the bone marrow-derived mast cell at a molecular ratio of IgE:$IgE_{TRAP}$ of 0.79 (Table 5).

[Table 5]

| Anti-DNP IgE | 1 ug/mL (= 5.26 nM) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| $IgE_{TRAP}$ (ug/mL) | 0 | 0.016 | 0.063 | 0.25 | 0.5 | 1 | 2 | 4 |
| $IgE_{TRAP}$ (nM) | 0 | 0.11 | 0.42 | 1.67 | 3.33 | 6.67 | 13.3 | 26.7 |
| [IgE/$IgE_{TRAP}$] molar ratio | 0 | 47.8 | 12.5 | 3.15 | 1.58 | 0.79 | 0.40 | 0.20 |
| Average inhibition ratio (%) | 0 | 2.12 | 12.4 | 13.8 | 49.4 | 99.3 | 99.4 | 99.8 |

Specifically, as shown in FIG. 15A, the polypeptide dimer of an embodiment of the present invention exhibited a mast cell inhibition ratio of about 49.4% in a case of having half (0.5 ug/mL) the concentration of mouse IgE, and exhibited a mast cell inhibition ratio of about 99.4% in a case of having the same concentration (1 ug/mL) of mouse IgE. That is, it can be seen that IgE-induced activity of bone marrow-derived mast cells is greatly suppressed by the FceRIa-ECD polypeptide dimer of the present invention.

**Experimental Example 4. Comparison of activity of FcεRIα ECD fusion protein and anti-human IgE antibody using β-hexosaminidase assay in human FcεRI-expressing bone marrow-derived mast cells**

β-Hexosaminidase assay was conducted to identify superiority of the FcεRIα ECD fusion protein relative to Xolair through *in vitro* activity analysis. The respective drugs, FcεRIαECD-Fc2ST ($IgE_{TRAP}$) and Xolair, were prepared at each concentration, and then mixed with human IgE (1 ug/mL). Then, incubation was performed at room temperature for 30 minutes. During pre-incubation of the drug, a human FcεRI gene was introduced, and mast cells derived from and differentiated from mouse bone marrow, in which the mouse FcεRI gene had been removed, were prepared. The prepared mast cells were washed with HBSS buffer, and then $5 \times 10^5$ cells were injected into 60 μL of HBSS buffer. 20 μL of the pre-incubated sample was added to the prepared mast cells, and then incubated in a 5% $CO_2$ incubator at 37°C for 30 minutes.

Subsequently, after 20 uL of anti-human IgE antibody (BioLegend, Cat No. 325502, 0.5 ug/mL) was added to induce a similar reaction to a foreign antigen, and then the resultant was incubated again in 5% $CO_2$ incubator at 37°C for 30 minutes. Subsequently, after centrifugation at 1,500 rpm at 4°C, 30 uL of the supernatant was separated. 30 uL of the separated supernatant and 30 uL of the substrate (4-nitrophenyl N-acetyl-β-glucosaminide, 5.84 mM) were mixed well, and then incubated in a 5% $CO_2$ incubator at 37°C for 25 minutes. Then, 140 uL of 0.1 M sodium carbonate buffer (pH 10) was added to terminate the reaction.

Subsequently, absorbance at 405 nm was measured to compare relative amounts of secreted β-hexosaminidase, and a mass cell-inhibitory effect depending on each drug concentration was identified. The results are shown in FIG. 15B. As shown in FIG. 15B, $IC_{50}$ of the FcεRIα ECD fusion protein was measured to be approximately 11.16 ng/mL, and $IC_{50}$ of the Xolair protein was measured to be approximately 649.8 ng/mL. Therefore, it was identified that the FcεRIα ECD fusion protein has a 58-fold higher inhibitory ability on mast cell activity than Xolair.

**Experimental Example 5. *In vivo* assay of FcεRIα ECD fusion protein: Food allergy model**

50 ug of ovalbumin (OVA) and 1 mg of alum were intraperitoneally administered to Balb/c mice (Orientbio Inc.) two times at a 14-day interval to induce sensitization. Thereafter, 50 mg of OVA was orally administered five times in total on days 28, 30, 32, 34, and 36, to induce food allergy in intestines.

After the OVA was orally administered two times, that is, on day 31, the mice were divided into three groups, each containing 7 mice. The three divided groups were as follows: The first group receiving the FcεRIαECD-Fc2ST fusion protein at a high concentration (200 ug), the second group receiving the FcεRIαECD-Fc2ST fusion protein at a low concentration (20 ug), and the third group receiving nothing. While orally administering the OVA, it was identified whether diarrhea occurs due to food allergy induction. The mice were sacrificed on day 37, and the number of mast cells in the small intestine, the IgE concentration in blood, and the concentration of enzyme (mast cell protease-1 (MCPT-1)) of mast cell degranulation in blood were analyzed for the mice belonging to each group.

As shown in FIG. 16, it was identified that the mice belonging to the group receiving the FcεRIαECD-Fc2ST, which is a polypeptide dimer, at a high concentration exhibits an effect of alleviating food allergy in a concentration-dependent manner, as compared with the mice belonging to the group receiving nothing.

**II. Preparation of combination of polypeptide with binding ability to IgE and probiotics, and identification of its effect**

**Example 3: Culture and administration of probiotics**

*L. casei* (*Lactobacillus casei*; KACC 12413), *Lc. lactis* (*Lactococcus lactis*; KACC 13877), *L. fermentum* (*Lactobacillus fermentum*; KACC 11441), and *L. rhamnosus* (*Lactobacillus rhamnosus*; KACC 11953) were inoculated on MRS broth or Brain Heart Infusion (BHI) medium and cultured in a 37°C incubator (N-Biotek Cat # NB201L) for 24 hours. *L. reuteri* (*Lactobacillus reuteri*; KACC 11452) and *S. thermophiles* (*Streptococcus thermophiles*; KACC 11857) were cultured in a shaking incubator (N-biotek) at 37°C and 50 rpm for 24 hours in view of their aerobic nature.

The cultured probiotics were dissolved in a lyophilization medium containing 10% skim milk and 10% sucrose, and lyophilized using a freeze dryer (Labcono). Then, the resultant was powdered. For the completed probiotics, a colony forming unit (cfu) present per gram was measured by serial dilution.

The lyophilized probiotics were continuously fed at $1 \times 10^9$ to $2.5 \times 10^9$ cfu per mouse using an oral zonde at 2- to 3-day intervals during the experiment. A negative control was fed an equal amount of lyophilized medium.

**Example 4: Preparation of composition comprising probiotics and polypeptide with binding ability to IgE**

The polypeptide with a binding ability to IgE which had been obtained in Example 1 and the probiotics obtained in Example 3 were mixed to prepare a composition for treating allergy.

**Experimental Example 6. Identification of effect of FcεRIα-Fc fusion protein on amelioration of allergy**

50 ug of ovalbumin (OVA) and 1 mg of alum were intraperitoneally administered to Balb/c mice (Orientbio Inc.) two times at a 14-day interval to induce sensitization. Thereafter,

50 mg of OVA was orally administered five times in total on days 28, 30, 32, 34, and 36, to induce food allergy in intestines. The mice in which food allergy had been induced were divided into five groups, each containing 7 mice. The five divided groups were as follows: The first group receiving an FcεRIαECD recombinant protein at a high concentration (200 ug), the second group receiving the FcεRIαECD recombinant protein at a low concentration (20 ug), the third group receiving the FcεRIαECD recombinant protein at a high concentration (200 ug) plus *B. longum*, the fourth group receiving the FcεRIαECD recombinant protein at a low concentration (20 ug) plus *B. longum*, and the fifth group receiving nothing.

While orally administering the OVA, it was identified whether diarrhea occurs due to food allergy induction. The mice were sacrificed on day 37, and the number of mast cells in the small intestine, the IgE concentration in blood, and the concentration of enzyme (mast cell protease-1 (MCPT-1)) with mast cell degranulation in blood were analyzed for the mice belonging to each group. As shown in FIG. 19B, it was found that the mice belonging to the group receiving a combination of the FcεRIαECD polypeptide dimer and *B. longum* exhibits an effect of alleviating food allergy as compared with the mice belonging to the group receiving nothing.

**Experimental Example 7. Identification of effect of combined administration of IgETRAP and probiotics on amelioration of allergy**

In order to evaluate an effect of $IgE_{TRAP}$ on food allergy, dose-dependent acute diarrhea was induced in BALB/c mice to produce a mouse model with allergen-induced food allergy. Specifically, experiments were carried out in the same manner as in Experimental Example 6, except that $IgE_{TRAP}$, an FcεRIαECD recombinant protein, was administered at 100 ug/head. In addition, the probiotics *B. longum* was lyophilized and mixed with powdered mouse feed at $3 \times 10^9$ cfu/g. The mice were allowed *ad libitum* access to the feed. In order to maintain freshness, the mouse feed mixed with *B. longum* was replaced every 2 to 3 days. As a result, as shown in the following Table 6 and FIG. 18, it was found that an excellent allergy-alleviating effect was exhibited in the experiment group simultaneously receiving the probiotics *B. longum* and $IgE_{TRAP}$, an FcεRIαECD polypeptide dimer.

[Table 6]

| Group | Final number of mice | Characteristic feature | 1st OVA challenge | 2nd OVA challenge | 3rd OVA challenge | 4th OVA challenge | 5th OVA challenge |
|---|---|---|---|---|---|---|---|
| 1 | 17 | Disease Ctrl | 0/17 (0.00%) | 0/17 (0.00%) | 2/17 (11.76%) | 14/17 (82.35%) | 15/17 (88.24%) |
| 2 | 17 | *B.longum* only | 0/17 (0.00%) | 0/17 (0.00%) | 2/17 (11.11%) | 11/17 (61.11%) | 11/17 (64.71%) |
| 3 | 18 | IgE trap + *B.longum* | 0/18 (0.00%) | 0/18 (0.00%) | 1/18 (5.56%) | 2/18 (11.11%) | 4/18 (22.22%) |
| 4 | 17 | IgE trap only | 0/17 (0.00%) | 0/17 (0.00%) | 3/17 (17.65%) | 6/17 (35.29%) | 9/17 (52.94%) |
| 5 | 16 | Normal Ctrl | 0/16 (0.00%) | 0/16 (0.00%) | 0/16 (0.00%) | 0/16 (0.00%) | 0/16 (0.00%) |

Using this model, the present inventors have found that administration of $IgE_{TRAP}$ alone effectively decreased occurrence of diarrhea, and have identified that administration of $IgE_{TRAP}$ in combination with *B. longum* remarkably decreased occurrence of diarrhea as compared with the administration of $IgE_{TRAP}$ alone (FIG. 18). It has been reported that *B. longum* decreased the number of mast cells and alleviated food allergic symptoms through apoptosis. However, the group receiving intraperitoneal injection of $IgE_{TRAP}$ exhibited a better therapeutic effect than the group daily receiving *B. longum* (FIG. 18). Interestingly, even in a case where $IgE_{TRAP}$ with a 10-fold decreased concentration was used, a combination therapy of $IgE_{TRAP}$ and *B. longum* exhibited a similar effect to the $IgE_{TRAP}$-alone therapy (FIG. 19B).

**Experimental Example 8. Identification of effects at time of combined administration of IgETRAP and *B. longum*: MCPT-1 and IgE levels**

In addition, a level of serum MCPT-1 was measured to identify mast cell degranulation. Administration of $IgE_{TRAP}$ and *B. longum* alone did not decrease a level of MCPT-1, but a combination of $IgE_{TRAP}$ and *B. longum* significantly decreased a level of MCPT-1 (FIG. 20). Thus, it can be seen that *B. longum* and $IgE_{TRAP}$ cooperate to inhibit mast cell degranulation. In a food allergic mouse model, in order to investigate efficacy of a therapeutic agent that decreases IgE, ELISA was used to analyze levels of total IgE and free IgE in serum. $IgE_{TRAP}$, and the combination of $IgE_{TRAP}$ and *B. longum* slightly increased the level of total IgE (FIG. 21). However, $IgE_{TRAP}$, and the combination of $IgE_{TRAP}$ and *B. longum* greatly decreased the level of free IgE (FIG. 22). In contrast, administration of *B. longum* alone did not affect levels of total IgE and free IgE (FIGS. 21 and 22). Thus, $IgE_{TRAP}$ and *B. longum* alleviate food allergic symptoms in several ways, suggesting that this

combination may exert an effective therapeutic effect on food allergy.

**Experimental Example 9. Identification of inhibitory effect of combination of IgETRAP and *B. longum* on number of mast cells and goblet cell hyperplasia**

In order to investigate whether $IgE_{TRAP}$ and *B. longum*, and a combination thereof decrease the number of mast cells, chloroacetate esterase activity was used to stain mast cells. The results showed that administration of $IgE_{TRAP}$ and *B. longum* alone remarkably decreased the number of mast cells, and a combination of the two was much more effective (FIGS. 23 and 24). It can be seen that the results for *B. longum* are consistent with those reported previously. Investigation was made on whether goblet cell hyperplasia induced by Th2 cytokine environment can be inhibited by $IgE_{TRAP}$, *B. longum*, and combination therapy thereof. As expected, it was identified that the size of goblet cells in the small intestine of the food allergic mice was increased, and that hyperplasia occurred as the number of cells increased (FIGS. 25 and 26).

It was identified that administration of $IgE_{TRAP}$ and *B. longum* remarkably decreased the size and number of goblet cells and this effect became greater in a case where $IgE_{TRAP}$ and *B. longum* were administered in combination (FIGS. 25 and 26). In addition, it was seen that mRNA expression of IL-33 tended to be decreased by $IgE_{TRAP}$ and *B. longum*, and this effect became significantly greater in a case of being administered in combination (FIG. 28). These results indicate that $IgE_{TRAP}$ and *B. longum* significantly inhibit the number of intestinal mast cells and goblet cell hyperplasia, and exhibit a further improved effect in a case of being administered in combination.

**Experimental Example 10. Identification of presence of IgETRAP in serum after oral administration of IgETRAP to mice**

$IgE_{TRAP}$ (300 ug) was orally administered to mice, and 2 hours later, serum is collected by retro-orbital blood collection. After being allowed to react at room temperature for 30 minutes, supernatant (serum) was obtained by centrifugation at 4°C and 1,300 rpm for 15 minutes. A 96-well immuno plate was coated with an anti-FcεRI antibody (Abcam, ab54411) and allowed to react overnight at 4°C. The plate was washed with washing buffer (PBS containing 0.05% Tween-20), and then blocking buffer (PBS containing 1% bovine serum albumin) was added thereto. The plate was allowed to react for 1 hour. The plate was

washed again with the washing buffer, and a standard sample and a diluted mouse serum sample were added to the plate. The plate was allowed to react for 2 hours, and washed again with the washing buffer. An anti-human IgG4 Fc antibody (Abcam, ab99823) was added thereto and the plate was allowed to react for 1 hour. The plate was washed again with the washing buffer, and TMB substrate (Supmodics) was added thereto. After being allowed to react for 20 minutes while blocking the light, a stop reaction (1 M $H_2SO_4$) was added to stop the reaction. A concentration value was measured with a microplate reader (Epoch microplate spectrophotometer) by setting a wavelength to 450 nm. As a result, $IgE_{TRAP}$ was detected in the serum of normal mice (FIG. 35). From these results, it can be seen that in a case where an $IgE_{TRAP}$ protein is orally administered, the $IgE_{TRAP}$ protein is delivered into the serum via binding with FcRn in the mucosa, and thus a therapeutic effect can be exhibited.

**Experimental Example 11. Identification of effect obtained by combined administration of IgETRAP and various probiotics in food allergy model**

In order to evaluate an effect of $IgE_{TRAP}$ and probiotics on food allergy, dose-dependent acute diarrhea was induced in BALB/c mice to produce a mouse model with allergen-induced food allergy. Specifically, experiments were carried out in the same manner as in Experimental Example 7, except that $IgE_{TRAP}$, an FcεRIαECD recombinant protein, was intraperitoneally administered to mice at 100 ug/head as in the experiment schedule of FIG. 17. In addition, lyophilized probiotics were continuously fed at $1 \times 10^9$ to $2.5 \times 10^9$ cfu per mouse using an oral zonde at 2- to 3-day intervals during the experiment, and a negative control was fed an equal amount of lyophilized medium.

The results obtained by identifying diarrhea frequency after administration of $IgE_{TRAP}$ and *L. casei* are illustrated in FIG. 29. The results obtained by identifying diarrhea frequency after administration of $IgE_{TRAP}$ and *Lc. lactis* are illustrated in FIG. 30. The results obtained by identifying diarrhea frequency after administration of $IgE_{TRAP}$ and *S. thermophilus* are illustrated in FIG. 31. The results obtained by identifying diarrhea frequency after administration of $IgE_{TRAP}$ and *L. rhamnosus* are illustrated in FIG. 32. The results obtained by identifying diarrhea frequency after administration of $IgE_{TRAP}$ and *L. reuteri* were illustrated in FIG. 33. The results obtained by identifying diarrhea frequency after administration of $IgE_{TRAP}$ and *L. fermentum* are illustrated in FIG. 34.

Reference Signs List

*B. longum: Bifidobacterium longum*

OIT: Oral immunotherapy

PSA: Passive systemic anaphylaxis

SPR: Surface plasmon resonance

BLI: Bio-layer interferometry

BMMC: Bone marrow-derived mast cell

cfu: Colony-forming unit

MCPT-1: Mast cell protease-1

OVA: Ovalbumin

Treg cell: Regulatory T cell

Th2 cell: Type 2 helper T cell

ILC2: Group 2 innate lymphoid cell

[Sequence List Text]

SEQ ID NO: 1

VPQKPKVSLN PPWNRIFKGE NVTLTCNGNN FFEVSSTKWF HNGSLSEETN SSLNIVNAKF EDSGEYKCQH QQVNESEPVY LEVFSDWLLL QASAEVVMEG QPLFLRCHGW RNWDVYKVIY YKDGEALKYW YENHNISITN ATVEDSGTYY CTGKVWQLDY ESEPLNITVI KAPREKYWLQ

SEQ ID NO: 2

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SEQ ID NO: 3

RNTGRGGEEK KGSKEKEEQE ERETKTPECP

SEQ ID NO: 4

AQPQAEGSLA KATTAPATTR NTGRGGEEKK GSKEKEEQEE RETKTPECP

SEQ ID NO: 5

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SEQ ID NO: 6

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SEQ ID NO: 7

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SEQ ID NO: 8

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SEQ ID NO: 9

MDAMLRGLCC VLLLCGAVFV SPSHA

SEQ ID NO: 10

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SEQ ID NO: 11

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SEQ ID NO: 12

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SEQ ID NO: 13

MDAMLRGLCC VLLLCGAVFV SPSHAVPQKP KVSLNPPWNR IFKGENVTLT CNGNNFFEVS STKWFHNGSL SEETNSSLNI VNAKFEDSGE YKCQHQQVNE SEPVYLEVFS DWLLLQASAE VVMEGQPLFL RCHGWRNWDV YKVIYYKDGE ALKYWYENHN ISITNATVED SGTYYCTGKV WQLDYESEPL NITVIKAPRE KYWLQAQPQA EGSLAKATTA PATTRNTGRG GEEKKGSKEK EEQEERETKT PECPSHTQPL GVFLFPPKPK DTLMISRTPE VTCVVVDVSQ EDPEVQFNWY VDGVEVHNAK TKPREEQFNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKGL PSSIEKTISK AKGQPREPQV YTLPPSQEEM TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS RLTVDKSRWQ EGNVFSCSVM HEALHNHYTQ KSLSLSLGK

SEQ ID NO: 14

atggacgcca tgctgagagg cctgtgctgt gtgctgctgc tgtgcggcgc cgtgttcgtg tcccctagcc acgccgtgcc ccagaagccc aaggtgagcc tgaaccctcc ctggaacaga atcttcaagg gcgagaacgt gaccctgacc tgcaacggca acaacttctt cgaggtgagc agcaccaagt ggttccacaa tggcagcctg agcgaggaga ccaacagctc cctgaacatc gtgaacgcca agttcgagga cagcggcgag tacaagtgcc agcaccagca ggtgaacgag agcgagcccg tgtacctgga ggtgttcagc gactggctgc tgctgcaggc cagcgccgag gtggtgatgg agggccagcc cctgttcctg agatgccacg gctggagaaa ctgggacgtg tacaaggtga tctactacaa ggatggcgag gccctgaagt actggtacga gaaccacaac atctccatca ccaacgccac cgtggaggac agcggcacct actactgcac aggcaaggtg tggcagctgg actacgagag cgagcccctg aacatcaccg tgatcaaggc tcccagagag aagtactggc tgcaggccca gccccaggcc gagggcagcc tggctaaggc caccacagct cccgccacca ccaggaacac cggcagagga ggcgaggaaa agaaaggaag caaggagaag gaggagcagg aggaaagaga aaccaagacc cccgagtgcc ccagccacac ccagcccctg ggcgtgttcc tgttcccccc caagcccaag gacaccctga tgatcagcag aacccccgag gtgacctgcg tggtcgtgga tgtgagccag gaagatcccg aagtgcagtt caactggtac gtggatggcg tggaagtgca caacgccaag accaagccca gagaagagca gttcaactcc acctacagag tggtgagcgt gctgaccgtg ctgcaccagg actggctgaa cggcaaggag tacaagtgca aggtgtccaa caaaggcctg cccagctcca tcgagaagac catcagcaaa gccaaaggcc agcccagaga accccaggtg tacaccctgc ctcccagcca ggaagagatg accaagaacc aggtgtccct gacctgcctg gtgaaaggct tctaccccag cgacatcgcc gtggagtggg aaagcaacgg ccagcccgag aacaattaca agacaacccc tcccgtgctg gatagcgatg gcagcttctt tctgtacagc agactgaccg tggacaagag cagatggcag gaaggcaacg tgttcagctg cagcgtgatg

cacgaagccc tgcacaacca ctacacccag aagagcctgt ccctgagcct gggcaag

SEQ ID NO: 15

MIHTNLKKKF SCCVLVFLLF AVICVWKEKK KGSYYDSFKL QTKEFQVLKS LGKLAMGSDS QSVSSSSTQD PHRGRQTLGS LRGLAKAKPE ASFQVWNKDS SSKNLIPRLQ KIWKNYLSMN KYKVSYKGPG PGIKFSAEAL RCHLRDHVNV SMVEVTDFPF NTSEWEGYLP KESIRTKAGP WGRCAVVSSA GSLKSSQLGR EIDDHDAVLR FNGAPTANFQ QDVGTKTTIR LMNSQLVTTE KRFLKDSLYN EGILIVWDPS VYHSDIPKWY QNPDYNFFNN YKTYRKLHPN QPFYILKPQM PWELWDILQE ISPEEIQPNP PSSGMLGIII MMTLCDQVDI YEFLPSKRKT DVCYYYQKFF DSACTMGAYH PLLYEKNLVK HLNQGTDEDI YLLGKATLPG FRTIHC

SEQ ID NO: 16

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SEQ ID NO: 17

RNTGRGGEEK KXXKEKEEQE ERETKTPECP

SEQ ID NO: 18

AQPQAEGSLA KATTAPATTR NTGRGGEEKK XXKEKEEQEE RETKTPECP

SEQ ID NO: 19

RNTGRGGEEK KKEKEKEEQE ERETKTPECP

SEQ ID NO: 20

VPQKPKVSLN PPWNRIFKGE NVTLTCNGNN FFEVSSTKWF HNGSLSEETN SSLNIVNAKF EDSGEYKCQH QQVNESEPVY LEVFSDWLLL QASAEVVMEG QPLFLRCHGW RNWDVYKVIY YKDGEALKYW YENHNISITN ATVEDSGTYY CTGKVWQLDY ESEPLNITVI KAPREKYWLQ RNTGRGGEEK KKEKEKEEQE ERETKTPECP SHTQPLGVFL FPPKPKDTLM ISRTPEVTCV VVDVSQEDPE VQFNWYVDGV EVHNAKTKPR EEQFNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKGLPSSI EKTISKAKGQ PREPQVYTLP PSQEEMTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSRLTV DKSRWQEGNV FSCSVMHEAL HNHYTQKSLS LSLGK

SEQ ID NO: 21

VPQKPKVSLN PPWNRIFKGE NVTLTCNGNN FFEVSSTKWF HNGSLSEETN SSLNIVNAKF EDSGEYKCQH QQVNESEPVY LEVFSDWLLL QASAEVVMEG QPLFLRCHGW RNWDVYKVIY YKDGEALKYW YENHNISITN ATVEDSGTYY CTGKVWQLDY ESEPLNITVI KAPREKYWLQ RNTGRGGEEK KGSKEKEEQE ERETKTPECP SHTQPLGVFL FPPKPKDTLM ISRTPEVTCV VVDVSQEDPE VQFNWYVDGV EVHNAKTKPR EEQFNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKGLPSSI EKTISKAKGQ PREPQVYTLP PSQEEMTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSRLTV DKSRWQEGNV FSCSVMHEAL HNHYTQKSLS LSLGK

SEQ ID NO: 22

VPQKPKVSLN PPWNRIFKGE NVTLTCNGNN FFEVSSTKWF HNGSLSEETN SSLNIVNAKF EDSGEYKCQH QQVNESEPVY LEVFSDWLLL QASAEVVMEG QPLFLRCHGW RNWDVYKVIY YKDGEALKYW YENHNISITN ATVEDSGTYY CTGKVWQLDY ESEPLNITVI KAPREKYWLQ AQPQAEGSLA KATTAPATTR NTGRGGEEKK GSKEKEEQEE RETKTPECPS HTQPLGVFLF PPKPKDTLMI SRTPEVTCVV VDVSQEDPEV QFNWYVDGVE VHNAKTKPRE EQFNSTYRVV

SVLTVLHQDW LNGKEYKCKV SNKGLPSSIE KTISKAKGQP REPQVYTLPP SQEEMTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSRLTVD KSRWQEGNVF SCSVMHEALH NHYTQKSLSL SLGK

## CLAIMS

1. A pharmaceutical composition for treating or preventing an allergic disease, comprising, as active ingredients, probiotics and a polypeptide with a binding ability to IgE,
wherein the probiotics is any one selected from the group consisting of *B. longum, L. casei, Lc. lactis, S. thermophilus, L. rhamnosus, L. reuteri,* and *L. fermentum,*
wherein the polypeptide with a binding ability to IgE is a polypeptide dimer containing two monomers, each of which contains an extracellular domain of an alpha subunit of an IgE Fc receptor (FcεRIαECD),
the monomer contains a modified Fc region, and
the modified Fc region and the FcεRIαECD are linked via a linker.

2. The pharmaceutical composition for treating or preventing an allergic disease according to claim 1,
wherein the extracellular domain of the alpha subunit of the IgE Fc receptor has the amino acid sequence of SEQ ID NO: 1.

3. The pharmaceutical composition for treating or preventing an allergic disease according to claim 1,
wherein the probiotics are in the form of a dried powder.

4. The pharmaceutical composition for treating or preventing an allergic disease according to claim 1,
wherein the allergic disease is any one selected from the group consisting of food allergy, atopic dermatitis, asthma, allergic rhinitis, allergic conjunctivitis, allergic dermatitis, chronic idiopathic urticaria, and allergic contact dermatitis.

5. A health functional food composition for ameliorating or alleviating an allergic symptom, comprising, as active ingredients, probiotics and a polypeptide with a binding ability to IgE,
wherein the probiotics is any one selected from the group consisting of *B. longum, L.*

*casei, Lc. lactis, S. thermophilus, L. rhamnosus, L. reuteri,* and *L. fermentum,*

wherein the polypeptide with a binding ability to IgE is a polypeptide dimer containing two monomers, each of which contains an extracellular domain of an alpha subunit of an IgE Fc receptor (FcεRIαECD),

the monomer contains a modified Fc region, and

the modified Fc region and the FcεRIαECD are linked via a linker.

6. A kit for treating or preventing an allergic disease, comprising:

a first composition that contains probiotics; and

a second composition that contains a polypeptide with a binding ability to IgE,

wherein the probiotics is any one selected from the group consisting of *B. longum, L. casei, Lc. lactis, S. thermophilus, L. rhamnosus, L. reuteri,* and *L. fermentum,*

wherein the polypeptide with a binding ability to IgE is a polypeptide dimer containing two monomers, each of which contains an extracellular domain of an alpha subunit of an IgE Fc receptor (FcεRIαECD),

the monomer contains a modified Fc region, and

the modified Fc region and the FcεRIαECD are linked via a linker.

7. The kit for treating or preventing an allergic disease according to claim 18,

wherein the second composition is a composition for subcutaneous or intravenous administration.