DESCRIPTION

Title of Invention: NUCLEIC ACID FOR TREATING MITE ALLERGY

Technical Field

[0001]

The present invention relates to a nucleic acid which is expected to be useful as an active ingredient of a pharmaceutical composition, for example, a nucleic acid which is expected to be useful for treating mite allergy.

Background Art

[0002]

Mite allergy is an allergic disease that occurs in response to mite-derived allergens. The allergic disease is caused by the following steps: 1) allergens taken into a body are phagocytosed by antigen-presenting cells and presented to naive T cells, 2) the naive T cells are differentiated into Th2 cells, 3) cytokine such as IL-4 is produced from an immune cell such as the Th2 cell, 4) B cells produce IgE by IL-4, and 5) IgE binding to the allergens binds to mast cells. It has been known that in allergic disease patients, antagonism between Th1-type immunity involving Th1 cells producing IFN-γ or the like and Th2-type immunity involving Th2 cells producing IL-4 or the like shifts to Th2-type dominant which results in Th2-type inflammatory immune response (Middleton's Allergy Seventh edition Principles & Practice, 2009). Thus, IFN-γ can be used as an indicator of Th1-type immunity, and IL-4 can be used as an indicator of Th2-type immunity. Further, in mice, IFN-γ causes a preferential class switch to IgG2a isotype in activated B cells, while suppresses responses to all the other isotypes. That is, IgG2a can also be used as an indicator of Th1-type immunity. For example, it has been known that production of IgG2a is promoted in IL-4-deficient mice and that IgG2a production is suppressed in IFN-γ-deficient mice (Arthritis Res., 2002, Vol. 4, p. 54-58). There is also a report that antibodies produced from B cells are involved in the mechanism of action of allergen immunotherapy. For example, it has been known that in humans, IgG antagonizes IgE binding to an allergen to inhibit formation of allergen-IgE complex and thereby inhibit histamine release from mast cells (J Allergy Clin Immunol., 2017, Vol. 140, p. 1485-1498).

[0003]

Until now, development of multiple immunotherapies for mite allergy has been advanced (J Allergy Clin Immunol., 2013, Vol. 132, p. 1322-1336; WO 2014/195803; Expert Rev Vaccines., 2014, Vol. 13, p. 1427-1438). Further, Der p 1, Der p 2, Der p 7, Der p 23, and the like have been known as allergens related to the mite allergy (Patent Documents 1 and 2, and Non-Patent Document 1). However, for example, subcutaneous

immunotherapy (SCIT) and sublingual immunotherapy (SLIT) have problems such as possibility of anaphylaxis and long treatment period over several years.

[0004]

As one of the techniques for nucleic acid vaccines, nucleic acid vaccines for treating allergy using lysosome-associated membrane proteins (LAMP) have been studied. Further, a plasmid comprising a nucleic acid encoding a chimeric protein comprising LAMP-1, which is a member of LAMP family, and Cry J1 and/or Cry J2, which are allergens of *Cryptomeria japonica*, was constructed (Patent Document 3 and Non-Patent Document 2). It has been reported that such a plasmid does not cause systemic release of free allergen which causes anaphylaxis, but induces a Th1-type immune response. Furthermore, it has been reported that a plasmid comprising a nucleic acid encoding a chimeric protein comprising LAMP-1 and peanut allergens Ara H1, Ara H2 and Ara H3 reduced production of IgE in a mouse model (Patent Document 4). In a field of mite allergy, a vaccine comprising a nucleic acid encoding a chimeric protein comprising Der p 1 and a transmembrane domain of LAMP-1 and an endosomal/lysosomal targeting domain has been constructed (Patent Document 5 and Non-Patent Document 3). However, a nucleic acid vaccine for treating mite allergy comprising multiple mite allergen antigens, and an intra-organelle stabilizing domain of LAMP-1 and an endosomal/lysosomal targeting domain has not been reported.

Related Art

Patent Document

[0005]

[Patent Document 1] WO 1988/010297

[Patent Document 2] WO 2007/124524

[Patent Document 3] WO 2013/187906

[Patent Document 4] WO 2015/200357

[Patent Document 5] WO 2004/019978

Non-Patent Document

[0006]

[Non-Patent Document 1] "Clinical & Experimental Allergy", (UK), 1995; 25: 416-422

[Non-Patent Document 2] "Journal of Immunology Research", (Egypt), 2016; Article ID 4857869

[Non-Patent Document 3] "Vaccine", (Netherlands), 2006; 24 (29-30): 5762-5771

Summary of Invention

Problems to Be Solved by the Invention

[0007]

An object of the present invention is to provide a nucleic acid which is expected to be useful for treating mite allergy.

Means for Solving the Problems

[0008]

As a result of repeated investigation with considerable creativity in the preparation of nucleic acids for treating mite allergy, the present inventors have prepared LAMP-Der p 1-Der p 2-Der p 23-Der p 7 plasmid (Example 1), confirmed that a chimeric protein is expressed from the plasmid (Example 2), and found that a Th1-type immune response is induced in mice to which the plasmid is administered (Examples 3 and 4). As a result, a nucleic acid which is expected to be useful for treating mite allergy is provided, and thereby the present invention has been completed.

[0009]

That is, the present invention relates to the following [1] to [17].

[1]

A nucleic acid comprising:

a nucleotide sequence encoding a chimeric protein,

wherein the nucleotide sequence is a nucleotide sequence comprising the following nucleotide sequences in this order:

a nucleotide sequence encoding a signal peptide;

a nucleotide sequence encoding an intra-organelle stabilizing domain of LAMP;

a nucleotide sequence encoding an allergen domain comprising Der p 1, Der p 2, Der p 23, and Der p 7;

a nucleotide sequence encoding a transmembrane domain; and

a nucleotide sequence encoding an endosomal/lysosomal targeting domain of LAMP.

[2]

A nucleic acid comprising:

a nucleotide sequence encoding a chimeric protein,

wherein the nucleotide sequence is a nucleotide sequence comprising the following nucleotide sequences in this order:

a nucleotide sequence encoding a signal peptide;

a nucleotide sequence encoding an intra-organelle stabilizing domain of LAMP;

a nucleotide sequence encoding an allergen domain comprising Der p 1, Der p 2, Der p 23, and Der p 7 in this order;

a nucleotide sequence encoding a transmembrane domain; and

a nucleotide sequence encoding an endosomal/lysosomal targeting domain of

LAMP.

[3]

The nucleic acid described in [1] or [2], wherein the signal peptide is a signal peptide of LAMP.

[4]

The nucleic acid described in any one of [1] to [3], wherein the transmembrane domain is a transmembrane domain of LAMP.

[5]

The nucleic acid described in any one of [1] to [4], wherein the signal peptide consists of the amino acid sequence of amino acid numbers 1 to 27 of SEQ ID NO: 2, the intra-organelle stabilizing domain consists of an amino acid sequence of amino acid numbers 28 to 380 of SEQ ID NO: 2, the allergen domain is an allergen domain comprising Der p 1 consisting of the amino acid sequence of amino acid numbers 383 to 594 of SEQ ID NO: 2, Der p 2 consisting of the amino acid sequence of amino acid numbers 599 to 727 of SEQ ID NO: 2, Der p 23 consisting of the amino acid sequence of amino acid numbers 732 to 800 of SEQ ID NO: 2, and Der p 7 consisting of the amino acid sequence of amino acid numbers 805 to 1002 of SEQ ID NO: 2, the transmembrane domain consists of the amino acid sequence of amino acid numbers 1006 to 1028 of SEQ ID NO: 2, and the endosomal/lysosomal targeting domain consists of the an amino acid sequence of amino acid numbers 1037 to 1040 of SEQ ID NO: 2.

[6]

A nucleic acid comprising:

a nucleotide sequence encoding a chimeric protein consisting of an amino acid sequence having at least 90% identity to the amino acid sequence shown by SEQ ID NO: 2, wherein the nucleic acid has an action of inducing Th1-type immunity to an allergen selected from the group consisting of Der p 1, Der p 2, Der p 23, and Der p 7.

[7]

A nucleic acid comprising:

a) a nucleotide sequence encoding a chimeric protein consisting of the amino acid sequence shown by SEQ ID NO: 2; or

b) a nucleotide sequence encoding a chimeric protein consisting of an amino acid sequence in which 1 to 10 amino acids are deleted, substituted, inserted and/or added in the amino acid sequence shown by SEQ ID NO: 2, wherein the nucleic acid has an action of inducing Th1-type immunity to an allergen selected from the group consisting of Der p 1, Der p 2, Der p 23, and Der p 7.

[8]

A nucleic acid comprising:

a nucleotide sequence encoding a chimeric protein consisting of an amino acid

sequence shown by SEQ ID NO: 2.

[9]

An expression vector comprising:

the nucleic described in any one of [1] to [8].

[10]

An expression vector comprising:

the nucleic acid described in [8].

[11]

A host cell transformed with the nucleic acid described in any one of [1] to [8].

[12]

A method for producing a nucleic acid, comprising:

culturing a host cell transformed with the nucleic acid described in any one of [1] to [8].

[13]

A pharmaceutical composition comprising:

the expression vector described in [10] and a pharmaceutically acceptable excipient.

[14]

The pharmaceutical composition described in [13], which is a pharmaceutical composition for preventing or treating mite allergy.

[15]

A method for preventing or treating mite allergy, comprising:

administering a prophylactically effective or therapeutically effective amount of the expression vector described in [10].

[16]

The expression vector described in [10], for use in preventing or treating mite allergy.

[17]

Use of the expression vector described in [10] for the manufacture of a pharmaceutical composition for preventing or treating mite allergy.

Effects of the Invention

[0010]

The nucleic acid of the present invention can be used for preventing or treating mite allergy.

Brief Description of Drawings

[0011]

[Fig. 1] Fig. 1 is a diagram illustrating production of IgG2a specific to Der p 1, Der p 2, Der p 23, and Der p 7, which is induced when the nucleic acid of the present invention is administered to a mouse. The vertical axis indicates absorbance at 450 nm, and the horizontal axis indicates each administration group. The horizontal lines indicate arithmetic mean values.

[Fig. 2] Fig. 2 illustrates IFN-γ production when spleen cells of mice to which the nucleic acid of the present invention has been administered were stimulated with Der p 1 protein, Der p 2 protein, Der p 7 protein, or Der p 23 protein. The vertical axis indicates the concentration of IFN-γ in the culture supernatant (pg/mL), and the horizontal axis indicates each administration group. The horizontal lines indicate arithmetic mean values. The dotted line indicates the value of lower limit of detection (LLOD).

[Fig. 3] Fig. 3 illustrates IL-4 production when spleen cells of mice to which the nucleic acid of the present invention has been administered were stimulated with Der p 1 protein, Der p 2 protein, Der p 7 protein, or Der p 23 protein. The vertical axis indicates the concentration of IL-4 in the culture supernatant (pg/mL), and the horizontal axis indicates each administration group. The horizontal lines indicate arithmetic mean values. The dotted line indicates the value of lower limit of detection (LLOD).

Embodiments for Carrying Out the Invention

[0012]

Hereinafter, the present invention will be described in detail.

[0013]

<Nucleic acid of the present invention>

Examples of the nucleic acid of the present invention include a nucleic acid having the following features:

a nucleic acid comprising a nucleotide sequence encoding a chimeric protein, wherein the nucleotide sequence is a nucleotide sequence comprising the following nucleotide sequences in this order:

a nucleotide sequence encoding a signal peptide,

a nucleotide sequence encoding an intra-organelle stabilizing domain of LAMP,

a nucleotide sequence encoding an allergen domain comprising Der p 1, Der p 2, Der p 23, and Der p 7,

a nucleotide sequence encoding a transmembrane domain, and

a nucleotide sequence encoding an endosomal/lysosomal targeting domain of LAMP.

[0014]

In the present invention, the nucleic acid is a polymer which is formed by polymerization of nucleotides and consists of a nucleotide sequence with an arbitrary

length. The nucleotides can include deoxyribonucleotides, ribonucleotides, and/or their analogs. The nucleic acid of the present invention is DNA, RNA or modified a nucleic acid thereof. In one embodiment, the nucleic acid of the present invention is DNA.

[0015]

In one embodiment, the nucleic acid of the present invention is a nucleic acid introduced into an expression vector. In one embodiment, the nucleic acid of the present invention is a nucleic acid introduced into a plasmid vector.

[0016]

In the specification, "chimeric protein" means a protein encoded by a nucleotide sequence in which two or more genes are fused by using genetic recombination technology. The nucleic acid of the present invention includes a nucleotide sequence encoding chimeric protein comprising a signal peptide, an intra-organelle stabilizing domain of LAMP, an allergen domain comprising Der p 1, Der p 2, Der p 23, and Der p 7, a transmembrane domain, and an endosomal/lysosomal targeting domain of LAMP in this order (hereinafter, referred to as "chimeric protein relating to the present invention").

[0017]

LAMP is well-known protein to those skilled in the art (J Biol Chem., 1991, Vol.266, p.21327-21330). In the present specification, LAMP is not particularly limited, but examples thereof include LAMP-1, LAMP-2, CD63/LAMP-3, DC-LAMP, and LIMP II, and homologs, orthologs, paralogs, variants, and modified proteins thereof. In one embodiment of the present invention, LAMP is LAMP-1. In the present invention, an animal from which LAMP is derived is not particularly limited, but in one embodiment, LAMP is human LAMP. In one embodiment, human LAMP is human LAMP-1. Examples of an amino acid sequence of human LAMP-1 include an amino acid sequence in which the amino acid sequence shown by amino acid numbers 1005 to 1040 of SEQ ID NO: 2 is bound to a C-terminal of the amino acid sequence shown by amino acid numbers 1 to 380 of SEQ ID NO: 2.

[0018]

The general structure of the signal peptide is well known to those skilled in the art (Annu Rev Biochem., 2003, Vol. 72, p. 395 to 447). The signal peptide has a function of directing transport and localization of a protein. As the signal peptide used in the present invention, any suitable signal peptide can be selected as long as it has a function of directing transport and localization of the protein. In one embodiment, the signal peptide used in the present invention is a signal peptide of LAMP. In one embodiment, the signal peptide of LAMP used in the present invention is a signal peptide of LAMP-1.

[0019]

In one embodiment, the signal peptide used in the present invention consists of the following amino acid sequence of (a) or (b):

(a) an amino acid sequence having at least 90% identity to the amino acid sequence of amino acid numbers 1 to 27 of SEQ ID NO: 2; or

(b) the amino acid sequence of amino acid numbers 1 to 27 of SEQ ID NO: 2, or an amino acid sequence in which 1 to 3 amino acids are deleted, substituted, inserted and/or added in the amino acid sequence of amino acid numbers 1 to 27 of SEQ ID NO: 2.

[0020]

The term of "identity" in the present specification means a value of Identity obtained by using an EMBOSS Needle (Nucleic Acids Res., 2015, Vol.43, p.W580-W584; https://www.ebi.ac.uk/Tools/psa/emboss_needle/) with a parameter prepared by default. The above parameters are as follows.

Gap Open Penalty = 10

Gap Extend Penalty = 0.5

Matrix = EBLOSUM62

End Gap Penalty = false

[0021]

In one embodiment, the signal peptide used in the present invention consists of the amino acid sequence of amino acid numbers 1 to 27 of SEQ ID NO: 2.

[0022]

The sequence of the intra-organelle stabilizing domain of LAMP is well known to those skilled in the art (WO 2013/187906). The intra-organelle stabilizing domain of LAMP has a function of protecting the allergen domain from proteases, low pH, and other substances and conditions that destabilize a protein. As the intra-organelle stabilizing domain of LAMP used in the present invention, any suitable intra-organelle stabilizing domain of LAMP can be selected as long as it has a function of protecting the allergen domain from proteases, low pH, and other substances and conditions that destabilize a protein. In one embodiment, the intra-organelle stabilizing domain of LAMP used in the present invention is an intra-organelle stabilizing domain of LAMP-1.

[0023]

In one embodiment, the intra-organelle stabilizing domain of LAMP used in the present invention consists of the following amino acid sequence of (a) or (b):

(a) an amino acid sequence having at least 90% identity to the amino acid sequence of amino acid numbers 28 to 380 of SEQ ID NO: 2; or

(b) the amino acid sequence of amino acid numbers 28 to 380 of SEQ ID NO: 2, or an amino acid sequence in which 1 to 10 amino acids are deleted, substituted, inserted and/or added in the amino acid sequence of amino acid numbers 28 to 380 of SEQ ID NO: 2.

[0024]

In one embodiment, the intra-organelle stabilizing domain of LAMP used in the

present invention consists of the amino acid sequence of amino acid numbers 28 to 380 of SEQ ID NO: 2.

[0025]

The allergen domain used in the present invention includes Der p 1, Der p 2, Der p 23, and Der p 7 as allergens. Der p 1, Der p 2, Der p 23, and Der p 7 are allergens that can be observed in mites (WO 1988/010297; WO 2007/124524; and Clin Exp Allergy., 1995, Vol. 25, p. 416-422). Der p 1, Der p 2, Der p 23, and Der p 7 used in the present invention may be variants thereof as long as they have antigenicity. The antigenicity of any protein can be confirmed, for example, by observing that administration to an animal elicits antibody production or T cell response to that protein (Bioanalysis., 2012, Vol. 4, p. 397-406). In one embodiment, Der p 1, Der p 2, Der p 23, and Der p 7 used in the present invention lack the signal peptide.

[0026]

In one embodiment, Der p 1 consists of the following amino acid sequence of (a) or (b):

(a) an amino acid sequence having at least 90% identity to the amino acid sequence of amino acid numbers 383 to 594 of SEQ ID NO: 2; or

(b) the amino acid sequence of amino acid numbers 383 to 594 of SEQ ID NO: 2, or an amino acid sequence in which 1 to 10 amino acids are deleted, substituted, inserted and/or added in the amino acid sequence of amino acid numbers 383 to 594 of SEQ ID NO: 2.

[0027]

In one embodiment, Der p 1 consists of the amino acid sequence of amino acid numbers 383 to 594 of SEQ ID NO: 2.

[0028]

In one embodiment, Der p 2 consists of the following amino acid sequence of (a) or (b):

(a) an amino acid sequence having at least 90% identity to the amino acid sequence of amino acid numbers 599 to 727 of SEQ ID NO: 2; or

(b) the amino acid sequence of amino acid numbers 599 to 727 of SEQ ID NO: 2, or an amino acid sequence in which 1 to 10 amino acids are deleted, substituted, inserted and/or added in the amino acid sequence of amino acid numbers 599 to 727 of SEQ ID NO: 2.

[0029]

In one embodiment, Der p 2 consists of the amino acid sequence of amino acid numbers 599 to 727 of SEQ ID NO: 2.

[0030]

In one embodiment, Der p 23 consists of the following amino acid sequence of (a)

or (b):

(a) an amino acid sequence having at least 90% identity to the amino acid sequence of amino acid numbers 732 to 800 of SEQ ID NO: 2; or

(b) the amino acid sequence of amino acid numbers 732 to 800 of SEQ ID NO: 2, or an amino acid sequence in which 1 to 10 amino acids are deleted, substituted, inserted and/or added in the amino acid sequence of amino acid numbers 732 to 800 of SEQ ID NO: 2.

[0031]

In one embodiment, Der p 23 consists of the amino acid sequence of amino acid numbers 732 to 800 of SEQ ID NO: 2.

[0032]

In one embodiment, Der p 7 consists of the following amino acid sequence of (a) or (b):

(a) an amino acid sequence having at least 90% identity to the amino acid sequence of amino acid numbers 805 to 1002 of SEQ ID NO: 2; or

(b) the amino acid sequence of amino acid numbers 805 to 1002 of SEQ ID NO: 2, or an amino acid sequence in which 1 to 10 amino acids are deleted, substituted, inserted and/or added in the amino acid sequence of amino acid numbers 805 to 1002 of SEQ ID NO: 2.

[0033]

In one embodiment, Der p 7 consists of the amino acid sequence of amino acid numbers 805 to 1002 of SEQ ID NO: 2.

[0034]

In one embodiment, the allergen domain used in the present invention comprises Der p 1, Der p 2, Der p 23, and Der p 7 in any order. In addition, in one embodiment, the allergen domain used in the present invention comprises Der p 1, Der p 2, Der p 23, and Der p 7 in this order.

[0035]

In one embodiment, the allergen domain used in the present invention consists of the amino acid sequence of amino acid numbers 383 to 1002 of SEQ ID NO: 2.

[0036]

The general structure of the transmembrane domain is well known to those skilled in the art (Annu Rev Biochem., 2007, Vol. 76, p. 125 to 140). The transmembrane domain has a function of anchoring proteins to biological membranes. As the transmembrane domain used in the present invention, any suitable transmembrane domain protein can be selected as long as it has a function of anchoring proteins to biological membranes. In one embodiment, the transmembrane domain used in the present invention is a transmembrane domain of LAMP. In one embodiment, the transmembrane

domain of LAMP used in the present invention is a transmembrane domain of LAMP-1.

[0037]

In one embodiment, the transmembrane domain used in the present invention consists of the following amino acid sequence of (a) or (b):

(a) an amino acid sequence having at least 90% identity to the amino acid sequence of amino acid numbers 1006 to 1028 of SEQ ID NO: 2; or

(b) the amino acid sequence of amino acid numbers 1006 to 1028 of SEQ ID NO: 2, or an amino acid sequence in which 1 to 2 amino acids are deleted, substituted, inserted and/or added in the amino acid sequence of amino acid numbers 1006 to 1028 of SEQ ID NO: 2.

[0038]

In one embodiment, the transmembrane domain used in the present invention consists of the amino acid sequence of amino acid numbers 1006 to 1028 of SEQ ID NO: 2.

[0039]

The structure of the endosomal/lysosomal targeting domain of LAMP is well known to those skilled in the art (WO 1994/017192). The endosomal/lysosomal targeting domain of LAMP has a function of transporting a protein to lysosome. As the endosomal/lysosomal targeting domain of LAMP used in the present invention, any suitable endosomal/lysosomal targeting domain of LAMP can be selected as long as it has a function of transporting the protein to lysosome. In one embodiment, endosomal/lysosomal targeting domain of LAMP used in the present invention is an endosomal/lysosomal targeting domain of LAMP-1.

[0040]

In one embodiment, the endosomal/lysosomal targeting domain of LAMP used in the present invention consists of the amino acid sequence of amino acid numbers 1037 to 1040 of SEQ ID NO: 2, or an amino acid sequence in which 1 amino acid is deleted, substituted, inserted and/or added in the amino acid sequence of amino acid numbers 1037 to 1040 of SEQ ID NO: 2.

[0041]

In one embodiment, the endosomal/lysosomal targeting domain of LAMP used in the present invention consists of an amino acid sequence in a range of amino acid numbers 1037 to 1040 of SEQ ID NO: 2.

[0042]

In the chimeric protein relating to the present invention, the signal peptide, the intra-organelle stabilizing domain of LAMP, each allergen comprised in the allergen domain, the transmembrane domain, and the endosomal/lysosomal targeting domain of LAMP may be directly linked or may be indirectly linked via a linker peptide. The linker

peptide to be used can be appropriately selected by those skilled in the art. In one embodiment, the linker peptide consists of 10 or less amino acids. In one embodiment, a linker peptide used between the intra-organelle stabilizing domain of LAMP and the allergen domain, between allergens, and between the allergen domain and the transmembrane domain is a linker peptide selected from the group consisting of LeuGlu, GlyGlyGlyGly, and GluPheThr. In one embodiment, the linker peptide used between the transmembrane domain and the endosomal/lysosomal targeting domain of LAMP is a linker peptide consisting of the amino acid sequence of amino acid numbers 1029 to 1036 of SEQ ID NO: 2.

[0043]

In one embodiment, the nucleic acid of the present invention is the following nucleic acid:

a nucleic acid comprising a nucleotide sequence encoding a chimeric protein, wherein the nucleotide sequence is a nucleotide sequence comprising the following nucleotide sequences in this order:

a nucleotide sequence encoding a signal peptide of LAMP,

a nucleotide sequence encoding an intra-organelle stabilizing domain of LAMP,

a nucleotide sequence encoding an allergen domain comprising Der p 1, Der p 2, Der p 23, and Der p 7,

a nucleotide sequence encoding a transmembrane domain of LAMP, and

a nucleotide sequence encoding an endosomal/lysosomal targeting domain of LAMP.

[0044]

In one embodiment, the nucleic acid of the present invention is the following nucleic acid:

a nucleic acid comprising a nucleotide sequence encoding a chimeric protein, wherein the nucleotide sequence is a nucleotide sequence comprising the following nucleotide sequences in this order:

a nucleotide sequence encoding a signal peptide of LAMP-1,

a nucleotide sequence encoding the intra-organelle stabilizing domain of LAMP-1,

a nucleotide sequence encoding an allergen domain comprising Der p 1, Der p 2, Der p 23, and Der p 7,

a nucleotide sequence encoding a transmembrane domain of LAMP-1, and

a nucleotide sequence encoding an endosomal/lysosomal targeting domain the of LAMP-1.

[0045]

In one embodiment, the nucleic acid of the present invention is the following nucleic acid:

a nucleic acid comprising a nucleotide sequence encoding a chimeric protein, wherein the nucleotide sequence is a nucleotide sequence comprising the following nucleotide sequences in this order:

a nucleotide sequence encoding a signal peptide consisting of the amino acid sequence of amino acid numbers 1 to 27 of SEQ ID NO: 2,

a nucleotide sequence encoding an intra-organelle stabilizing domain of LAMP consisting of the amino acid sequence of amino acid numbers 28 to 380 of SEQ ID NO: 2,

a nucleotide sequence encoding an allergen domain comprising Der p 1 consisting of the amino acid sequence of amino acid numbers 383 to 594 of SEQ ID NO: 2, Der p 2 consisting of the amino acid sequence of amino acid numbers 599 to 727 of SEQ ID NO: 2, Der p 23 consisting of the amino acid sequence of amino acid numbers 732 to 800 of SEQ ID NO: 2, and Der p 7 consisting of the amino acid sequence of amino acid numbers 805 to 1002 of SEQ ID NO: 2,

a nucleotide sequence encoding a transmembrane domain consisting of the amino acid sequence of amino acid numbers 1006 to 1028 of SEQ ID NO: 2, and

a nucleotide sequence encoding an endosomal/lysosomal targeting domain of LAMP consisting of the amino acid sequence of amino acid numbers 1037 to 1040 of SEQ ID NO: 2.

[0046]

In one embodiment, the nucleic acid of the present invention is the following nucleic acid:

a nucleic acid comprising a nucleotide sequence encoding a chimeric protein, wherein the nucleotide sequence is a nucleotide sequence comprising the following nucleotide sequences in this order:

a nucleotide sequence encoding a signal peptide consisting of the amino acid sequence of amino acid numbers 1 to 27 of SEQ ID NO: 2,

a nucleotide sequence encoding an intra-organelle stabilizing domain of LAMP consisting of the amino acid sequence of amino acid numbers 28 to 380 of SEQ ID NO: 2,

a nucleotide sequence encoding an allergen domain comprising Der p 1 consisting of the amino acid sequence of amino acid numbers 383 to 594 of SEQ ID NO: 2, Der p 2 consisting of the amino acid sequence of amino acid numbers 599 to 727 of SEQ ID NO: 2, Der p 23 consisting of the amino acid sequence of amino acid numbers 732 to 800 of SEQ ID NO: 2, and Der p 7 consisting of the amino acid sequence of amino acid numbers 805 to 1002 of SEQ ID NO: 2,

a nucleotide sequence encoding a transmembrane domain consisting of the amino acid sequence of amino acid numbers 1006 to 1028 of SEQ ID NO: 2,

a nucleotide sequence encoding a peptide linker consisting of the amino acid sequence of amino acid numbers 1029 to 1036 of SEQ ID NO: 2, and

a nucleotide sequence encoding an endosomal/lysosomal targeting domain of LAMP consisting of the amino acid sequence of amino acid numbers 1037 to 1040 of SEQ ID NO: 2.

[0047]

In one embodiment, the nucleic acid of the present invention is the following nucleic acid:

a nucleic acid comprising a nucleotide sequence encoding a chimeric protein, wherein the nucleotide sequence is a nucleotide sequence comprising the following nucleotide sequences in this order:

a nucleotide sequence encoding a signal peptide,

a nucleotide sequence encoding an intra-organelle stabilizing domain of LAMP,

a nucleotide sequence encoding an allergen domain comprising Der p 1, Der p 2, Der p 23, and Der p 7 in this order,

a nucleotide sequence encoding a transmembrane domain, and

a nucleotide sequence encoding an endosomal/lysosomal targeting domain of LAMP.

[0048]

In one embodiment, the nucleic acid of the present invention is the following nucleic acid:

a nucleic acid comprising a nucleotide sequence encoding a chimeric protein, wherein the nucleotide sequence is a nucleotide sequence comprising the following nucleotide sequences in this order:

a nucleotide sequence encoding a signal peptide of LAMP,

a nucleotide sequence encoding an intra-organelle stabilizing domain of LAMP,

a nucleotide sequence encoding an allergen domain comprising Der p 1, Der p 2, Der p 23, and Der p 7 in this order,

a nucleotide sequence encoding a transmembrane domain of LAMP, and

a nucleotide sequence encoding an endosomal/lysosomal targeting domain of LAMP.

[0049]

In one embodiment, the nucleic acid of the present invention is the following nucleic acid:

a nucleic acid comprising a nucleotide sequence encoding a chimeric protein, wherein the nucleotide sequence is a nucleotide sequence comprising the following nucleotide sequences in this order:

a nucleotide sequence encoding a signal peptide of LAMP-1,

a nucleotide sequence encoding the intra-organelle stabilizing domain of LAMP-1,

a nucleotide sequence encoding an allergen domain comprising Der p 1, Der p 2,

Der p 23, and Der p 7 in this order,

a nucleotide sequence encoding a transmembrane domain of LAMP-1, and

a nucleotide sequence encoding an endosomal/lysosomal targeting domain of LAMP-1.

[0050]

In one embodiment, the nucleic acid of the present invention is the following nucleic acid:

a nucleic acid comprising a nucleotide sequence encoding a chimeric protein, wherein the nucleotide sequence is a nucleotide sequence comprising the following nucleotide sequences in this order:

a nucleotide sequence encoding a signal peptide consisting of the amino acid sequence of amino acid numbers 1 to 27 of SEQ ID NO: 2,

a nucleotide sequence encoding an intra-organelle stabilizing domain of LAMP consisting of the amino acid sequence of amino acid numbers 28 to 380 of SEQ ID NO: 2,

a nucleotide sequence encoding an allergen domain comprising Der p 1 consisting of the amino acid sequence of amino acid numbers 383 to 594 of SEQ ID NO: 2, Der p 2 consisting of the amino acid sequence of amino acid numbers 599 to 727 of SEQ ID NO: 2, Der p 23 consisting of the amino acid sequence of amino acid numbers 732 to 800 of SEQ ID NO: 2, and Der p 7 consisting of the amino acid sequence of amino acid numbers 805 to 1002 of SEQ ID NO: 2 in this order,

a nucleotide sequence encoding a transmembrane domain consisting of the amino acid sequence of amino acid numbers 1006 to 1028 of SEQ ID NO: 2, and

a nucleotide sequence encoding an endosomal/lysosomal targeting domain of LAMP consisting of the amino acid sequence of amino acid numbers 1037 to 1040 of SEQ ID NO: 2.

[0051]

In one embodiment, the nucleic acid of the present invention is the following nucleic acid:

a nucleic acid comprising a nucleotide sequence encoding a chimeric protein, wherein the nucleotide sequence is a nucleotide sequence comprising the following nucleotide sequences in this order:

a nucleotide sequence encoding a signal peptide consisting of the amino acid sequence of amino acid numbers 1 to 27 of SEQ ID NO: 2,

a nucleotide sequence encoding an intra-organelle stabilizing domain of LAMP consisting of the amino acid sequence of amino acid numbers 28 to 380 of SEQ ID NO: 2,

a nucleotide sequence encoding an allergen domain comprising Der p 1 consisting of the amino acid sequence of amino acid numbers 383 to 594 of SEQ ID NO: 2, Der p 2 consisting of the amino acid sequence of amino acid numbers 599 to 727 of SEQ ID NO: 2,

Der p 23 consisting of the amino acid sequence of amino acid numbers 732 to 800 of SEQ ID NO: 2, and Der p 7 consisting of the amino acid sequence of amino acid numbers 805 to 1002 of SEQ ID NO: 2 in this order,

a nucleotide sequence encoding a transmembrane domain consisting of the amino acid sequence of amino acid numbers 1006 to 1028 of SEQ ID NO: 2,

a nucleotide sequence encoding a peptide linker consisting of the amino acid sequence of amino acid numbers 1029 to 1036 of SEQ ID NO: 2, and

a nucleotide sequence encoding an endosomal/lysosomal targeting domain of LAMP consisting of the amino acid sequence of amino acid numbers 1037 to 1040 of SEQ ID NO: 2.

[0052]

The nucleic acid of the present invention is not particularly limited as long as it encodes the chimeric protein relating to the present invention, and has an action of inducing Th1-type immunity with respect to the allergen selected from the group consisting of Der p 1, Der p 2, Der p 23, and Der p 7, when the nucleic acid is administered to a human or an animal. One can confirm whether or not a certain nucleic acid has an action of inducing Th1-type immunity, when the nucleic acid is administered to a human or an animal, by the method described in, for example, Example 3 and/or Example 4. In addition, the nucleic acid of the present invention may be a nucleic acid having an action of inducing Th1 cell dominant immune response, when the nucleic acid is administered to a human or an animal. One can confirm whether or not a certain nucleic acid has an action of inducing Th1 cell dominant immune response, when the nucleic acid is administered to human or animal, by the method described in, for example, Example 4.

[0053]

In one embodiment, the nucleic acid of the present invention is the following nucleic acid:

a nucleic acid comprising a nucleotide sequence encoding a chimeric protein consisting of an amino acid sequence having at least 90%, 92%, 94%, 96%, 98%, or 99% identity to the amino acid sequence shown by SEQ ID NO: 2.

[0054]

In one embodiment, the nucleic acid of the present invention is the following nucleic acid:

a nucleic acid comprising a nucleotide sequence encoding a chimeric protein consisting of an amino acid sequence having at least 90% identity to the amino acid sequence shown by SEQ ID NO: 2.

[0055]

In one embodiment, the nucleic acid of the present invention is the following nucleic acid:

a nucleic acid comprising a nucleotide sequence encoding a chimeric protein consisting of the amino acid sequence shown by SEQ ID NO: 2, or a chimeric protein consisting of an amino acid sequence in which 1 to 10 amino acids are deleted, substituted, inserted and/or added in the amino acid sequence shown by SEQ ID NO: 2.

[0056]

In one embodiment, the nucleic acid of the present invention is the following nucleic acid:

a nucleic acid comprising a nucleotide sequence encoding a chimeric protein consisting of an amino acid sequence having at least 90% identity to the amino acid sequence shown by SEQ ID NO: 2,

wherein the nucleic acid has an action of inducing Th1-type immunity to the allergen selected from the group consisting of Der p 1, Der p 2, Der p 23, and Der p 7.

[0057]

In one embodiment, the nucleic acid of the present invention is the following nucleic acid:

a) a nucleic acid comprising a nucleotide sequence encoding a chimeric protein consisting of the amino acid sequence shown by SEQ ID NO: 2 or

b) a nucleic acid comprising a nucleotide sequence encoding a chimeric protein consisting of an amino acid sequence in which 1 to 10 amino acids are deleted, substituted, inserted and/or added in the amino acid sequence shown by SEQ ID NO: 2, wherein the nucleic acid has an action of inducing Th1-type immunity to an allergen selected from the group consisting of Der p 1, Der p 2, Der p 23, and Der p 7.

[0058]

In one embodiment, the nucleic acid of the present invention is the following nucleic acid:

a nucleic acid comprising a nucleotide sequence encoding a chimeric protein consisting of an amino acid sequence having at least 90% identity to the amino acid sequence shown by SEQ ID NO: 2, wherein the nucleic acid has an action of inducing Th1-type immunity to Der p 1, Der p 2, Der p 23, and Der p 7.

[0059]

In one embodiment, the nucleic acid of the present invention is the following nucleic acid:

a) a nucleic acid comprising a nucleotide sequence encoding a chimeric protein consisting of the amino acid sequence shown by SEQ ID NO: 2 or

b) a nucleic acid comprising a nucleotide sequence encoding a chimeric protein consisting of an amino acid sequence in which 1 to 10 amino acids are deleted, substituted, inserted and/or added in the amino acid sequence shown by SEQ ID NO: 2, wherein the nucleic acid has an action of inducing Th1-type immunity to Der p 1, Der p 2, Der p 23,

and Der p 7.

[0060]

In one embodiment, the nucleic acid of the present invention is the following nucleic acids

a nucleic acid comprising a nucleotide sequence encoding a chimeric protein consisting of the amino acid sequence shown by SEQ ID NO: 2.

[0061]

In one embodiment, the nucleotide sequence encoding the chimeric protein consisting of the amino acid sequence shown by SEQ ID NO: 2 means the nucleotide sequence shown by SEQ ID NO: 1.

[0062]

Based on the nucleotide sequence, the nucleic acid of the present invention can be easily prepared by those skilled in the art by using methods known in the art. For example, the nucleic acid of the present invention can be synthesized by using gene synthesis methods known in the art. As such a gene synthesis method, various methods known to those skilled in the art such as a method for synthesizing an antibody gene described in WO 90/07861 can be used.

[0063]

Once being synthesized, the nucleic acid of the present invention can be easily replicated by those skilled in the art using methods known in the art. For example, the nucleic acid of the present invention can be replicated by the method described later in <Method for producing the nucleic acid of the present invention and nucleic acid which can be produced by the method>.

[0064]

<Expression vector of the present invention>

The expression vector of the present invention includes an expression vector comprising the nucleic acid of the present invention.

[0065]

The expression vector used to express a chimeric protein from the nucleic acid of the present invention is not particularly limited as long as it can express the chimeric protein from the nucleic acid of the present invention in the animal cells. In one embodiment, the expression vector used to express a chimeric protein from the nucleic acid of the present invention is an expression vector which can be used for expressing the chimeric protein in a human body. Examples of the expression vector used in the present invention include a plasmid vector, a viral vector (for example, adenovirus, retrovirus, adeno-associated virus) and the like. In one embodiment, the expression vector of the present invention is a plasmid vector. In the present specification, "plasmid" means the plasmid vector.

[0066]

The expression vector of the present invention may comprise a promoter operably linked to the nucleic acid of the present invention. Examples of the promoter for expressing the chimeric protein from the nucleic acid of the present invention in animal cells include a virus-derived promoter such as CMV (cytomegalovirus), RSV (respiratory syncytial virus), and SV40 (simian virus 40), an actin promoter, EF (elongation factor) 1α promoter, a heat shock promoter and the like. In one embodiment, the promoter comprised in the expression vector of the present invention is a CMV promoter. The expression vector of the present invention may comprise a start codon and a stop codon. In this case, an enhancer sequence, an untranslated region, a splicing junction, a polyadenylation site, or a replicable unit may be comprised.

[0067]

In one embodiment, the expression vector of the present invention is an expression vector comprising the following nucleic acid:

a nucleic acid comprising a nucleotide sequence encoding a chimeric protein, wherein the nucleotide sequence is a nucleotide sequence comprising the following nucleotide sequences in this order:

a nucleotide sequence encoding a signal peptide consisting of the amino acid sequence of amino acid numbers 1 to 27 of SEQ ID NO: 2,

a nucleotide sequence encoding an intra-organelle stabilizing domain of LAMP consisting of the amino acid sequence of amino acid numbers 28 to 380 of SEQ ID NO: 2,

a nucleotide sequence encoding an allergen domain comprising Der p 1 consisting of the amino acid sequence of amino acid numbers 383 to 594 of SEQ ID NO: 2, Der p 2 consisting of the amino acid sequence of amino acid numbers 599 to 727 of SEQ ID NO: 2, Der p 23 consisting of the amino acid sequence of amino acid numbers 732 to 800 of SEQ ID NO: 2, and Der p 7 consisting of the amino acid sequence of amino acid numbers 805 to 1002 of SEQ ID NO: 2,

a nucleotide sequence encoding a transmembrane domain consisting of the amino acid sequence of amino acid numbers 1006 to 1028 of SEQ ID NO: 2, and

a nucleotide sequence encoding an endosomal/lysosomal targeting domain of LAMP consisting of the amino acid sequence of amino acid numbers 1037 to 1040 of SEQ ID NO: 2.

[0068]

In one embodiment, the expression vector of the present invention is an expression vector comprising the following nucleic acid:

a nucleic acid comprising a nucleotide sequence encoding a chimeric protein, wherein the nucleotide sequence is a nucleotide sequence comprising the following nucleotide sequences in this order:

a nucleotide sequence encoding a signal peptide consisting of the amino acid sequence of amino acid numbers 1 to 27 of SEQ ID NO: 2,

a nucleotide sequence encoding an intra-organelle stabilizing domain of LAMP consisting of the amino acid sequence of amino acid numbers 28 to 380 of SEQ ID NO: 2,

a nucleotide sequence encoding an allergen domain comprising Der p 1 consisting of the amino acid sequence of amino acid numbers 383 to 594 of SEQ ID NO: 2, Der p 2 consisting of the amino acid sequence of amino acid numbers 599 to 727 of SEQ ID NO: 2, Der p 23 consisting of the amino acid sequence of amino acid numbers 732 to 800 of SEQ ID NO: 2, and Der p 7 consisting of the amino acid sequence of amino acid numbers 805 to 1002 of SEQ ID NO: 2,

a nucleotide sequence encoding a transmembrane domain consisting of the amino acid sequence of amino acid numbers 1006 to 1028 of SEQ ID NO: 2,

a nucleotide sequence encoding a peptide linker consisting of the amino acid sequence of amino acid numbers 1029 to 1036 of SEQ ID NO: 2, and

a nucleotide sequence encoding an endosomal/lysosomal targeting domain of LAMP consisting of the amino acid sequence of amino acid numbers 1037 to 1040 of SEQ ID NO: 2.

[0069]

In one embodiment, the expression vector of the present invention is an expression vector comprising the following nucleic acid:

a nucleic acid comprising a nucleotide sequence encoding a chimeric protein, wherein the nucleotide sequence is a nucleotide sequence comprising the following nucleotide sequences in this order:

a nucleotide sequence encoding a signal peptide consisting of the amino acid sequence of amino acid numbers 1 to 27 of SEQ ID NO: 2,

a nucleotide sequence encoding an intra-organelle stabilizing domain of LAMP consisting of the amino acid sequence of amino acid numbers 28 to 380 of SEQ ID NO: 2,

a nucleotide sequence encoding an allergen domain comprising Der p 1 consisting of the amino acid sequence of amino acid numbers 383 to 594 of SEQ ID NO: 2, Der p 2 consisting of the amino acid sequence of amino acid numbers 599 to 727 of SEQ ID NO: 2, Der p 23 consisting of the amino acid sequence of amino acid numbers 732 to 800 of SEQ ID NO: 2, and Der p 7 consisting of the amino acid sequence of amino acid numbers 805 to 1002 of SEQ ID NO: 2 in this order,

a nucleotide sequence encoding a transmembrane domain consisting of the amino acid sequence of amino acid numbers 1006 to 1028 of SEQ ID NO: 2, and

a nucleotide sequence encoding an endosomal/lysosomal targeting domain of LAMP consisting of the amino acid sequence of amino acid numbers 1037 to 1040 of SEQ ID NO: 2.

[0070]

In one embodiment, the expression vector of the present invention is an expression vector comprising the following nucleic acid:

a nucleic acid comprising a nucleotide sequence encoding a chimeric protein, wherein the nucleotide sequence is a nucleotide sequence comprising the following nucleotide sequences in this order:

a nucleotide sequence encoding a signal peptide consisting of the amino acid sequence of amino acid numbers 1 to 27 of SEQ ID NO: 2,

a nucleotide sequence encoding an intra-organelle stabilizing domain of LAMP consisting of the amino acid sequence of amino acid numbers 28 to 380 of SEQ ID NO: 2,

a nucleotide sequence encoding an allergen domain comprising Der p 1 consisting of the amino acid sequence of amino acid numbers 383 to 594 of SEQ ID NO: 2, Der p 2 consisting of the amino acid sequence of amino acid numbers 599 to 727 of SEQ ID NO: 2, Der p 23 consisting of the amino acid sequence of amino acid numbers 732 to 800 of SEQ ID NO: 2, and Der p 7 consisting of the amino acid sequence of amino acid numbers 805 to 1002 of SEQ ID NO: 2 in this order,

a nucleotide sequence encoding a transmembrane domain consisting of the amino acid sequence of amino acid numbers 1006 to 1028 of SEQ ID NO: 2,

a nucleotide sequence encoding a peptide linker consisting of the amino acid sequence of amino acid numbers 1029 to 1036 of SEQ ID NO: 2, and

a nucleotide sequence encoding an endosomal/lysosomal targeting domain of LAMP consisting of the amino acid sequence of amino acid numbers 1037 to 1040 of SEQ ID NO: 2.

[0071]

In one embodiment, the expression vector of the present invention is an expression vector comprising a nucleic acid comprising a nucleotide sequence encoding a chimeric protein consisting of the amino acid sequence shown by SEQ ID NO: 2.

[0072]

In one embodiment, the expression vector of the present invention is an expression vector comprising a nucleic acid comprising the nucleotide sequence shown by SEQ ID NO: 1.

[0073]

In one embodiment, the expression vector of the present invention is an expression vector comprising a nucleic acid consisting of the nucleotide sequence shown by SEQ ID NO: 3.

[0074]

<Host cell of the present invention>

The host cell of the present invention includes a host cell transformed with the

nucleic acid of the present invention. In one embodiment, the host cell of the present invention is a host cell transformed with the expression vector of the present invention. In one embodiment, the host cell of the present invention is a host cell transformed with the expression vector of the present invention which is a plasmid vector.

[0075]

The host cell transformed with the nucleic acid of the present invention is not particularly limited, and any cell known in the art can be selected as long as it is a cell that can be used for nucleic acid replication.

[0076]

Examples of the host cell that can be used for nucleic acid replication include various cells such as natural cells or artificially established cells commonly used in the technical field of the present invention (for example, animal cells (for example, CHOK1SV cells), insect cells (for example, Sf9), bacteria (for example, *E. coli*), and yeasts (for example, *Saccharomyces* and *Pichia*)). In one embodiment, *E. coli* can be used as a host cell. Transformation itself can be carried out by known methods.

[0077]

<Method for producing the nucleic acid of the present invention and nucleic acid which can be produced by the method>

Examples of the method for producing the nucleic acid of the present invention include a method for producing a nucleic acid or an expression vector, which comprises a step of culturing host cells transformed with the nucleic acid or the expression vector of the present invention. In one embodiment, the method for producing the nucleic acid of the present invention comprises a step of culturing the host cell transformed with the nucleic acid of the present invention, and replicating the nucleic acid of the present invention. In one embodiment, the method for producing the nucleic acid of the present invention comprises a step of culturing the host cell transformed with the expression vector of the present invention, and replicating the expression vector of the present invention.

[0078]

In one embodiment, the host cell used in the method for producing the nucleic acid of the present invention is *E. coli*. For culture of *E. coli*, an appropriate culture medium such as LB medium, M9 medium, Terrific Broth medium, SOB medium, SOC medium, or 2 × YT medium can be selected. In addition, the culturing of *E. coli* can be carried out in an environment where carbon (it is not particularly limited as long as it is an assimilable carbon compound; for example, polyols such as glycerin, or organic acids such as pyruvic acid, succinic acid, or citric acid), nitrogen (it is not particularly limited as long as it is a nitrogen compound that can be used by *E. coli*; for example, peptone, meat extract, yeast extract, casein hydrolysate, soybean meal alkaline extract, or ammonia or a salt thereof), inorganics and inorganic ions (it is not particularly limited, and examples thereof include

phosphate, carbonate, sulfate, magnesium, calcium, potassium, iron, manganese and zinc), a vitamin source, and an antifoaming agent are controlled to an appropriate concentration. In addition, the control of culturing includes control of parameters such as pH, temperature, stir, air flow and dissolved oxygen. In one embodiment, the conditions of culturing include pH of 6.7 to 7.5, temperature of 20°C to 37°C, and a stirring speed of 200 to 300 rpm.

[0079]

The method for producing the nucleic acid of the present invention may comprise a step of obtaining lysate from collected culture solutions. The lysate can be obtained, for example, by treating the collected culture solutions with an alkaline lysis method or boiling method. Also, the step of obtaining the lysate may include a step of sterile filtration of a final lysate material.

[0080]

The method for producing the nucleic acid of the present invention may further comprise a step of purifying nucleic acid or an expression vector from lysate. Ion exchange chromatography and/or hydrophobic interaction chromatography can be used to purify the nucleic acid or the expression vector from the lysate. The step of purifying the nucleic acid or the expression vector from the lysate may include a step of ultrafiltration and/or diafiltration. In addition, as a final treatment of the purification step, a sterile filtration step may be comprised.

[0081]

In one embodiment, the nucleic acid of the present invention is a nucleic acid produced by the method for producing the nucleic acid of the present invention.

[0082]

In one embodiment, the expression vector of the present invention is an expression vector produced by the method for producing the nucleic acid of the present invention.

[0083]

<Pharmaceutical composition of the present invention>

The pharmaceutical composition of the present invention includes a pharmaceutical composition comprising the nucleic acid of the present invention and a pharmaceutically acceptable excipient. In one embodiment, the pharmaceutical composition of the present invention is a pharmaceutical composition comprising the vector of the present invention and the pharmaceutically acceptable excipient. The pharmaceutical composition of the present invention can be prepared by a generally used method with an excipient generally used in the field, that is, a pharmaceutical excipient, a pharmaceutical carrier or the like. Examples of dosage forms of these pharmaceutical compositions include, for example, parenteral agents such as injections and drip agents, which can be administered by intravenous administration, subcutaneous administration,

intradermal administration, and intramuscular administration. In formulating, excipients, carriers, additives, and the like can be used according to these dosage forms within the pharmaceutically acceptable range.

[0084]

In one embodiment, the pharmaceutical composition of the present invention is a pharmaceutical composition comprising the nucleic acid or the expression vector of the present invention and the pharmaceutically acceptable excipient.

[0085]

While the administration amount of the nucleic acid of the present invention or the expression vector varies depending on the degree of symptoms and age of the patient, and the dosage form of the preparation used, for example, the amount in a range of 0.001 mg/kg to 100 mg/kg can be used. Further, it is possible to prepare a formulation by adding the nucleic acid or the expression vector of the present invention in an amount corresponding to such administration amount.

[0086]

The pharmaceutical composition of the present invention can be used as an agent for preventing or treating allergy caused by an allergen selected from Der p 1, Der p 2, Der p 23, and Der p 7. Further, the pharmaceutical composition of the present invention can be used as an agent for prevention or treating the mite allergy.

[0087]

The present invention includes a pharmaceutical composition for preventing or treating allergy, comprising the nucleic acid of the present invention. In addition, the present invention includes a method for preventing or treating allergy, comprising administering a prophylactically effective or therapeutically effective amount of the nucleic acid of the present invention. The present invention also includes the nucleic acid of the present invention for use in preventing or treating allergy. In addition, the present invention includes use of the nucleic acid of the present invention for the manufacture of a pharmaceutical composition for preventing or treating allergy. In one embodiment, the above-described allergy is allergy caused by an allergen selected from the group consisting of Der p 1, Der p 2, Der p 23, and Der p 7. In addition, in one embodiment, the above-described allergy is allergy affecting an allergy patient having an antibody that responds to an allergen selected from the group consisting of Der p 1, Der p 2, Der p 23, and Der p 7. Further, in one embodiment, the above-described allergy is mite allergy.

[0088]

In one embodiment, the pharmaceutical composition of the present invention is a pharmaceutical composition for preventing or treating allergy, comprising the following nucleic acid and a pharmaceutically acceptable excipient:

a nucleic acid comprising a nucleotide sequence encoding a chimeric protein,

wherein the nucleotide sequence is a nucleotide sequence comprising the following nucleotide sequences in this order:

a nucleotide sequence encoding a signal peptide consisting of the amino acid sequence of amino acid numbers 1 to 27 of SEQ ID NO: 2,

a nucleotide sequence encoding an intra-organelle stabilizing domain of LAMP consisting of the amino acid sequence of amino acid numbers 28 to 380 of SEQ ID NO: 2,

a nucleotide sequence encoding an allergen domain comprising Der p 1 consisting of the amino acid sequence of amino acid numbers 383 to 594 of SEQ ID NO: 2, Der p 2 consisting of the amino acid sequence of amino acid numbers 599 to 727 of SEQ ID NO: 2, Der p 23 consisting of the amino acid sequence of amino acid numbers 732 to 800 of SEQ ID NO: 2, and Der p 7 consisting of the amino acid sequence of amino acid numbers 805 to 1002 of SEQ ID NO: 2,

a nucleotide sequence encoding a transmembrane domain consisting of the amino acid sequence of amino acid numbers 1006 to 1028 of SEQ ID NO: 2, and

a nucleotide sequence encoding an endosomal/lysosomal targeting domain of LAMP consisting of the amino acid sequence of amino acid numbers 1037 to 1040 of SEQ ID NO: 2.

[0089]

In one embodiment, the pharmaceutical composition of the present invention is a pharmaceutical composition for preventing or treating allergy, comprising the following nucleic acid and a pharmaceutically acceptable excipient:

a nucleic acid comprising a nucleotide sequence encoding a chimeric protein, wherein the nucleotide sequence is a nucleotide sequence comprising the following nucleotide sequences in this order:

a nucleotide sequence encoding a signal peptide consisting of the amino acid sequence of amino acid numbers 1 to 27 of SEQ ID NO: 2,

a nucleotide sequence encoding an intra-organelle stabilizing domain of LAMP consisting of the amino acid sequence of amino acid numbers 28 to 380 of SEQ ID NO: 2,

a nucleotide sequence encoding an allergen domain comprising Der p 1 consisting of the amino acid sequence of amino acid numbers 383 to 594 of SEQ ID NO: 2, Der p 2 consisting of the amino acid sequence of amino acid numbers 599 to 727 of SEQ ID NO: 2, Der p 23 consisting of the amino acid sequence of amino acid numbers 732 to 800 of SEQ ID NO: 2, and Der p 7 consisting of the amino acid sequence of amino acid numbers 805 to 1002 of SEQ ID NO: 2 in this order,

a nucleotide sequence encoding a transmembrane domain consisting of the amino acid sequence of amino acid numbers 1006 to 1028 of SEQ ID NO: 2, and

a nucleotide sequence encoding an endosomal/lysosomal targeting domain of LAMP consisting of the amino acid sequence of amino acid numbers 1037 to 1040 of SEQ

ID NO: 2.

[0090]

In one embodiment, the pharmaceutical composition of the present invention is a pharmaceutical composition for preventing or treating allergy, comprising a nucleic acid comprising a nucleotide sequence encoding a chimeric protein consisting of the amino acid sequence shown by SEQ ID NO: 2 and a pharmaceutically acceptable excipient.

[0091]

The present invention includes a pharmaceutical composition for preventing or treating allergy, comprising the expression vector of the present invention. In addition, the present invention includes a method for preventing or treating allergy, comprising administering a prophylactically effective or therapeutically effective amount of the expression vector of the present invention. The present invention also includes the expression vector of the present invention for use in preventing or treating allergy. In addition, the present invention includes use of the expression vector of the present invention for the manufacture of a pharmaceutical composition for preventing or treating allergy. In one embodiment, the above-described allergy is allergy caused by an allergen selected from the group consisting of Der p 1, Der p 2, Der p 23, and Der p 7. In addition, in one embodiment, the above-described allergy is allergy affecting an allergy patient having an antibody that responds to an allergen selected from the group consisting of Der p 1, Der p 2, Der p 23, and Der p 7. Further, in one embodiment, the above-described allergy is mite allergy.

[0092]

In one embodiment, the pharmaceutical composition of the present invention is a pharmaceutical composition for preventing or treating allergy, comprising an expression vector comprising the following nucleic acid and a pharmaceutically acceptable excipient:

a nucleic acid comprising a nucleotide sequence encoding a chimeric protein, wherein the nucleotide sequence is a nucleotide sequence comprising the following nucleotide sequences in this order:

a nucleotide sequence encoding a signal peptide consisting of the amino acid sequence of amino acid numbers 1 to 27 of SEQ ID NO: 2,

a nucleotide sequence encoding an intra-organelle stabilizing domain of LAMP consisting of the amino acid sequence of amino acid numbers 28 to 380 of SEQ ID NO: 2,

a nucleotide sequence encoding an allergen domain comprising Der p 1 consisting of the amino acid sequence of amino acid numbers 383 to 594 of SEQ ID NO: 2, Der p 2 consisting of the amino acid sequence of amino acid numbers 599 to 727 of SEQ ID NO: 2, Der p 23 consisting of the amino acid sequence of amino acid numbers 732 to 800 of SEQ ID NO: 2, and Der p 7 consisting of the amino acid sequence of amino acid numbers 805 to 1002 of SEQ ID NO: 2,

a nucleotide sequence encoding a transmembrane domain consisting of the amino acid sequence of amino acid numbers 1006 to 1028 of SEQ ID NO: 2, and

a nucleotide sequence encoding an endosomal/lysosomal targeting domain of LAMP consisting of the amino acid sequence of amino acid numbers 1037 to 1040 of SEQ ID NO: 2.

[0093]

In one embodiment, the pharmaceutical composition of the present invention is a pharmaceutical composition for preventing or treating allergy, comprising an expression vector comprising the following nucleic acid and a pharmaceutically acceptable excipient:

a nucleic acid comprising a nucleotide sequence encoding a chimeric protein, wherein the nucleotide sequence is a nucleotide sequence comprising the following nucleotide sequences in this order:

a nucleotide sequence encoding a signal peptide consisting of the amino acid sequence of amino acid numbers 1 to 27 of SEQ ID NO: 2,

a nucleotide sequence encoding an intra-organelle stabilizing domain of LAMP consisting of the amino acid sequence of amino acid numbers 28 to 380 of SEQ ID NO: 2,

a nucleotide sequence encoding an allergen domain comprising Der p 1 consisting of the amino acid sequence of amino acid numbers 383 to 594 of SEQ ID NO: 2, Der p 2 consisting of the amino acid sequence of amino acid numbers 599 to 727 of SEQ ID NO: 2, Der p 23 consisting of the amino acid sequence of amino acid numbers 732 to 800 of SEQ ID NO: 2, and Der p 7 consisting of the amino acid sequence of amino acid numbers 805 to 1002 of SEQ ID NO: 2 in this order,

a nucleotide sequence encoding a transmembrane domain consisting of the amino acid sequence of amino acid numbers 1006 to 1028 of SEQ ID NO: 2, and

a nucleotide sequence encoding an endosomal/lysosomal targeting domain of LAMP consisting of the amino acid sequence of amino acid numbers 1037 to 1040 of SEQ ID NO: 2.

[0094]

In one embodiment, the pharmaceutical composition of the present invention is a pharmaceutical composition for preventing or treating allergy, comprising an expression vector comprising a nucleic acid comprising a nucleotide sequence encoding a chimeric protein consisting of the amino acid sequence shown by SEQ ID NO: 2 and a pharmaceutically acceptable excipient.

[0095]

Specific examples are provided herein for reference in order to obtain further understanding of the present invention; however, these examples are for the purpose of illustration and the present invention is not limited thereto.

[Examples]

[0096]

[Example 1: Construction of LAMP-Der p 1-Der p 2-Der p 23-Der p 7 plasmid]

LAMP-Der p 1-Der p 2-Der p 23-Der p 7 plasmid consisting of the nucleotide sequence shown by SEQ ID NO: 3 (an expression vector comprising a nucleic acid comprising a nucleotide sequence comprising the following nucleotide sequences in this order (that is, a nucleotide sequence encoding a chimeric protein consisting of the amino acid sequence shown by SEQ ID NO: 2): a nucleotide sequence encoding a signal peptide of LAMP-1 (the amino acid sequence of 1 to 27 of SEQ ID NO: 2), a nucleotide sequence encoding an intra-organelle stabilizing domain of LAMP-1 (the amino acid sequence of 28 to 380 of SEQ ID NO: 2), a nucleotide sequence encoding an allergen domain comprising Der p 1, Der p 2, Der p 23, and Der p 7 in this order (the amino acid sequence of 383 to 1002 of SEQ ID NO: 2), a nucleotide sequence encoding a transmembrane domain of LAMP-1 (the amino acid sequence of 1006 to 1028 of SEQ ID NO: 2), and a nucleotide sequence encoding an endosomal/lysosomal targeting domain of LAMP-1 (the amino acid sequence of 1037 to 1040 of SEQ ID NO: 2)) was constructed. The plasmid can be constructed by inserting synthetic DNA, in which Xho I recognition sequence is added to 5' end of the nucleotide sequence of 1147 to 3006 of SEQ ID NO: 1 (a nucleotide sequence encoding an allergen domain comprising Der p 1, Der p 2, Der p 23, and Der p 7 in this order) and Eco RI recognition sequence is added to the 3' end of the nucleic acid sequence, into Eco RI-Xho I site of the plasmid shown by SEQ ID NO: 6 of Japanese Patent No. 5807994. *E. coli* was transformed with the constructed LAMP-Der p 1-Der p 2-Der p 23-Der p 7 plasmid and cultured in a liquid medium. The amplified LAMP-Der p 1-Der p 2-Der p 23-Der p 7 plasmid was obtained by a method of centrifuging the culture solution and collecting the cells based on a general plasmid extraction and purification method (miniprep method).

[0097]

[Example 2: Expression of LAMP-Der p 1-Der p 2-Der p 23-Der p 7 chimeric protein]

In vitro expression of the LAMP-Der p 1-Der p 2-Der p 23-Der p 7 chimeric protein (a chimeric protein consisting of an amino acid sequence encoded by the nucleotide sequence shown by SEQ ID NO: 1 (that is, the amino acid sequence shown by SEQ ID NO: 2)) by using human fetal kidney-derived 293T cell line was evaluated.

[0098]

(1) Cell culture and plasmid introduction

Human fetal kidney-derived 293T cells (Thermo Fisher Scientific, Cat. HCL4517) were seeded in 6-well plates (Cat. 3810-006 manufactured by IWAKI) at 3×10^5 cells/well in D-MEM medium (Sigma-Aldrich, Cat. D5796) containing 10% fetal bovine serum (Hyclone, Cat. SH30070.03) and 100-fold diluted penicillin-streptomycin (Thermo Fisher Scientific, Cat. 15070063). After overnight culture of the seeded cells at 37°C in the

presence of 5% CO_2, a mixed solution having a ratio of LAMP-Der p 1-Der p 2-Der p 23-Der p 7 plasmid : Lipofectamine 2000 (Thermo Fisher Scientific, Cat. 11668027) = 2.5 (μg) : 10 (μL) was added. After overnight culture of the seeded cells at 37°C in the presence of 5% CO_2 again, the medium was removed and washed once with PBS, and then western blotting was performed.

[0099]

(2) Western blotting

Pretreatment: Cells were lysed in RIPA buffer (Pierce, Cat. 89900) containing a protease inhibitor (Sigma-Aldrich, Cat. 1873580), and the protein concentration of the supernatant after centrifugation at 20,000 × g for 5 minutes was measured. To 5 μL of the cell lysate diluted with PBS containing protease inhibitor, 5 μL of LDS sample buffer (Thermo Fisher Scientific, Cat. NP0007) containing 100 mM DTT was added so that the protein concentration would be 200 μg/mL, and heat-treated at 70°C for 10 minutes.

SDS-PAGE: Using NuPAGE (Registered trademark) MOPS SDS Running buffer (Thermo Fisher Scientific, Cat. NP0001) and NuPAGE (Registered trademark) 4%-12% Bis-Tris Gel (Thermo Fisher Scientific, Cat. NP0323), the above-mentioned pretreated cell lysate was applied to the gel and electrophoresis was performed at a constant voltage of 200 V.

Blotting: Blotting was performed by bringing PVDF membrane (Thermo Fisher Scientific, Cat. LC2005) into contact with the gel after SDS-PAGE, and electrifying for 90 minutes at 180 mA in XCell II Blot Module (Thermo Fisher Scientific, Cat. EI9051) filled with NuPAGE (Registered trademark) Transfer buffer (Thermo Fisher Scientific, Cat. NP0006) containing 20% of methanol.

Blocking: The membrane after electrification was immersed in Blocking One (Nacalai Tesque, Cat. 03953-95) and shaken at room temperature for one hour.

Primary antibody: Anti-human LAMP-1 antibody (Sino biological, Cat. 11215-RP01) was added at 1000-fold dilution in TBS Tween-20 buffer (Thermo Fisher Scientific, Cat. 28360) containing 10% of Blocking One. The membrane was immersed in this buffer and shaken overnight at 4°C.

Secondary antibody: The membrane was washed with TBS Tween-20 buffer. Anti-rabbit IgG (H + L chain) pAb-HRP (MBL, Cat. 458) was added at 3000-fold dilution in TBS Tween-20 buffer containing 10% of Blocking One. The membrane was immersed in this buffer and shaken at room temperature for one hour.

Detection: The membrane was washed with TBS Tween-20 buffer. The membrane was immersed in ECL prime western blotting detection reagent (GE Healthcare, Cat. RPN2232), and an image was detected with LumiVision PRO 400EX (Aisin Seiki Co., Ltd.). In the image, a band responsive to the anti-human LAMP-1 antibody corresponding to the chimeric protein was detected.

[0100]

As the result of the above-mentioned tests, it was confirmed that LAMP-Der p 1-Der p 2-Der p 23-Der p 7 chimeric protein in the cell was expressed by introducing the LAMP-Der p 1-Der p 2-Der p 23-Der p 7 plasmid to the human fetal kidney-derived 293T cell line.

[0101]

[Example 3: Induction of IgG2a production by administration of LAMP-Der p 1-Der p 2-Der p 23-Der p 7 plasmid]

Evaluation of induction of antibody production *in vivo* was performed. In eight examples in each group, 25 μL of a PBS solution containing 50 μg of LAMP-Der p 1-Der p 2-Der p 23-Der p 7 plasmid was administered in the ear of 7-week-old BALB/c female mice (Charles River Laboratories Japan, Inc.) at the start of administration intradermally three times every week (Day 0, 7 and 14). One week after the final administration, blood was collected and plasma samples were obtained (Day 21). As a control, LAMP-Der p 23-Der p 7-Der p 2-Der p 1 plasmid (an expression vector comprising a nucleic acid comprising a nucleotide sequence comprising the following nucleotide sequences in this order: a nucleotide sequence encoding the amino acid sequence of amino acid numbers 1 to 380 of SEQ ID NO: 2 (hereinafter, refer to as N-terminal of LAMP-1 in Examples 3 and 4), a nucleotide sequence encoding an allergen domain comprising Der p 23 consisting of the amino acid sequence of amino acid numbers 732 to 800 of SEQ ID NO: 2 (hereinafter, refer to as Der p 23 domain in Examples 3 and 4), Der p 7 consisting of the amino acid sequence of amino acid numbers 805 to 1002 of SEQ ID NO: 2 (hereinafter, refer to as Der p 7 domain in Examples 3 and 4), Der p 2 consisting of the amino acid sequence of amino acid numbers 599 to 727 of SEQ ID NO: 2 (hereinafter, refer to as Der p 2 domain in Examples 3 and 4), and Der p 1 consisting of the amino acid sequence of amino acid numbers 383 to 594 of SEQ ID NO: 2 (hereinafter, refer to as Der p 1 domain in Examples 3 and 4) in this order, and a nucleotide sequence encoding the amino acid sequence of amino acid numbers 1006 to 1040 of SEQ ID NO: 2 (hereinafter, refer to as C-terminal of LAMP-1 in Examples 3 and 4)); a mixture of LAMP-Der p 1-Der p 2 plasmid (an expression vector comprising a nucleic acid comprising a nucleotide sequence comprising the following nucleotide sequences in this order: a nucleotide sequence encoding N-terminal of LAMP-1, a nucleotide sequence encoding an allergen domain comprising Der p 1 domain and Der p 2 domain in this order, and a nucleotide sequence encoding C-terminal of LAMP-1) and LAMP-Der p 23-Der p 7 plasmid (an expression vector comprising a nucleic acid comprising a nucleotide sequence comprising the following nucleotide sequences in this order: a nucleotide sequence encoding N-terminal of LAMP-1, a nucleotide sequence encoding allergen domain containing Der p 23 domain and Der p 7 domain in this order, and a nucleotide sequence encoding C-terminal of LAMP-1); and a

mixture of LAMP-Der p 1 plasmid (an expression vector comprising a nucleic acid comprising a nucleotide sequences comprising the following nucleotide sequences in this order: a nucleotide sequence encoding N-terminal of LAMP-1, a nucleotide sequence encoding an allergen domain comprising Der p 1 domain, and a nucleotide sequence encoding C-terminal of LAMP-1), LAMP-Der p 2 plasmid (an expression vector comprising a nucleic acid comprising a nucleotide sequence comprising the following nucleotide sequences in this order: a nucleotide sequence encoding N-terminal of LAMP-1, a nucleotide sequence encoding an allergen domain comprising Der p 2 domain, and a nucleotide sequence encoding C-terminal of LAMP-1), LAMP-Der p 7 plasmid (an expression vector comprising a nucleic acid comprising a nucleotide sequence comprising the following nucleotide sequences in this order: a nucleotide sequence encoding N-terminal of LAMP-1, a nucleotide sequence encoding an allergen domain comprising Der p 7 domain, and a nucleotide sequence encoding C-terminal of LAMP-1), and LAMP-Der p 23 plasmid (an expression vector comprising a nucleic acid comprising a nucleotide sequence comprising the following nucleotide sequences in this order: a nucleotide sequence encoding N-terminal of LAMP-1, a nucleotide sequence encoding an allergen domain comprising Der p 23 domain, and a nucleotide sequence encoding C-terminal of LAMP-1) were prepared. Each control plasmid can be prepared by the same method as the method described in Example 1. To mice, 25 μL of PBS solution containing 50 μg of the above plasmid or the above plasmid mixture or 25 μL of PBS was administered. An antibody titer was measured by ELISA using a 100-fold or 1000-fold diluted plasma sample, and the absorbance at 450 nm was measured. ELISA measurement was performed based on a general ELISA method using F96 MAXISORP NUNC-IMMUNO PLATE (Nunc, Cat. 439454) as a test plate. Der p 1 which is a purified protein (Indoor biotechnologies, NA-DP1-1, lot: 38052), Der p 2 which is a purified protein (Indoor biotechnologies, NA-DP2-1, lot: 36118), Der p 7 which is a recombinant purified protein (Indoor biotechnologies, RP-DP7-1, lot: 34033), or Der p 23 which is a recombinant purified protein (Sysmex, UniProtKB: A0A0K2DQU8) is prepared to 1 μg/mL with PBS, added at 50 μL/well and allowed to stand overnight at 4°C. After washing a test plate three times with a washing buffer (PBS Tween-20 buffer; Thermo Fisher Scientific, Cat. 28352), 100 μL/well of PBS containing 1% of BSA (Sigma-Aldrich, Cat. A8022) was added and allowed to stand at room temperature for one hour. After washing three times with the washing buffer, 50 μL/well of a 100-fold or 1000-fold diluted plasma sample in PBS containing 1% of BSA was added and allowed to stand at room temperature for one hour. After washing three times with the washing buffer, 50 μL/well of a 50000-fold diluted secondary antibody, Goat anti-mouse IgG2a HRP Conjugated (Bethyl Laboratories, Cat. A90-107P), in PBS containing 1% of BSA was added, and the test plate was allowed to stand at room temperature for one hour. After washing three

times with the washing buffer, 50 μL/well of TMB Microwell Peroxidase Substrate System (SeraCare Life Sciences, Inc., Cat.50-76-03) which is a substrate solution was added and the plate was allowed to stand at room temperature for 15 minutes with blocking light. A reaction stop solution (2N H_2SO_4) was added at 50 μL/well and absorbance at 450 nm was measured.

[0102]

As the result of the above-mentioned tests, the production of Der p 1, Der p 2, Der p 23 and Der p 7 specific IgG2a was detected by administering LAMP-Der p 1-Der p 2-Der p 23-Der p 7 plasmid (Der p1-p2-p23-p7) to mice (Fig. 1). On the other hand, even when LAMP-Der p 23-Der p 7-Der p 2-Der p 1 plasmid (Der p23-p7-p2-p1), a mixture of LAMP-Der p 1-Der p 2 plasmid and LAMP-Der p 23-Der p 7 plasmid (Der p1-p2 + Der p23-p7), and a mixture of LAMP-Der p 1 plasmid, LAMP-Der p 2 plasmid, LAMP-Der p 7 plasmid and LAMP-Der p 23 plasmid (4 plasmid mix) were administered to the mice, the production of Der p 1-specific IgG2a was not detected. In addition, when a mixture of LAMP-Der p 1-Der p 2 plasmid and LAMP-Der p 23-Der p 7 plasmid (Der p1-p2 + Der p23-p7) is administered to mice, the production of Der p 2 specific IgG 2a was not detected as well. That is, it was only the LAMP-Der p 1-Der p 2-Der p 23-Der p 7 plasmid that the production of IgG2a specific for all allergens encoded in the plasmid was detected. From the above results, it has been suggested that among the tested plasmids, only LAMP-Der p 1-Der p 2-Der p 23-Der p 7 plasmid induces Th1 immune responses for all the allergens and causes class switch of activated B cells to the IgG2a isotype.

[0103]

[Example 4: Induction of IFN-γ and IL-4 production by LAMP-Der p 1-Der p 2-Der p 23-Der p 7 Plasmid]

Evaluation of cytokine production induction upon stimulation with allergen was performed on splenocytes collected from mice administered with LAMP-Der p 1-Der p 2-Der p 23-Der p 7 plasmid. Splenocytes were prepared according to a general method from the mice used in Example 3 and the mice administered with a control plasmid (an expression vector comprising a nucleic acid comprising a nucleotide sequence comprising the following nucleotide sequences in this order: a nucleotide sequence encoding N-terminal of LAMP-1 and a nucleotide sequence encoding C-terminal of LAMP-1) with the same protocol as in Example 3 on Day 63. The control plasmid can be prepared by deleting Eco RI-Xho I site of the plasmid shown by SEQ ID NO: 6 of Japanese Patent No. 5807994. Splenocytes were seeded in 96-well plates (Cat. 3860-096 manufactured by IWAKI) at 8×10^5 cells/well in RPMI-1640 medium (Sigma-Aldrich, Cat. R8758) containing 10% fetal bovine serum (Hyclone, Cat. SH30070.03) and 100-fold diluted penicillin-streptomycin (ThermoFisher Scientific, Cat. 15070063). Der p 1 (Indoor biotechnologies, NA-DP1-1, lot: 38052), Der p 2 (Indoor biotechnologies, NA-DP2-1, lot:

36118), Der p 7 (Indoor biotechnologies, RP-DP7-1, lot: 34033), or Der p 23 (Sysmex, UniProtKB: A0A0K2DQU8) were added such that the final concentrations thereof were respectively 3, 3, 3, and 1.3 μg/mL. Culturing was performed at 37°C under 5% of CO_2 for 72 hours. The concentrations of IFN-γ and IL-4 in the culture supernatant were measured by ELISA method. A supernatant sample diluted 10-fold with TBS containing 0.1% BSA and 0.05% Tween 20 was used for the measurement of IFN-γ, and a supernatant undiluted sample was used for the measurement of IL-4. As a test plate for ELISA measurement, F96 MAXISORP NUNC-IMMUNO PLATE (Nunc, Cat. 439454) was used. The measurement was carried out using mouse IFN-γ DuoSet ELISA (R&D Systems, Cat. DY485) and mouse IL-4 DuoSet ELISA (R&D Systems, Cat. DY 404) according to attached protocol. As the result of the above-mentioned test, a mite-derived allergen-specific IFN-γ production was induced by administering 50 μg of LAMP-Der p 1-Der p 2-Der p 23-Der p 7 plasmid (Der p 1-p2-p 23-p 7) to mice three times (Fig. 2). Also, even in a case where LAMP-Der p 23-Der p 7-Der p 2-Der p 1 plasmid (Der p23-p7-p2-p1), a mixture of LAMP-Der p 1-Der p 2 plasmid and LAMP-Der p 23-Der p 7 plasmid (Der p1-p2 + Der p23-p7), and a mixture of LAMP-Der p 1 plasmid, LAMP-Der p 2 plasmid, LAMP-Der p 7 plasmid,and LAMP-Der p 23 plasmid (4 plasmid mix) were administered to the mice three times, comparable mite-derived allergen-specific IFN-γ production was induced. On the other hand, in the mice administered three times with 50 μg of LAMP-Der p 1-Der p 2-Der p 23-Der p 7 plasmid (Der p 1-p2-p23-p7), the mite-derived allergen-specific IL-4 production was the lower limit of detection (Fig. 3). Even in a case where LAMP-Der p 23-Der p 7-Der p 2-Der p 1 plasmid (Der p23-p7-p2-p1), a mixture of LAMP-Der p 1-Der p 2 plasmid and LAMP-Der p 23-Der p 7 plasmid (Der p1-p2 + Der p23-p7), and a mixture of LAMP-Der p 1 plasmid, LAMP-Der p 2 plasmid, LAMP-Der p 7 plasmid, and LAMP-Der p 23 plasmid (4 plasmid mix) were administered to the mice three times, the mite-derived allergen-specific IL-4 production was below the lower limit of detection.

[0104]

As the result of the above-mentioned tests, a mixture of LAMP-Der p 1-Der p 2-Der p 23-Der p 7 plasmid, LAMP-Der p 23-Der p 7-Der p 2-Der p 1 plasmid, LAMP-Der p 1-Der p 2 plasmid, and LAMP-Der p 23-Der p 7 plasmid, and a mixture of LAMP-Der p 1 plasmid, LAMP-Der p 2 plasmid, LAMP-Der p 7 plasmid, and LAMP-Der p 23 plasmid have been shown to induce Th1 cell dominant immune responses.

Industrial Applicability

[0105]

The nucleic acid of the present invention is expected to be useful for the prevention or treatment of mite allergy. In addition, the method for producing the nucleic

acid of the present invention is useful for producing the nucleic acid.

Sequence Listing Free Text

[0106]

The numerical heading <223> in the following sequence listing describes the description of "Artificial Sequence". Specifically, the nucleotide sequence shown by SEQ ID NO: 1 in the sequence listing is a nucleotide sequence encoding LAMP-Der p 1-Der p 2-Der p 23-Der p 7 chimeric protein, and the amino acid sequence shown by SEQ ID NO: 2 in the sequence listing is the amino acid sequence encoded by SEQ ID NO: 1. In addition, the nucleotide sequence shown by SEQ ID NO: 3 is the nucleotide sequence of LAMP-Der p 1-Der p 2-Der p 23-Der p 7 plasmid.

Claims

[Claim 1] A nucleic acid comprising:
a nucleotide sequence encoding a chimeric protein,
wherein the nucleotide sequence is a nucleotide sequence comprising the following nucleotide sequences in this order:
a nucleotide sequence encoding a signal peptide;
a nucleotide sequence encoding an intra-organelle stabilizing domain of LAMP;
a nucleotide sequence encoding an allergen domain comprising Der p 1, Der p 2, Der p 23, and Der p 7;
a nucleotide sequence encoding a transmembrane domain; and
a nucleotide sequence encoding an endosomal/lysosomal targeting domain of LAMP.

[Claim 2] A nucleic acid comprising:
a nucleotide sequence encoding a chimeric protein,
wherein the nucleotide sequence is a nucleotide sequence comprising the following nucleotide sequences in this order:
a nucleotide sequence encoding a signal peptide;
a nucleotide sequence encoding an intra-organelle stabilizing domain of LAMP;
a nucleotide sequence encoding an allergen domain comprising Der p 1, Der p 2, Der p 23, and Der p 7 in this order;
a nucleotide sequence encoding a transmembrane domain; and
a nucleotide sequence encoding an endosomal/lysosomal targeting domain of LAMP.

[Claim 3] The nucleic acid according to claim 1 or 2, wherein the signal peptide is a signal peptide of LAMP.

[Claim 4] The nucleic acid according to any one of claims 1 to 3, wherein the transmembrane domain is a transmembrane domain of LAMP.

[Claim 5] The nucleic acid according to any one of claims 1 to 4, wherein the signal peptide consists of the amino acid sequence of amino acid numbers 1 to 27 of SEQ ID NO: 2, the intra-organelle stabilizing domain consists of an amino acid sequence of amino acid numbers 28 to 380 of SEQ ID NO: 2, the allergen domain is an allergen domain comprising Der p 1 consisting of the amino acid sequence of amino acid numbers 383 to 594 of SEQ ID NO: 2, Der p 2 consisting of the amino acid sequence of amino acid

numbers 599 to 727 of SEQ ID NO: 2, Der p 23 consisting of the amino acid sequence of amino acid numbers 732 to 800 of SEQ ID NO: 2, and Der p 7 consisting of the amino acid sequence of amino acid numbers 805 to 1002 of SEQ ID NO: 2, the transmembrane domain consists of the amino acid sequence of amino acid numbers 1006 to 1028 of SEQ ID NO: 2, and the endosomal/lysosomal targeting domain consists of the an amino acid sequence of amino acid numbers 1037 to 1040 of SEQ ID NO: 2.

[Claim 6] A nucleic acid comprising:

a nucleotide sequence encoding a chimeric protein consisting of an amino acid sequence having at least 90% identity to the amino acid sequence shown by SEQ ID NO: 2, wherein the nucleic acid has an action of inducing Th1-type immunity to an allergen selected from the group consisting of Der p 1, Der p 2, Der p 23, and Der p 7.

[Claim 7] A nucleic acid comprising:

a) a nucleotide sequence encoding a chimeric protein consisting of the amino acid sequence shown by SEQ ID NO: 2; or

b) a nucleotide sequence encoding a chimeric protein consisting of an amino acid sequence in which 1 to 10 amino acids are deleted, substituted, inserted and/or added in the amino acid sequence shown by SEQ ID NO: 2, wherein the nucleic acid has an action of inducing Th1-type immunity to an allergen selected from the group consisting of Der p 1, Der p 2, Der p 23, and Der p 7.

[Claim 8] A nucleic acid comprising:

a nucleotide sequence encoding a chimeric protein consisting of an amino acid sequence shown by SEQ ID NO: 2.

[Claim 9] An expression vector comprising:

the nucleic acid according to any one of claims 1 to 8.

[Claim 10] An expression vector comprising:

the nucleic acid according to claim 8.

[Claim 11] A host cell transformed with the nucleic acid according to any one of claims 1 to 8.

[Claim 12] A method for producing a nucleic acid, comprising:

culturing a host cell transformed with the nucleic acid according to any one of claims 1 to 8.

[Claim 13] A pharmaceutical composition comprising:
the expression vector according to claim 10 and a pharmaceutically acceptable excipient.

[Claim 14] The pharmaceutical composition according to claim 13, which is a pharmaceutical composition for preventing or treating mite allergy.

[Claim 15] A method for preventing or treating mite allergy, comprising:
administering a prophylactically effective or therapeutically effective amount of the expression vector according to claim 10.

[Claim 16] The expression vector according to claim 10, for use in preventing or treating mite allergy.

[Claim 17] Use of the expression vector according to claim 10 for the manufacture of a pharmaceutical composition for preventing or treating mite allergy.