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(54) **CYTOTOXIC T CELL EPITOPE PEPTIDE FOR SARS CORONAVIRUS, AND USE THEREOF**

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C07K 14/005	(2006.01)
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CPC ***A61K 9/127*** (2013.01); *C12N 2770/20034* (2013.01); *A61K 2039/60* (2013.01); ***A61K 39/215*** (2013.01); *A61K 2039/55555* (2013.01); ***C07K 14/005*** (2013.01); *C12N 2770/20022* (2013.01)
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(57) **ABSTRACT**

The present invention aims to provide a novel CTL epitope peptide of the SARS coronavirus. The present invention provides a peptide having an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 11, 12, 13, 15, 17, 18, 23 and 24.

14 Claims, 3 Drawing Sheets

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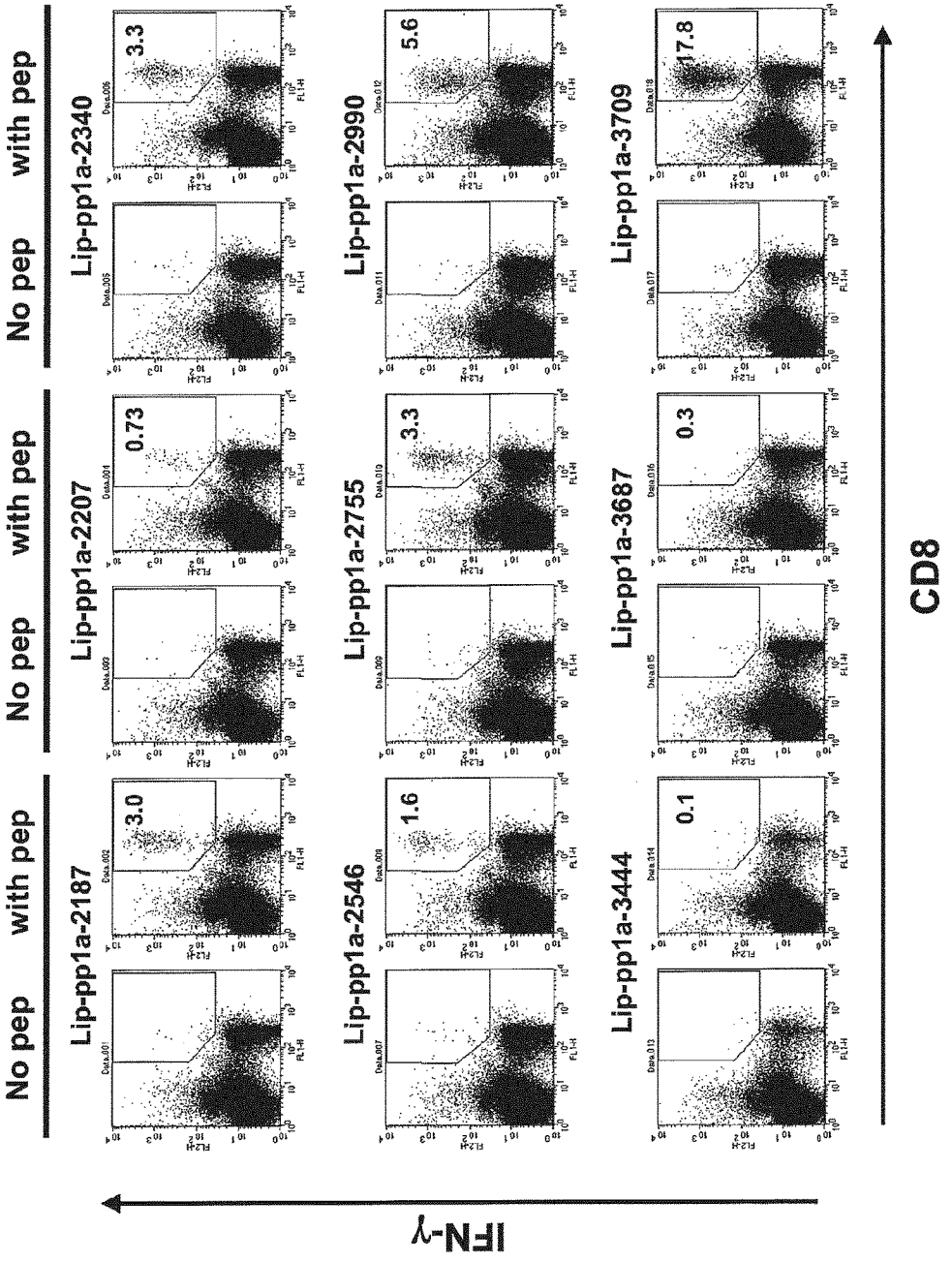
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with pep
No pep
with pep
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Lip-pp1a-2207
0.73
Lip-pp1a-2546
1.6
Lip-pp1a-2755
3.3
Lip-pp1a-3444
0.1
Lip-pp1a-3687
0.3
Lip-pp1a-2340
3.3
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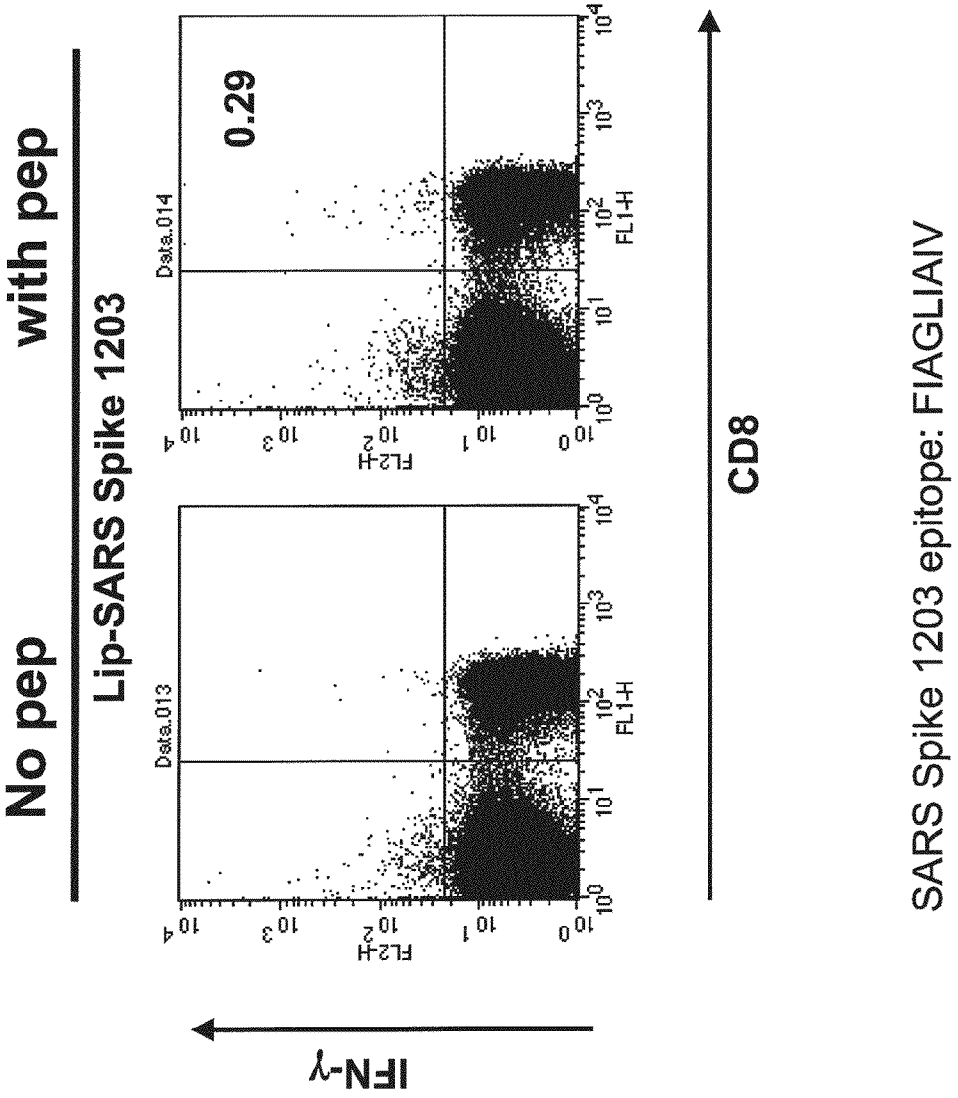
5.6
Lip-pp1a-3709
17.8
IFN-γ
CD8

Fig.1


No pep
with pep
Lip-SARS Spike 1203
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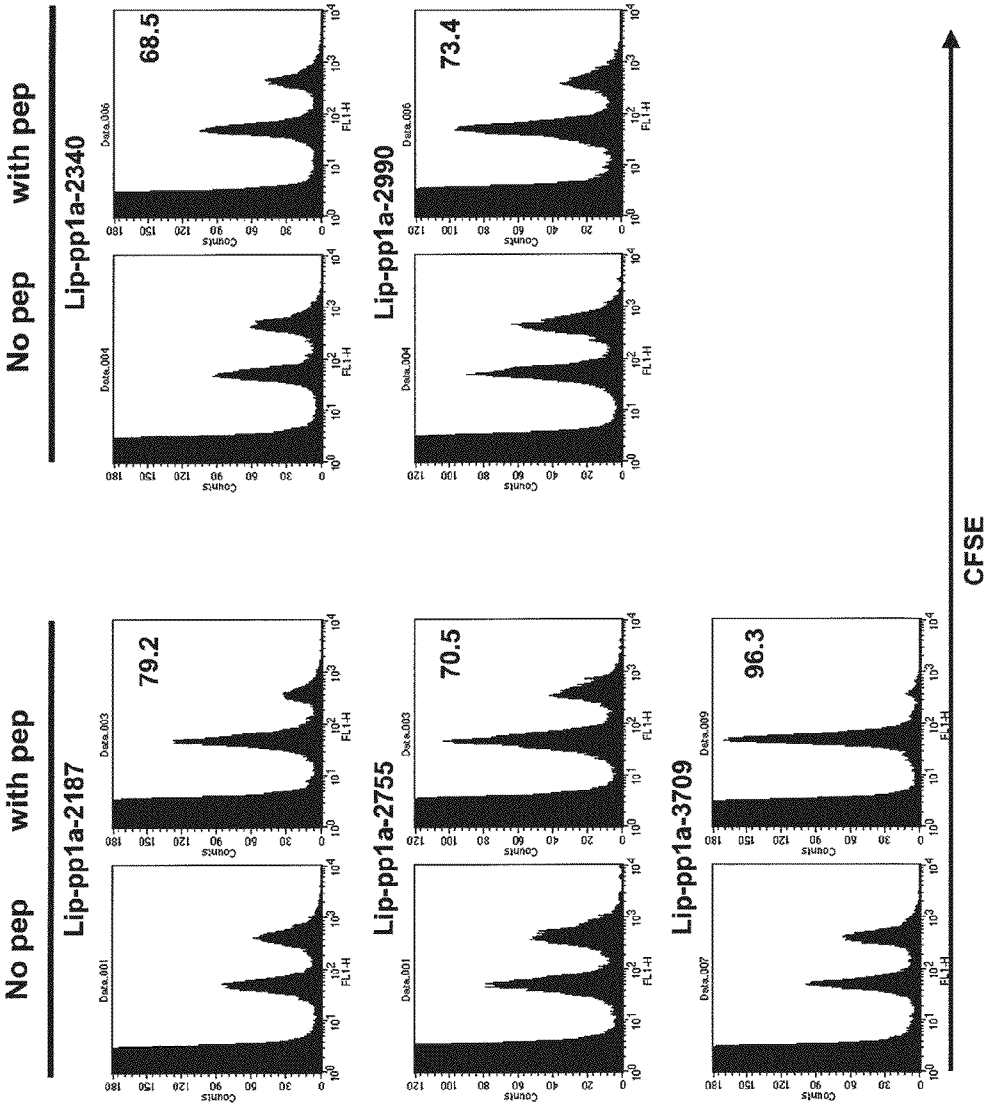
Data.013
FL2-H
FL1-H
IFN-γ
CD8
SARS Spike 1203 epitope: FIAGLIAIV

Fig.2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with pep
Lip-pp1a-2187
79.2
Lip-pp1a-2755
70.5
Lip-pp1a-3709
96.3
Lip-pp1a-2340
68.5
Lip-pp1a-2990
73.4
CFSE

Fig.3

CYTOTOXIC T CELL EPITOPE PEPTIDE FOR SARS CORONAVIRUS, AND USE THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a National Stage of International Application No. PCT/JP2009/070043 filed Nov. 27, 2009, claiming priority based on Japanese Patent Application No. 2008-304965, filed Nov. 28, 2008, the contents of all of which are incorporated herein by reference in their entirety.

TECHNICAL FIELD

The present invention relates to a cytotoxic T cell epitope peptide for the SARS coronavirus and use thereof.

BACKGROUND ART

Severe Acute Respiratory Syndrome (SARS) is an emerging infectious disease with a high lethality caused by the novel SARS coronavirus (SARS-CoV). Since the outbreak of the disease in 2003 in China, not less than 8000 people have been infected and about 800 people have died. However, no effective prophylactic or therapeutic method for the disease exists so far. After the outbreak of SARS, the SARS virus, which is the causative virus of SARS, was identified (Non-patent Document 1), and its base sequence has been determined (Non-patent Document 2).

The SARS coronavirus is a novel species of coronavirus belonging to Coronaviridae, a group of single-stranded (+) RNA viruses. The genome size of the SARS coronavirus is 29.7 kb, which is very large (Non-patent Document 2), and the genome encodes 23 putative proteins. As major structural proteins, there are Spike (1256 aa), Nucleocapsid (423 aa), Membrane (222 aa) and Small Envelope (77 aa). As nonstructural proteins, there are two polyproteins pp1a (4382 aa, SEQ ID NO:31; GenBank Accession No. AAP13439) and pp1b (2696 aa), and from these polyproteins, individual proteins are cleaved out by proteases in a site-specific manner.

CITATION LIST

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Non-patent Document 4: Wang. Y. D. et al., J. Virol. 78:5612-5618 (2004)

Non-patent Document 5: Chen, H. et al., J. Immunol. 175: 591-598 (2005)

Non-patent Document 6: Zhou, M. et al., J. Immunol. 177: 2138-2145 (2006)

SUMMARY OF INVENTION

Technical Problem

It has been reported so far that a virus-neutralizing antibody is induced by a DNA vaccine encoding the Spike protein (Non-patent Document 3). Although humoral immunity and cell-mediated immunity are necessary for effective defense reaction, cell-mediated immunity has been less studied compared to humoral immunity in the field of the SARS coronavirus. Cytotoxic T lymphocytes (CTLs) play an important role for protection against viruses in cell-mediated immunity, and therefore a new therapeutic method may be provided by controlling the CTL activity specific to the SARS coronavirus. From this viewpoint, identification of a strong CTL epitope peptide having a high antigenicity among the SARS coronavirus proteins has been demanded. Several types of partial peptides derived from the Spike protein, which is a structural protein, have been identified so far as CTL epitope peptides specific to the SARS coronavirus (Non-patent Documents 4 to 6). However, CTL epitope peptides derived from nonstructural proteins have not been reported yet.

The present invention aims to provide a novel CTL epitope peptide of the SARS coronavirus. More particularly, the present invention aims to provide a peptide comprising a novel CTL epitope derived from the SARS coronavirus pp1a protein, a peptide-bound liposome and an antigen presenting cell. Furthermore, the present invention aims to provide an inducing agent for HLA-A2-restricted CTLs, which comprises the peptide, the peptide-bound liposome or the antigen presenting cell as an active ingredient and is specific to the SARS coronavirus. The present invention also aims to provide a vaccine and the like for therapy or prophylaxis of infection by the SARS coronavirus.

Solution to Problem

The present inventors carried out the following studies in order to identify a CTL epitope derived from the SARS coronavirus, which has not been reported so far. First, by focusing on the pp1a protein, which is a nonstructural protein of the SARS coronavirus, 30 kinds of peptides were selected among peptides predicted as candidates of the epitope. Subsequently, the binding affinity to the HLA-A2 molecule, which is a MHC class I molecule, was confirmed for the peptides, and 9 kinds of peptides among the epitope candidate peptides were found to have significant CTL inducing activities. Furthermore, it was revealed that, by immunization with peptide-bound liposomes containing the peptides, the CTL induction is initiated and the CTL response is activated in vivo. Based on these findings, the present inventors succeeded in providing peptides comprising novel CTL epitopes derived from the SARS coronavirus pp1a protein, thereby completing the present invention.

That is, the present invention provides a peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 11, 12, 13, 15, 17, 18, 23 and 24. Furthermore, the peptide of the present invention preferably comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 12, 15, 17 and 24, and more preferably comprises the amino acid sequence shown in SEQ ID NO:24. The peptide of the present invention is characterized in that it comprises a cytotoxic T cell epitope specific to the SARS coronavirus, and that it comprises an HLA-A2-restricted cytotoxic T cell epitope.

The present invention provides a peptide-bound liposome, wherein a peptide is bound to the surface of a liposome, wherein the liposome comprises a phospholipid comprising a C_{14}-C_{24} acyl group containing one unsaturated bond, or a C_{14}-C_{24} hydrocarbon group containing one unsaturated bond, and a stabilizer, and wherein the peptide is at least one peptide selected from the above-described peptide.

The above-described phospholipid preferably comprises a C_{14}-C_{24} acyl group containing one unsaturated bond, and more preferably comprising an oleoyl group. Furthermore,

the above phospholipid is preferably at least one selected from the group consisting of diacylphosphatidylserine, diacylphosphatidylglycerol, diacylphosphatidic acid, diacylphosphatidylcholine, diacylphosphatidylethanolamine, succinimidyl-diacylphosphatidylethanolamine and maleimide-diacylphosphatidylethanolamine.

If the phospholipid comprised in the liposome has such a constitution, CTLs for killing pathogen-infected cells can be efficiently enhanced, and prophylaxis and therapy of infectious diseases becomes possible.

The above-described stabilizer is preferably a cholesterol. By this constitution, the above liposome can be more stabilized.

The above-described peptide is preferably bound to the phospholipid comprised in the liposome. By this way, the peptide can be presented on the surface of the liposome, thereby CTLs specific to the SARS coronavirus can be induced more effectively.

Furthermore, the peptide-bound liposome of the present invention preferably comprises the following constituents:

(A) 1 to 99.8 mol % of a phospholipid comprising a C_{14}-C_{24} acyl group containing one unsaturated bond, or a C_{14}-C_{24} hydrocarbon group containing one unsaturated bond; and

(B) 0.2 to 75 mol % of a stabilizer.

Furthermore, the peptide-bound liposome of the present invention preferably comprises the following constituents:

(I) 1 to 85 mol % of an acidic phospholipid comprising a C_{14}-C_{24} acyl group containing one unsaturated bond, or a C_{14}-C_{24} hydrocarbon group containing one unsaturated bond;

(II) 0.01 to 80 mol % of a neutral phospholipid comprising a C_{14}-C_{24} acyl group containing one unsaturated bond, or a C_{14}-C_{24} hydrocarbon group containing one unsaturated bond, at a concentration of;

(III) 0.2 to 80 mol % of a phospholipid comprising a C_{14}-C_{24} acyl group containing one unsaturated bond, or a C_{14}-C_{24} hydrocarbon group containing one unsaturated bond, wherein the phospholipid is bound to at least one peptide selected from the above-described peptide; and

(IV) 0.2 to 75 mol % of a stabilizer.

The present invention provides an antigen presenting cell prepared by contacting a cell expressing a cell surface antigen HLA-A2 with at least one peptide selected from the above-described peptide in vitro. The cell is preferably autologous, and more preferably allogenic.

Furthermore, the present invention provides an inducing agent for HLA-A2-restricted CTLs specific to the SARS coronavirus comprising at least one peptide selected from the above-described peptide, the peptide-bound liposome or the antigen presenting cell as an active ingredient.

The present invention provides a vaccine for prophylaxis of infection by the SARS coronavirus comprising at least one peptide selected from the above-described peptide, the peptide-bound liposome or the antigen presenting cell as an active ingredient.

Furthermore, the present invention provides a method for providing immunity to a subject who needs to be given immunity against the SARS coronavirus comprising administering at least one peptide selected from the above-described peptide, the peptide-bound liposome or the antigen presenting cell to the subject.

The present invention provides a method for providing immunity to a subject who needs to be given immunity against the SARS coronavirus comprising: collecting cells from the subject; preparing antigen presenting cells by contacting the cells with at least one peptide selected from the above-described peptide in vitro; and reinjecting the antigen presenting cells to the subject. The cells are preferably lymphoid monocytes, and more preferably dendritic cells.

Advantageous Effects of Invention

The present invention provides a peptide comprising a novel CTL epitope derived from the SARS coronavirus pp1a protein, a peptide-bound liposome and an antigen presenting cell. Since the CTL induction is initiated and the CTL response is activated in vivo by the peptide, the peptide-bound liposome or the antigen presenting cell, any of the peptide, the peptide-bound liposome and the antigen presenting cell of the present invention can be used as a CTL inducing agent and/or a vaccine for elimination of the SARS coronavirus. Furthermore, since nonstructural proteins are synthesized earlier than structural proteins during the process of virus infection, a virus elimination effect at the initial stage of virus infection can be expected by activating the immune reaction using a CTL epitope derived from the pp1a protein, which is a nonstructural protein.

BRIEF DESCRIPTION OF DRAWINGS

FIG. **1** shows staining plots for CD8 and IFN-γ by flow cytometry in mice immunized with a peptide-bound liposome. In FIG. **1**, the legends "No pep" and "with pep" each indicate that the immunization was conducted using a liposome alone and a peptide-bound liposome, respectively. In FIG. **1**, the legends "Lip-pp1a-xxxx" each indicate a peptide-bound liposome wherein the peptide is a SARS coronavirus pp1a epitope having a sequence shown in Table 1.

FIG. **2** shows staining plots for CD8 and IFN-γ by flow cytometry in mice immunized with a spike-1203-peptide-bound liposome. In FIG. **2**, the legends "No pep" and "with pep" each indicate that the immunization was conducted using a liposome alone and a peptide-bound liposome, respectively. In FIG. **2**, the legend "Lip-SARS Spike 1203" indicates a peptide-bound liposome wherein the peptide is a SARS Spike 1203 epitope of SEQ ID NO: 34 (FIAGLIAIV).

FIG. **3** shows staining plots for CFSE (carboxy fluorescein diacetate succinimidyl ester) by flow cytometry in mice which were booster-immunized with CFSE-labeled antigen presenting cells. In FIG. **3**, the legends "No pep" and "with pep" each indicate that the immunization was conducted using a liposome alone and a peptide-bound liposome, respectively. In FIG. **3**, the legends "Lip-pp1a-xxxx" each indicate a peptide-bound liposome wherein the peptide is a SARS coronavirus pp1a epitope having a sequence shown in Table 1.

DESCRIPTION OF EMBODIMENTS

The best mode for carrying out the invention will be described in detail below.

The peptide in the present invention comprises a CTL epitope derived from the SARS coronavirus pp1a protein, and particular examples thereof include peptides containing an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 11, 12, 13, 15, 17, 18, 23 and 24, and peptides comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 11, 12, 13, 15, 17, 18, 23 and 24. Furthermore, an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 12, 15, 17 and 24 is preferred, and the amino acid sequence shown in SEQ ID NO:24 is more preferred. These peptides comprise an HLA-A2-restricted CTL epitope, more particularly, an HLA-A*0201-restricted CTL epitope. The peptide of the present

invention may be used in various forms (e.g., unmodified, fusion, glycosylated and nonglycosylated), and may contain a C-terminal modification (amidation, esterification, modification with aldehyde or the like), N-terminal modification (acetylation, biotinylation, fluorescent labeling or the like) and chemical modification of a functional group (phosphorylation, sulfation, biotinylation or the like).

The peptide may be synthesized according to a method used in conventional peptide chemistry. Examples of the known method of peptide synthesis include those described in literatures (Peptide Synthesis, Interscience, New York, 1966; The Proteins, Vol 2, Academic Press Inc., New York, 1976; Peptide Synthesis, MARUZEN Co., Ltd., 1975; Fundamentals and Experiments of Peptide Synthesis, MARUZEN Co., Ltd., 1985; Development of Pharmaceuticals, Continued Edition, Vol. 14, Peptide Synthesis, Hirokawa Shoten, 1991) and the like.

The peptide of the present invention as described above may be used solely or in combination of plural types of peptides as an inducing agent for HLA-A2-restricted CTLs specific to the SARS coronavirus and/or as a vaccine for therapy or prophylaxis of infection of the SARS coronavirus. That is, since the peptide of the present invention can bind to a HLA-A2 molecule and is presented by cells that express the HLA-A2 molecule, thereby strongly induce CTLs, the peptide of the present invention can be used as a CTL inducing agent and/or a vaccine for elimination of the SARS coronavirus.

The liposome used for the peptide-bound liposome of the present invention is a phospholipid bilayer membrane having a closed space.

The liposome used for the peptide-bound liposome of the present invention comprises: a phospholipid comprising a C_{14}-C_{24} acyl group containing one unsaturated bond, or a C_{14}-C_{24} hydrocarbon group containing one unsaturated bond; and a stabilizer. A phospholipid comprising a C_{14}-C_{24} acyl group containing one unsaturated bond is preferably used as the phospholipid.

The carbon number of the acyl group in the phospholipid comprising a C_{14}-C_{24} acyl group containing one unsaturated bond is preferably 16 to 22, more preferably 18 to 22, and most preferably 18. Particular examples of the acyl group include palmitoleoyl, oleoyl and erucoyl, and most preferably oleoyl. The carbon number of the hydrocarbon group in the phospholipid comprising a C_{14}-C_{24} hydrocarbon group containing one unsaturated bond is preferably 16 to 22, more preferably 18 to 22, and most preferably 18. Particular examples of the hydrocarbon group include tetradecenyl, hexadecenyl, octadecenyl, C_{20} monoene, C_{22} monoene and C_{24} monoene and the like. The unsaturated acyl groups or unsaturated hydrocarbon groups bound to the 1-position and the 2-position of the glycerin residue comprised in the phospholipid may be either the same or different. In view of industrial productivity, the groups at the 1-position and the 2-position are preferably the same.

In view of enhancement of the CTL activity to a practically sufficient level, the phospholipid preferably comprises a C_{14}-C_{24} acyl group containing one unsaturated bond. In cases where the carbon number of the acyl group is less than 13, the liposome may be unstable, or the CTL activity enhancement effect may be insufficient. In cases where the carbon number of the acyl group is more than 24, the liposome may be unstable.

Examples of the phospholipid comprising a C_{14}-C_{24} acyl group containing one unsaturated bond, or comprising a C_{14}-C_{24} hydrocarbon group containing one unsaturated bond include acidic phospholipids, neutral phospholipids, and reactive phospholipids comprising a functional group to which a peptide can be bound. Their types and ratios may be selected depending on various demands.

Examples of the acidic phospholipids which may be used include phosphatidylserine, phosphatidylglycerol, phosphatidic acid and phosphatidylinositol. In view of enhancement of the CTL activity to a practically sufficient level, industrial supply capacity, quality for use as a pharmaceutical, and the like, diacylphosphatidylserine, diacylphosphatidylglycerol, diacylphosphatidic acid and diacylphosphatidylinositol comprising a C_{14}-C_{24} acyl group containing one unsaturated bond are preferably used. Since an acidic phospholipid gives an anionic group on the surface of a liposome, a negative zeta potential is given on the surface of the liposome. Therefore, the liposome gains charge repulsion and can exist as a stable formulation in an aqueous solvent. Thus, an acidic phospholipid is important in view of ensuring the stability of the liposome in an aqueous solvent.

Examples of the neutral phospholipids include phosphatidylcholine. The neutral phospholipids which may be employed in the present invention may be used by selecting their types and amounts appropriately within the ranges in which enhancement of the CTL activity can be achieved. Compared to an acidic phospholipid and a phospholipid to which a peptide is bound, a neutral phospholipid has a higher function to stabilize a liposome and hence can enhance the stability of the membrane. From this viewpoint, the liposome to be used for the peptide-bound liposome of the present invention preferably contains a neutral phospholipid. The amount of the neutral phospholipid to be used can be determined after securing the contents of the acidic phospholipid for achievement of the CTL activity enhancement effect, the reactive phospholipid for the peptide bond and the stabilizer.

The peptide of the present invention is bound to the surface of the liposome by being bound to a phospholipid comprising a C_{14}-C_{24} acyl group containing one unsaturated bond, or a C_{14}-C_{24} hydrocarbon group containing one unsaturated bond, wherein the phospholipid is comprised in the liposome. As the phospholipid for such binding of the peptide, a reactive phospholipid comprising a functional group to which the peptide can be bound is used. The type and the amount of the reactive phospholipid comprising a C_{14}-C_{24} acyl group containing one unsaturated bond, or comprising a C_{14}-C_{24} hydrocarbon group containing one unsaturated bond are appropriately selected depending on various demands. Similarly to the case of the above-described phospholipid, it is also not preferred that the carbon number of the unsaturated acyl group or the unsaturated hydrocarbon group comprised in the phospholipid is more than 24 or less than 14 in the case of the reactive phospholipid.

Examples of the reactive phospholipid include phosphatidylethanolamine and terminally modified derivatives thereof. Furthermore, phosphatidylglycerol, phosphatidylserine, phosphatidic acid and phosphatidylinositol, and terminally modified derivatives thereof may also be used as the reactive phospholipid. In view of industrial availability, simplicity of the process of binding with a peptide, yield and the like, phosphatidylethanolamine or a terminally modified derivatives thereof is preferably used. Phosphatidylethanolamine has an amino group to which an antibody can be bound at its terminus. Furthermore, in view of enhancement of the CTL activity to a practically sufficient level, stability in a liposome, industrial supply capacity, quality for use as a pharmaceutical, and the like, diacylphosphatidylethanolamine comprising a C_{14}-C_{24} acyl group containing one unsaturated bond or a terminally modified derivatives thereof is most preferably used.

Diacylphosphatidylethanolamine can be obtained by, for example, using diacylphosphatidylcholine as a crude material and carrying out a base-exchange reaction of choline and ethanolamine using phospholipase D. More particularly, a solution of diacylphosphatidylcholine in chloroform is mixed at an appropriate ratio with water in which phospholipase D and ethanolamine are dissolved, to obtain a crude reaction product. The crude reaction product is purified with a silica gel column using a chloroform/methanol/water solvent, thereby obtaining the diacylphosphatidylethanolamine of interest. Those skilled in the art can carry out the purification by appropriately selecting the conditions for purification by the column, such as the composition ratio of the solvent.

Examples of the terminally modified derivatives include a terminally modified diacylphosphatidylethanolamine produced by binding one of the termini of a divalent reactive compound to the amino group of diacylphosphatidylethanolamine. Examples of the divalent reactive compound which may be used include compounds comprising, at least one terminus, an aldehyde group or a succinimide group which can react with the amino group of diacylphosphatidylethanolamine. Examples of the divalent reactive compound comprising an aldehyde group include glyoxal, glutaraldehyde, succindialdehyde and terephthalaldehyde. Preferred examples thereof include glutaraldehyde. Examples of the divalent reactive compound comprising a succinimide group include dithiobis(succinimidylpropionate), ethylene glycolbis(succinimidylsuccinate), disuccinimidyl succinate, disuccinimidyl suberate and disuccinimidyl glutarate.

Furthermore, examples of the divalent reactive compound comprising a succinimide group at one terminus and a maleimide group at the other terminus include N-succinimidyl4-(p-maleimidophenyl)butyrate, sulfosuccinimidyl-4-(p-maleimidophenyl)butyrate, N-succinimidyl-4-(p-maleimidophenyl)acetate, N-succinimidyl-4-(p-maleimidophenyl)propionate, succinimidyl-4-(N-maleimidoethyl)-cyclohexane-1-carboxylate, sulfosuccinimidyl-4-(N-maleimidoethyl)-cyclohexane-1-carboxylate, N-(γ-maleimidobutyryloxy)succinimide and N-(ϵ-maleimidocaproyloxy)succinimide. By using such a divalent reactive compound, a terminally modified diacylphosphatidylethanolamine comprising a maleimide group as a functional group is obtained. By binding a functional group at one terminus of such a divalent reactive compound to the amino group of diacylphosphatidylethanolamine, a terminally modified diacylphosphatidylethanolamine can be obtained.

Examples of the method for binding the peptide to the surface of the liposome include a method wherein a liposome comprising the above reactive phospholipid is prepared and the peptide is then added thereto to bind the peptide to the reactive phospholipid in the liposome. Furthermore, by preliminarily binding the peptide to the reactive phospholipid and mixing the obtained peptide-bound reactive phospholipid with a phospholipid other than a reactive phospholipid and a stabilizer, a liposome in which the peptide is bound to its surface can also be obtained. The method for binding a peptide to a reactive phospholipid is well-known in the art.

The liposome to be used for the peptide-bound liposome of the present invention comprises at least 1 type, for example, 2 or more types, preferably 3 or more types of phospholipid(s) comprising a C_{14}-C_{24} acyl group containing one unsaturated bond, or a C_{14}-C_{24} hydrocarbon group containing one unsaturated bond. For example, the liposome to be used in the peptide-bound liposome of the present invention comprises at least 1 type, for example, 2 or more types, preferably 3 or more types of phospholipid(s) comprising a C_{14}-C_{24} acyl group containing one unsaturated bond, or a C_{14}-C_{24} hydrocarbon group containing one unsaturated bond selected from diacylphosphatidylserine, diacylphosphatidylglycerol, diacylphosphatidic acid, diacylphosphatidylcholine, diacylphosphatidylethanolamine, succinimidyl-diacylphosphatidylethanolamine and maleimide-diacylphosphatidylethanolamine.

Furthermore, the liposome to be used for the peptide-bound liposome of the present invention preferably comprises at least one type of each of:

acidic phospholipid comprising a C_{14}-C_{24} acyl group containing one unsaturated bond, or a C_{14}-C_{24} hydrocarbon group containing one unsaturated bond;

neutral phospholipid comprising a C_{14}-C_{24} acyl group containing one unsaturated bond, or a C_{14}-C_{24} hydrocarbon group containing one unsaturated bond; and

reactive phospholipid comprising a C_{14}-C_{24} acyl group containing one unsaturated bond, or a C_{14}-C_{24} hydrocarbon group containing one unsaturated bond.

In the present invention, sterols and tocopherols may be used as the stabilizer of the liposome. The sterols may be those generally known as sterols, and examples thereof include cholesterol, sitosterol, campesterol, stigmasterol and brassicasterol. In view of availability and the like, cholesterol is especially preferably used. The tocopherols may be those generally known as tocopherols, and preferred examples thereof include commercially available α-tocopherol in view of availability and the like.

Furthermore, as long as the effect of the present invention is not adversely affected, the liposome to be used for the peptide-bound liposome of the present invention may contain known liposome constituting components that can constitute a liposome.

Examples of the composition of the liposome to be used for the peptide-bound liposome of the present invention include the following:

(A) 1 to 99.8 mol % of a phospholipid comprising: a C_{14}-C_{24} acyl group containing one unsaturated bond, or a C_{14}-C_{24} hydrocarbon group containing one unsaturated bond; and

(B) 0.2 to 75 mol % of a stabilizer.

The content of each component is represented as mol % with respect to the total constituting components of the peptide-bound liposome.

In view of the stability of the liposome, the content of the component (A) is preferably 10 to 90 mol %, more preferably 30 to 80 mol %, still more preferably 50 to 70 mol %.

In view of the stability of the liposome, the content of the component (B) is preferably 5 to 70 mol %, more preferably 10 to 60 mol %, still more preferably 20 to 50 mol %. In cases where the content of the stabilizer is more than 75 mol %, the stability of the liposome is deteriorated, which is not preferred.

The component (A) comprises the followings:

(a) a phospholipid, to which a peptide is not bound, comprising a C_{14}-C_{24} acyl group containing one unsaturated bond, or a C_{14}-C_{24} hydrocarbon group containing one unsaturated bond; and

(b) a phospholipid, to which a peptide is bound, comprising a C_{14}-C_{24} acyl group containing one unsaturated bond, or a C_{14}-C_{24} hydrocarbon group containing one unsaturated bond.

The content of the component (a) is usually 0.01 to 85 mol %, preferably 0.1 to 80 mol %, more preferably 0.1 to 60 mol %, still more preferably 0.1 to 50 mol %.

The content of the component (b) is usually 0.2 to 80 mol %, preferably 0.3 to 60 mol %, more preferably 0.4 to 50 mol %, still more preferably 0.5 to 25 mol %. In cases where the

content is less than 0.2 mol %, the amount of the peptide becomes low, and therefore it is difficult to activate CTLs to a practically sufficient level. In cases where the content is more than 80 mol %, the stability of the liposome becomes low.

The phospholipid of the component (a) usually includes the above-mentioned acidic phospholipid and neutral phospholipid. The phospholipid of the component (b) includes the above-mentioned reactive phospholipid.

The content of the acidic phospholipid is usually 1 to 85 mol %, preferably 2 to 80 mol %, more preferably 4 to 60 mol %, still more preferably 5 to 40 mol %. In cases where the content is less than 1 mol %, the zeta potential becomes small and the stability of the liposome becomes low, and it is difficult to activate CTLs to a practically sufficient level. On the other hand, in cases where the content is more than 85%, the content of the peptide-bound phospholipid in the liposome becomes low as a result, and therefore it is difficult to activate CTLs to a practically sufficient level.

The content of the neutral phospholipid is usually 0.01 to 80 mol %, preferably 0.1 to 70 mol %, more preferably 0.1 to 60 mol %, still more preferably 0.1 to 50 mol %. In cases where the content is more than 80.0 mol %, the contents of the acidic phospholipid, the peptide-bound phospholipid and the liposome stabilizer comprised in the liposome become low, and it is difficult to activate CTLs to a practically sufficient level.

The peptide-bound phospholipid is obtained by binding a peptide to the reactive phospholipid, and the ratio of binding of the reactive phospholipid to the peptide may be selected within the range in which the effect of the present invention is not adversely affected, by taking the type of the functional group used in the binding, conditions of the binding treatment, and the like into consideration appropriately. For example, in cases where the terminally modified diacylphosphatidylethanolamine obtained by binding one terminus of a divalent reactive compound disuccinimidyl succinate to the terminal amino group of diacylphosphatidylethanolamine is used as the reactive phospholipid, 10 to 99% of the reactive phospholipid can be bound to the peptide depending on selection of various conditions of the binding treatment. In such cases, the reactive phospholipid which is not bound to the peptide is comprised in the liposome as an acidic phospholipid.

Examples of a preferred mode of the peptide-bound liposome of the present invention include the following composition:

(I) 1 to 85 mol % of an acidic phospholipid comprising a C_{14}-C_{24} acyl group containing one unsaturated bond, or a C_{14}-C_{24} hydrocarbon group containing one unsaturated bond;

(II) 0.01 to 80 mol % of a neutral phospholipid comprising a C_{14}-C_{24} acyl group containing one unsaturated bond, or a C_{14}-C_{24} hydrocarbon group containing one unsaturated bond;

(III) 0.2 to 80 mol % of a phospholipid, to which at least one peptide selected from the above-described peptide is bound, comprising a C_{14}-C_{24} acyl group containing one unsaturated bond, or a C_{14}-C_{24} hydrocarbon group containing one unsaturated bond; and

(IV) 0.2 to 75 mol % of a stabilizer.

(100 mol % in total)

Examples of a more preferred mode of the liposome to be used for the peptide-bound liposome of the present invention include the following composition:

the component (I) at a concentration of 2 to 80 mol %;

the component (II) at a concentration of 0.1 to 70 mol %;

the component (III) at a concentration of 0.3 to 60 mol %; and

the component (IV) at a concentration of 10 to 70 mol %.

(100 mol % in total)

Examples of a still more preferred mode of the liposome to be used for the peptide-bound liposome of the present invention include the following composition:

the component (I) at a concentration of 4 to 60 mol %;

the component (II) at a concentration of 0.1 to 60 mol %;

the component (III) at a concentration of 0.4 to 50 mol %; and

the component (IV) at a concentration of 20 to 60 mol %.

(100 mol % in total)

Examples of an especially preferred mode of the liposome to be used for the peptide-bound liposome of the present invention include the following composition:

the component (I) at a concentration of 5 to 40 mol %;

the component (II) at a concentration of 0.1 to 50 mol %;

the component (III) at a concentration of 0.5 to 25 mol %; and

the component (IV) at a concentration of 25 to 55 mol %.

(100 mol % in total)

The carbon number of the unsaturated acyl group or the unsaturated hydrocarbon group to be comprised in the phospholipid in the liposome used in the peptide-bound liposome of the present invention is characteristically 14 to 24, but a phospholipid comprising an unsaturated acyl group or unsaturated hydrocarbon group comprising a carbon number of less than 14 or more than 24 may also be comprised to an extent at which the effect of the present invention is not adversely affected. The ratio of the number of the C_{14}-C_{24} unsaturated acyl group or unsaturated hydrocarbon group with respect to the total number of all the unsaturated acyl groups or unsaturated hydrocarbon groups comprised in the phospholipid in the liposome used in the peptide-bound liposome of the present invention is, for example, 50% or more, preferably 60% or more, more preferably 75% or more, still more preferably 90% or more, most preferably 97% or more (for example, substantially 100%).

The liposome to be used for the peptide-bound liposome of the present invention may also comprise a lipid other than a phospholipid, comprising a C_{14}-C_{24} acyl group or hydrocarbon group, as long as the effect of the present invention is not adversely affected. The content of the lipid is usually 40 mol % or less, preferably 20 mol % or less, more preferably 10 mol % or less, still more preferably 5 mol % or less (for example, substantially 0 mol %).

The liposome to be used in the present invention can be obtained by, for example, a method wherein a phospholipid as a constituting component, reactive phospholipid, stabilizer, peptide and the like are used and are blended and processed in an appropriate way, followed by adding the resulting product to an appropriate solvent. Examples of the production process include the extrusion method, vortex mixer method, ultrasonic method, surfactant removal method, reverse-phase evaporation method, ethanol injection method, prevesicle method, French press method, W/O/W emulsion method, annealing method and freeze-thaw method. The particle diameter of the liposome is not restricted, however, in view of the stability during storage, the particle diameter is, for example, 20 to 600 nm, preferably 30 to 500 nm, more preferably 40 to 400 nm, still more preferably 50 to 300 nm, most preferably 70 to 230 nm.

In the present invention, in order to enhance the physicochemical stability of the liposome, sugars or polyols may be added to the internal aqueous phase and/or the external aqueous phase during or after preparation of the liposome. In

particular, in cases where the liposome needs to be stored for a long time or during formulation, a sugar or a polyol may be added/dissolved as a protective agent for the liposome, followed by removing water by freeze-drying to prepare a lyophilized product of the phospholipid composition.

Examples of the sugars include monosaccharides such as glucose, galactose, mannose, fructose, inositol, ribose and xylose; disaccharides such as saccharose, lactose, cellobiose, trehalose and maltose; trisaccharides such as raffinose and melezitose; oligosaccharides such as cyclodextrin; polysaccharides such as dextrin; and sugar alcohols such as xylitol, sorbitol, mannitol and maltitol. Among these sugars, monosaccharides and disaccharides are preferred, and glucose and saccharose are especially preferred in view of availability and the like.

Examples of the polyols include glycerin compounds such as glycerin, diglycerin, triglycerin, tetraglycerin, pentaglycerin, hexaglycerin, heptaglycerin, octaglycerin, nonaglycerin, decaglycerin and polyglycerin; sugar alcohol compounds such as sorbitol and mannitol; ethylene glycol; diethylene glycol; triethylene glycol; tetraethylene glycol; pentaethylene glycol; hexaethylene glycol; heptaethylene glycol; octaethylene glycol and nonaethylene glycol. Among these, glycerin, diglycerin, triglycerin, sorbitol, mannitol, and polyethylene glycols having molecular weights of 400 to 10,000 are preferred in view of availability. The concentration of the sugars or polyols to be contained in the internal aqueous phase and/or the external aqueous phase is, for example, 1 to 20% by weight, preferably 2 to 10% by weight.

When the peptide-bound liposome of the present invention is produced, the peptide-bound liposome can be simply obtained by preparing a liposome to which the peptide has not been bound yet and then binding the peptide thereto. For example, a liposome which contains a phospholipid, stabilizer and a reactive phospholipid for binding the peptide on the surface of the membrane is prepared as, for example, a liposome liquid, and sucrose, which is one of the sugars, is added to its external aqueous phase to about 2 to 10% by weight and dissolved. This sugar-added formulation is transferred to a 10 ml glass vial and the vial is placed in a shelf freeze dryer, followed by cooling to, for example, −40° C. to freeze the sample, thereby obtaining a lyophilized product by a conventional method. The obtained lyophilized product can be stored for a long time since water has been removed, and, when necessary, by adding a particular peptide and carrying out the following steps, the peptide-bound liposome of the present invention can be simply and rapidly obtained. In cases where the interaction between the peptide and the liposome is strong and strong instability is caused, it is a very simple way to store the liposome at the stage of a lyophilized product like this and to use after binding the peptide when necessary.

The liposome to be used for the peptide-bound liposome of the present invention may comprise a phospholipid to which a peptide is bound. Examples of the method to obtain the liposome containing a phospholipid to which a peptide is bound include the methods by the following (A) and (B).

(A) A liposome containing a phospholipid, a reactive phospholipid and a stabilizer is prepared, and a peptide and a divalent reactive compound are added thereto, followed by linking a functional group of the reactive phospholipid to a functional group of the peptide via the divalent reactive compound. As the divalent reactive compound, the one used for preparation of the terminally modified derivatives of the reactive phospholipid may be similarly used. Particular examples of the divalent reactive compound comprising an aldehyde group include glyoxal, glutaraldehyde, succindialdehyde and terephthalaldehyde. Preferred examples thereof include glutaraldehyde. Examples of the divalent reactive compound comprising a succinimide group include dithiobis(succinimidylpropionate), ethylene glycol-bis(succinimidylsuccinate), disuccinimidyl succinate, disuccinimidyl suberate and disuccinimidyl glutarate. Furthermore, examples of the divalent reactive compound comprising a succinimide group at one terminus and a maleimide group at the other terminus include N-succinimidyl-4-(p-maleimidophenyl)butyrate, sulfosuccinimidyl-4-(p-maleimidophenyl)butyrate, N-succinimidyl-4-(p-maleimidophenyl)acetate, N-succinimidyl-4-(p-maleimidophenyl)propionate, succinimidyl-4-(N-maleimidoethyl)-cyclohexane-1-carboxylate, sulfosuccinimidyl-4-(N-maleimidoethyl)-cyclohexane-1-carboxylate, N-(γ-maleimidobutyryloxy)succinimide and N-(ε-maleimidocaproyloxy)succinimide. By using such a divalent reactive compound, a terminally modified derivatives of a reactive phospholipid comprising a maleimide group as a functional group (e.g., phosphatidylethanolamine) is obtained.

(B) A method wherein a liposome containing a phospholipid, a reactive phospholipid and a stabilizer is prepared, and a peptide is added thereto, followed by linking a functional group of the reactive phospholipid contained in the liposome to a functional group of the peptide, and thereby binding the peptide to the liposome.

Examples of the type of the bond in the above (A) and (B) include an ionic bond, hydrophobic bond and covalent bond, and the type of the bond is preferably a covalent bond. Furthermore, particular examples of the covalent bond include a Schiff base bond, amide bond, thioether bond and ester bond. Both of the above two methods allow binding of the peptide to the reactive phospholipid contained in the liposome, thereby forming a phospholipid to which the peptide is bound in the liposome.

In the method (A), particular examples of the method to bind the liposome as a crude material to the peptide via a divalent reactive compound include a method using a Schiff base bond. Examples of the method to bind the liposome to the peptide via a Schiff base bond include a method wherein a liposome comprising an amino group on its surface is prepared and the peptide is added to a suspension of the liposome, followed by adding dialdehyde as the divalent reactive compound to the resulting mixture and binding the amino group on the surface of the liposome to the amino group in the peptide via a Schiff base.

Particular examples of this binding procedure include the following method.

(A-1) In order to obtain a liposome comprising an amino group on its surface, a reactive phospholipid comprising a C_{14}-C_{24} acyl group containing one unsaturated bond, or a C_{14}-C_{24} hydrocarbon group containing one unsaturated bond (e.g., phosphatidylethanolamine) is mixed with lipids as crude materials for the liposome (e.g., a phospholipid and a stabilizer for a liposome), to prepare a liposome wherein amino groups exist on the surface of the liposome in a certain amount.

(A-2) A peptide is added to the liposome suspension.

(A-3) Subsequently, glutaraldehyde is added as the divalent reactive compound, and the reaction is allowed to proceed for a certain length of time, thereby allowing a Schiff base bond to be formed between the liposome and the peptide.

(A-4) Thereafter, in order to inactivate the reactivity of the excess glutaraldehyde, glycine as an amino group-containing water-soluble compound is added to the liposome suspension and allowed to react therewith.

(A-5) By a method such as gel filtration, dialysis, ultrafiltration or centrifugation, the peptide unbound to the liposome, the reaction product between glutaraldehyde and glycine, and excess glycine are removed, to obtain a peptide-bound liposome suspension.

Particular examples of the method (B) include a method wherein a reactive phospholipid comprising a functional group that can form an amide bond, thioether bond, Schiff base bond, ester bond or the like is introduced to the phospholipid membrane. Particular examples of such a functional base include succinimide, maleimide, amino, imino, carboxyl, hydroxyl and thiol. Examples of the reactive phospholipid to be introduced to the liposome include the reactive phospholipid comprising a C_{14}-C_{24} acyl group containing one unsaturated bond, or a C_{14}-C_{24} hydrocarbon group containing one unsaturated bond (e.g., phosphatidylethanolamine) whose amino terminus is modified.

A particular example of the binding procedure will now be described referring to a case in which diacylphosphatidylethanolamine is used.

(B-1) Diacylphosphatidylethanolamine comprising a C_{14}-C_{24} acyl group containing one unsaturated bond is reacted with disuccinimidyl succinate by a known method at only one terminus, to obtain disuccinimidyl succinate-bound diacylphosphatidylethanolamine comprising a succinimide group as a functional group at the terminus.

(B-2) The disuccinimidyl succinate-bound diacylphosphatidylethanolamine is mixed with other liposome constituting components (e.g., a phospholipid and a stabilizer) by a known method, to prepare a liposome composition comprising a succinimide group on its surface as a functional group.

(B-3) To the liposome composition suspension, a peptide is added, and the amino group in the peptide is reacted with the succinimide group on the surface of the phospholipid membrane.

(B-4) The unreacted peptides, reaction byproducts and the like are removed by a method such as gel filtration, dialysis, ultrafiltration or centrifugation, to obtain a liposome suspension containing a peptide-bound phospholipid.

In cases where a liposome is bound to a peptide, it is practically preferred to use an amino group or a thiol group, which is often comprised as a functional group. In cases where an amino group is used, a Schiff base bond can be formed by reacting it with a succinimide group. In cases where a thiol group is used, a thioether bond can be formed by reacting it with a maleimide group.

The antigen presenting cell in the present invention is a cell prepared by bringing a cell that expresses the cell surface antigen HLA-A2 into contact with one or more types of peptides in vitro. The cell is preferably autologous and/or allogenic. Examples of the cell include cells that express the cell surface antigen HLA-A2 (e.g., HLA-A*0201). The cell is preferably a lymphoid monocyte (T cell, macrophage, B cell, dendric cell or the like), more preferably a dendritic cell.

The antigen presenting cell of the present invention can be used as an inducer of HLA-A2-restricted CTLs specific to the SARS coronavirus, and/or as a vaccine for therapy or prophylaxis of infection of the SARS coronavirus, by administering (injecting) the cell to a subject. That is, the antigen presenting cell of the present invention presents the peptide of the present invention on its surface and can strongly induce CTLs, so that the cell may be used as a CTL inducer and/or as a vaccine for the purpose of elimination of the SARS coronavirus. The antigen presenting cell is preferably prepared using a peptide at a concentration of preferably 1 to 100 μM, more preferably 5 to 50 μM, for example, 10 μM, per 10^7 cells.

The peptide, the peptide-bound liposome and the antigen presenting cell of the present invention can be used as inducers of HLA-A2-restricted CTLs specific to the SARS coronavirus, and/or as vaccines for therapy or prophylaxis of infection of the SARS coronavirus. The subject may be any animal including human. In a certain mode, the subject is human. The inducer and/or vaccine of the present invention is/are made into a common form as a pharmaceutical composition depending on the substance regarded as the active ingredient, and direct delivery of the composition is generally achieved by parenteral injection (e.g., subcutaneous injection, intraperitoneal injection, intravenous injection, intramuscular injection, or injection to the space between tissues). Examples of other administration methods include mucosal administration (e.g., oral, transnasal or pulmonary), transocular administration, percutaneous administration and administration by suppositories.

That is, in cases where the composition is administered parenterally, it may be administered in a dosage form such as an injection solution, transnasal agent, formulation for topical administration (e.g., percutaneous preparation), or formulation for rectal administration. In cases where the composition is orally administered, it may be administered in a dosage form usually used in the art. Examples of the injection solution include sterile solutions or suspensions, and emulsions, and particular examples thereof include water, water-propylene glycol solutions, buffers and 0.4% physiological saline. Furthermore, in cases where the composition is made into a liquid formulation, it can be stored frozen, or stored after removing water by freeze-drying or the like. When the freeze-dried formulation is to be used, it can be used by adding distilled water for injection or the like thereto and redissolving it. Examples of the formulation for topical administration include creams, ointments, lotions and percutaneous preparations. Examples of the oral preparation or the formulation for rectal administration include capsules, tablets, pills, powders, drops, suppositories and liquids.

The above dosage forms are formulated by methods usually used in the art, together with pharmaceutically acceptable vehicles and additives. Examples of the pharmaceutically acceptable vehicles and additives include carriers, binders, flavoring agents, buffering agents, thickening agents, coloring agents, stabilizers, emulsifiers, dispersants, suspending agents, antiseptics, pH adjusting agents, tonicity adjusting agents and wetting agents. Furthermore, examples of the pharmaceutically acceptable carriers include magnesium carbonate, lactose, pectin, starch and methyl cellulose.

The inducer of the present invention containing as an active ingredient a peptide, a peptide-bound liposome or an antigen presenting cell, and the vaccine of the present invention containing as an active ingredient a peptide, a peptide-bound liposome or an antigen presenting cell may further contain an adjuvant for enhancement of its effect. Examples of the adjuvant include aluminum hydroxide gel, Freund's complete adjuvant, Freund's incomplete adjuvant, pertussis adjuvant, poly(I,C) and CpG-DNA. Among these, CpG-DNA is preferred. CpG-DNA is a DNA containing an unmethylated CpG motif, and it can activate dendritic cells and enhance the CTL induction by the peptide, peptide-bound liposome or antigen presenting cell of the present invention.

The dose of the peptide of the present invention in the formulation, and the number of doses of the formulation vary depending on the symptoms, age, body weight, dosage form and the like, and it is preferred to administer usually 0.01 μg to 1 mg, preferably 0.1 μg to 500 μg, more preferably 1.0 μg to 100 μg of the peptide once in every several days or several months. For example, for the primary immune response (that

is, therapeutic or prophylactic administration), 1.0 μg to 500 μg of the peptide is administered to an adult patient, and depending on the response and the conditions of the patient assayed by measurement of the specific CTL activity in blood of the patient, boosting administration of 1.0 μg to 100 μg of the peptide is subsequently carried out according to boosting therapy that continues for several weeks to several months. The number of the antigen presenting cells of the present invention to be administered is preferably 10^9 to 10^6, more preferably 10^8 to 10^7, and the number of the cells may be appropriately controlled based on the symptoms, age, body weight, dosage form and the like.

EXAMPLES

The present invention will now be described more concretely, but the present invention is not restricted to the Examples below.

Example 1

Prediction of CTL Epitopes

Using two kinds of computer software for prediction of epitopes, BIMAS <http://www-bimas.cit.nih.gov/molbio/hla_bind>) and SYFPEITHI (<http://www.syfpeithi.de>), epitope candidates for pp1a were searched. The search was carried out with the following settings: HLA Molecule, HLA-A*0201; and Peptide Length, 9 to 10 amino acid residues. Thirty kinds of epitope candidate peptides showing high prediction scores in the both analytic methods were selected by the search. The amino acid sequences of these 30 kinds of epitope candidate peptides are shown in Table 1. For each of the 30 kinds of epitope candidate peptides, a synthetic peptide was prepared. Peptides that can actually function as epitopes were searched, and 9 kinds of epitopes were identified. In order to further analyze functions of the 9 kinds of peptides, the Examples 1 to 6 below were carried out.

TABLE 1

Epitope	Sequence	SEQ ID No:	BIMAS	SYFPEITHI
1) pp1a-15	QLSLPVLQV	1	160.0	26
2) pp1a-103	TLGVLVPHV	2	160.0	26
3) pp1a-445	TLNEDLLEI	3	98.4	28
4) pp1a-634	KLSAGVEFL	4	463.5	27
5) pp1a-651	FLITGVFDI	5	640.2	27
6) pp1a-1121	ILLAPLLSA	6	71.9	26
7) pp1a-1139	SLQVCVQTV	7	160.0	28
8) pp1a-1288	MLSRALKKV	8	272.0	25
9) pp1a-1652	YLSSVLLAL	9	226.0	28
10) pp1a-2187	CLDAGINYV	10	351.9	27
11) pp1a-2207	AMWLLLLSI	11	143.8	27
12) pp1a-2340	WLMWFIISI	12	1551.9	26
13) pp1a-2546	ILLLDQVLV	13	437.5	26
14) pp1a-2754	TLLCVLAAL	14	181.8	29
15) pp1a-2755	LLCVLAALV	15	118.2	25
16) pp1a-2758	VLAALVCYI	16	224.4	26
17) pp1a-2990	ALSGVFCGV	17	132.1	25
18) pp1a-3444	VLAWLYAAV	18	177.4	27
19) pp1a-3459	FLNRFTTTL	19	373.4	27
20) pp1a-3560	MLLTFLTSL	20	1174.4	29
21) pp1a-3564	FLTSLLILV	21	735.9	25
22) pp1a-3616	FLLPSLATV	22	2722.7	33
23) pp1a-3687	TLMNVITLV	23	591.9	25
24) pp1a-3709	SMWALVISV	24	958.9	28
25) pp1a-3730	FLARAIVFV	25	4047.2	29
26) pp1a-3745	LLFITGNTL	26	134.4	26
27) pp1a-3816	KLNIKLLGI	27	84.0	27
28) pp1a-3848	VLLSVLQQL	28	309.1	27
29) pp1a-4071	ALWEIQQVV	29	970.0	25
30) pp1a-4219	VLGSLAATV	30	118.2	26

Example 2

Measurement of Binding Affinity of Peptide to HLA-A*0201 Molecule

For the 30 kinds of epitope candidate peptides listed in Table 1, the binding affinity to HLA-A*0201, which is a major histocompatibility complex (MHC) class I molecule, was measured. The measurement was carried out using T2 cells, which are human lymphoid cells. T2 cells lack the TAP gene and hence cannot transport self-peptides derived from autoantigens. Therefore, these cells express HLA-A*0201, to which peptides are not bound, on the cell surfaces. When a peptide added to the outside of the cell is bound to HLA-A*0201, an HLA-A*0201 complex is formed and becomes stable. Using this principle, the binding affinity was calculated based on the relationship between the amount of the HLA-A*0201 complex formed and the concentration of the peptide added. As the antibody for detection of the HLA-A*0201 complex, an anti-HLA-A2 monoclonal antibody BB7.2 (ATCC) was used. Furthermore, an epitope of hepatitis C virus (NS3-1585) was used as a control.

More particularly, T2 cells were incubated at 37° C. overnight together with various concentrations of peptides. Thereafter, the cells were allowed to react with the anti-HLA-A2 antibody BB7.2, and then with a FITC-labeled secondary antibody, followed by analyzing the cells by flow cytometry. Using the mean fluorescence intensity (MFI) of the T2 cells pulsed with NS3-1585 as a standard (100%), the peptide concentration at which a mean fluorescence intensity of 50% was achieved was represented as BL_{50} (half-maximal binding level) for each peptide and shown in Table 2. BL_{50} values of less than 100 μM, 100 to 200 μM, and more than 200 μM were grouped into 3 categories "High", "Medium" and "Low", respectively, and 24 kinds of peptides showed high binding affinities.

TABLE 2

Epitope	SEQ ID No:	BL_{50} (μM)	Affinity
1) pp1a-15	1	75.7	High
2) pp1a-103	2	3.1	High
3) pp1a-445	3	19.2	High
4) pp1a-634	4	59.8	High
5) pp1a-651	5	8.4	High
6) pp1a-1121	6	40.3	High
7) pp1a-1139	7	4.9	High
8) pp1a-1288	8	65.1	High
9) pp1a-1652	9	6.7	High
10) pp1a-2187	10	3.0	High
11) pp1a-2207	11	323.0	Low
12) pp1a-2340	12	2432.8	Low
13) pp1a-2546	13	7.6	High
14) pp1a-2754	14	96.7	High
15) pp1a-2755	15	187.2	Medium
16) pp1a-2758	16	97.3	High
17) pp1a-2990	17	6.2	High
18) pp1a-3444	18	53.0	High
19) pp1a-3459	19	39.6	High
20) pp1a-3560	20	47.1	High
21) pp1a-3564	21	100.4	Medium
22) pp1a-3616	22	31.8	High
23) pp1a-3687	23	22.8	High
24) pp1a-3709	24	6.4	High
25) pp1a-3730	25	25.7	High
26) pp1a-3745	26	153.7	Medium
27) pp1a-3816	27	68.1	High
28) pp1a-3848	28	102.5	Medium
29) pp1a-4071	29	8.3	High
30) pp1a-4219	30	53.3	High

Example 3

Induction of Peptide-Specific CTLs in Mice Immunized Using Antigen Presenting Cells Pulsed with Peptides

In order to investigate whether or not CTLs are induced specifically to the 30 kinds of epitope candidate peptides listed in Table 1, mice were immunized with mouse spleen cells pulsed with the peptides in vitro, and the spleen cells were stimulated with the peptides followed by measuring the CTL induction activities. As the mouse, the HLA-A2 transgenic mouse (HDD II mouse, Institut Pasteur, France; provided by Dr. F. Lemonnier) prepared by knocking out mouse MHC class I and β2-microglobulin (β2-m) in a mouse and introducing HLA-A*0201, which is a type of human MHC class I, and the human β2-m gene was used. Using as an index the ratio of cells in which production of interferon-γ (IFN-γ) was promoted among CD8-positive cells, the CTL induction activity was measured.

Spleen cells prepared from a naive HLA-A2 transgenic mouse were incubated in vitro with each peptide at a concentration of 10 μM at 37° C. for hour. Another individual of a naive HLA-A2 transgenic mouse was immunized with the spleen cells pulsed with the peptide, by intravenous injection.

One week after the immunization, spleen cells were prepared from the immunized mouse, and the cells were suspended in a medium supplemented with 10% fetal calf serum (FCS), followed by plating the cells in a 96-well plate at the cell number of 2×10^6 cells/well. To each well, each peptide (10 μM, final concentration) and 5 μL of 25-fold diluted GOLGIPLUS™ solution (Japan BD) were added, and the resulting mixture was incubated at 37° C. for 5 hours. Here, GOLGIPLUS™ was used to inhibit secretion of produced IFN-γ by stopping intracellular transport. After washing the cells, 1 μg/10^6 cells of FcBlock antibody (Japan BD) suspended in 100 μL of FACS buffer (PBS containing 2% FCS and 0.1% sodium azide) was added to the cells and the resulting mixture was incubated at 4° C. for 10 minutes in order to suppress nonspecific reaction by blocking the Fc receptors on the cell surfaces.

Subsequently, for detecting CD8-positive cells, cells were stained by adding 0.5 μg of a fluorescein isothiocyanate (FITC)-labeled CD8 antibody to the cell suspension, and incubating the resulting mixture at 4° C. for 30 minutes, followed by washing the cells twice. Furthermore, using intracellular cytokine staining (ICS), IFN-γ in the cells was stained by the following procedure. First, 100 μL/well of CYTOFIX/CYTOPERM™ solution (Japan BD) was added to the cells, and the resulting mixture was left to stand at 4° C. for 20 minutes to fix the cells and permeabilize the cell membrane. The cells were washed twice, and then collected. Subsequently, 0.5 μg of a phycoerythrin (PE)-labeled anti-IFN-γ antibody was added to the cells, and the cells were incubated at 4° C. for 30 minutes. The cells were washed, and suspended in 100 μL of FACS fix buffer (PBS containing 2% FCS, 0.1% sodium azide and 1% formaldehyde), after which the cells were subjected to flow cytometry analysis.

For each epitope candidate peptide, the ratio (%) of IFN-γ-positive cells among CD8-positive cells is shown in Table 3.

TABLE 3

Epitope	SEQ ID No:	ICS (% in CD8+ cells)
1) pp1a-15	1	0.05
2) pp1a-103	2	0.07
3) pp1a-445	3	0.08
4) pp1a-634	4	0.08
5) pp1a-651	5	0.02
6) pp1a-1121	6	0.05
7) pp1a-1139	7	0.07
8) pp1a-1288	8	0.05
9) pp1a-1652	9	0.05
10) pp1a-2187	10	*0.19
11) pp1a-2207	11	*0.48
12) pp1a-2340	12	*0.21
13) pp1a-2546	13	*0.17
14) pp1a-2754	14	0.04
15) pp1a-2755	15	*0.18
16) pp1a-2758	16	0.03
17) pp1a-2990	17	*0.16
18) pp1a-3444	18	*0.12
19) pp1a-3459	19	0.03
20) pp1a-3560	20	0.04
21) pp1a-3564	21	0.06
22) pp1a-3616	22	0.07
23) pp1a-3687	23	*0.20
24) pp1a-3709	24	*0.50
25) pp1a-3730	25	0.06
26) pp1a-3745	26	0.03
27) pp1a-3816	27	0.07
28) pp1a-3848	28	0.07
29) pp1a-4071	29	0.06
30) pp1a-4219	30	0.01

*ICS of 0.1% or more

As a result, among the 30 kinds of candidate peptides, 9 kinds of peptides (pp1a-2187, pp1a-2207, pp1a-2340, pp1a-2546, pp1a-2755, pp1a-2990, pp1a-3444, pp1a-3687 and pp1a-3709; SEQ ID NOs: 10, 11, 12, 13, 15, 17, 18, 23 and 24) significantly induced IFN-γ-positive cells among CD8-positive cells, so that these 9 kinds of peptides were determined as the epitopes. These included peptides having low binding affinities to HLA-A*0201 (pp1a-2207 and pp1a-2340) and a peptide having a medium binding affinity thereto (pp1a-2755), as shown in Example 2.

Example 4

Preparation of Liposomes and Peptide-Bound Liposomes

For the 9 kinds of CTL epitope peptides that showed especially high CTL induction activities in Example 3 (pp1a-2187, pp1a-2207, pp1a-2340, pp1a-2546, pp1a-2755, pp1a-2990, pp1a-3444, pp1a-3687 and pp1a-3709; SEQ ID NOs: 10, 11, 12, 13, 15, 17, 18, 23 and 24), peptide-bound liposomes were prepared by the following method. Furthermore, in the same manner, liposomes to which a helper peptide (amino acid sequence: TPPAYRPPNAPIL; SEQ ID NO:32) was bound were prepared. As the helper peptide, one synthesized based on the HBV core 128 helper peptide (Operon Biotechnologies) used by Dr. A. Sette et al. (e.g., Glenn Y et al., J. Immunol. 162:3915-3925 (1999)) was used.

(4-1) Synthesis of Reactive Phospholipid Composed of Terminally Modified Phosphatidylethanolamine (Succinimidyl Group-Dioleoylphosphatidylethanolamine)

In 50 ml of chloroform, 2 g of dioleoylphosphatidylethanolamine and 180 μl of triethylamine were dissolved/added, and the resulting solution was placed in a 300 ml four-necked flask. While stirring the solution in the flask with a magnetic stirrer at room temperature, a solution separately prepared by dissolving 3 g of disuccinimidyl suberate, which is a divalent reactive compound, in 80 ml of chloroform was added dropwise to the solution according to a conventional method for 4 hours, thereby allowing one terminus of disuccinimidyl suberate to react with the amino group of dioleoylphosphatidylethanolamine. This crude reaction solution was transferred to an eggplant type flask, and the solvent was evaporated with an evaporator. Subsequently, a small amount of chloroform which is enough for dissolving the crude reaction product was added to the flask to obtain a high concentration crude reaction product solution, and this solution was subjected to column chromatography according to a conventional method using silica gel equilibrated with chloroform/methanol/water (65/25/1, volume ratio). Only the fraction of interest that contained dioleoylphosphatidylethanolamine whose amino group is bound to one terminus of disuccinimidyl suberate was recovered, and the solvent was evaporated, to obtain the succinimidyl group-dioleoylphosphatidylethanolamine of interest.

(4-2) Preparation of Lipid Mixture Powder

In an eggplant type flask, 1.3354 g (1.6987 mmol) of dioleoylphosphatidylcholine, 0.2886 g (0.2831 mmol) of succinimidyl group-dioleoylphosphatidylethanolamine prepared in Example 4-1, 0.7663 g (1.9818 mmol) of cholesterol and 0.4513 g (0.5662 mmol) of dioleoylphosphatidylglycerol sodium salt were placed, and 50 ml of a chloroform/methanol/water (65/25/4, volume ratio)-mixed solvent was added thereto, followed by dissolving the reagents at 40° C. Subsequently, the solvent was evaporated under reduced pressure using a rotary evaporator, to prepare a thin lipid membrane. Furthermore, 30 ml of distilled water for injection was added thereto, and the resulting mixture was stirred, to obtain a uniform slurry. This slurry was frozen, and dried in a freeze dryer for 24 hours, to obtain a lipid mixture powder.

(4-3) Preparation of Liposomes

Subsequently, 60 ml of separately prepared buffer A (1.0 mM Na_2HPO_4/KH_2PO_4, 0.25 M saccharose, pH7.4) was placed in the eggplant type flask containing the lipid mixture powder, and the lipids were hydrated while stirring the resulting mixture at 40° C., thereby liposomes were obtained. Thereafter, the particle diameters of the liposomes were adjusted using an extruder. The liposomes were first allowed to pass through an 8-μm polycarbonate filter, and then through 5 μm, 3 μm, 1 μm, 0.65 μm, 0.4 μm and 0.2 μm filters in this order. As a result, liposome particles having an average particle diameter of 206 nm (measured by dynamic light scattering) were obtained.

(4-4) Preparation of Peptide-Bound Liposomes

In a 1.5 ml test tube, the liposomes obtained in Example 4-3 were collected, and 3 ml of each peptide solution (1.25 mM)/buffer A separately prepared was added thereto, followed by stirring the resulting mixture at 5° C. for 48 hours to allow the reaction to proceed. This reaction liquid was subjected to gel filtration according to a conventional method using Sepharose CL-4B equilibrated with buffer A. Since the liposome fraction is turbid, the fraction of interest can be easily recognized, but may also be confirmed using a UV detector or the like. The phosphorus concentration of the thus obtained liposome suspension was measured (Phospholipid Test, Wako), and the suspension was diluted with buffer A such that the concentration of phosphorus derived from the phospholipid is 2 mM, thereby obtaining a suspension of each peptide-bound liposome.

Example 5

Induction of Peptide-Specific CTLs in Mice Immunized Using Peptide-Bound Liposomes

For the 9 kinds of CTL epitope peptides that showed especially high CTL induction activities in Example 3 (pp1a-2187, pp1a-2207, pp1a-2340, pp1a-2546, pp1a-2755, pp1a-2990, pp1a-3444, pp1a-3687 and pp1a-3709; SEQ ID NOs: 10, 11, 12, 13, 15, 17, 18, 23 and 24), the CTL induction activity in a mouse immunized using the peptide-bound liposome was measured.

A naive HLA-A2 transgenic mouse was immunized at its foot pad with an immunization solution obtained by mixing the peptide-bound liposomes (25 μl) and the helper peptide-bound liposomes prepared in Example 4 (25 μl) and an oligonucleic acid comprising a CpG motif (5 μg, base sequence: 5'-tccatgacgt tctgatgtt-3'; SEQ ID NO:33) together. After the immunization, the mouse was kept for 1 week, and the CTL induction activity was then measured by the same method as in Example 3. As a control, liposomes to which the peptide is not bound were used instead of the peptide-bound liposomes. As the oligonucleic acid comprising a CpG motif, one prepared by gene synthesis (Hokkaido System Science Co., Ltd.) based on Nagata. T. et al., Vaccine 25:4914-4921 (2007) was used.

Staining plots for CD8 and IFN-γ by flow cytometry in mice immunized using peptide-bound liposomes are shown in FIG. **1**. Each dot inside the boxed area in the upper right of each graph corresponds to an IFN-γ-positive cell among CD8-positive cells, and the value shown in the boxed area indicates the ratio (%) of the IFN-γ-positive cells among the CD8-positive cells. As a result, it was revealed that immunization with the contained peptide-bound liposomes causes CTL induction.

Comparative Example

As a comparative control experiment for Example 5, the following experiment was carried out. Measurement of the

CTL induction activity was carried out by the same method as in Example 4 except that, instead of a CTL epitope peptide of pp1a, a CTL epitope peptide of the Spike protein of SARS-CoV (spike-1203, amino acid sequence: FIAGLIAIV; SEQ ID NO:34) was used and that a naive HLA-A2 transgenic mouse was immunized by intramuscular injection at thigh muscle.

Staining plots for CD8 and IFN-γ by flow cytometry in mice immunized using the spike-1203-peptide-bound liposomes are shown in FIG. **2**. As a result of the analysis, the ratio (%) of IFN-γ-positive cells among CD8-positive cells was 0.29 when the known CTL epitope peptide of the Spike protein (spike-1203) was used. When compared to the results in Example 4, it was revealed that 8 kinds of CTL epitope peptides derived from pp1a (pp1a-2187, pp1a-2207, pp1a-2340, pp1a-2546, pp1a-2755, pp1a-2990, pp1a-3687 and pp1a-3709; SEQ ID NOs: 10, 11, 12, 13, 15, 17, 23 and 24) show CTL induction activities higher than that of the CTL epitope peptide of the Spike protein.

Example 6

In Vivo CTL assay in Mice Immunized Using Peptide-Bound Liposomes

For 5 kinds of CTL epitope peptides that showed high CTL induction activities in Example 4 (pp1a-2187, pp1a-2340, pp1a-2755, pp1a-2990 and pp1a-3709; SEQ ID NOs: 10, 12, 15, 17 and 24), the CTL response activity in a mouse immunized using the peptide-bound liposome was measured in vivo.

The principle of measurement of the CTL response activity in vivo was as follows. Target cells which were not pulsed with the peptide (negative control) were labeled with carboxy fluorescein diacetate succinimidyl ester (CFSE) at a low concentration, and, on the other hand, target cells pulsed with the peptide were labeled with CFSE at a high concentration (10-fold). The same number of cells of the 2 kinds of cell populations were mixed together, and transferred to a mouse preliminarily immunized using the peptide-bound liposomes. Thereafter, spleen cells were collected from the mouse to which the mixed cells were transferred, and a spleen cell suspension was prepared, followed by measuring the ratio of the transferred cells labeled with CFSE by flow cytometry analysis. In a mouse having no CTL response activity to the antigen peptide, equal amounts of the CFSE-labeled transferred cells are collected. On the other hand, since, in a mouse having the CTL response activity to the antigen peptide, the target cells covered with the antigen peptide are lysed, the degree of the lysis in vivo can be measured by quantifying decrease in the cells highly labeled with CFSE by flow cytometry.

More particularly, the measurement was carried out by the following method.

A mouse for the transfer was preliminarily prepared by immunizing a mouse with the peptide-bound liposomes prepared using each peptide and inducing the peptide-specific CTLs. As a control, a mouse immunized with liposomes to which the peptide was not bound, instead of the peptide-bound liposomes, was used. One week after the immunization, spleen cells were prepared from another individual of the naive mouse, and the cells were suspended in RPMI-1640 (Sigma) at the cell number of the medium is 2×10^8 cells/2 mL. A 1 mL aliquot of the resulting suspension was taken into each of two tubes. To only one of the tubes, the peptide was added to a final concentration of 10 μM, and the two tubes were incubated at 37° C. for 1 to 2 hours. After washing the cells once, the cells were suspended in 20 mL of PBS/0.1% BSA and then briefly vortexed. Thereafter, to the tube containing the cells pulsed with the peptide, 10 μL of 5 mM CFSE was added, immediately followed by adding 1 μL of 5 mM CFSE to the tube containing the cells which were not incubated with the peptide. The both tubes were vortexed to suspend the cells, and incubated in a water bath at 37° C. for 10 minutes. The both cell populations were centrifuged and washed once, followed by counting the live cell numbers and suspending each cell population in HBSS to a concentration of 5×10^7 cells/mL. Equal amounts of the cell suspensions were mixed together to prepare an immunization liquid, and 200 μL (1×10^7 cells/individual) of the resulting cell suspension was intravenously injected to a mouse preliminarily immunized, thereby carrying out the cell transfer. Twelve hours after the transfer, spleen cells were collected from the mouse, and suspended in 1 mL/spleen of PBS/1% FBS/5 mM EDTA. The cells were centrifuged and suspended in 1 mL of FACS fix buffer, and 100 μL of this spleen cell suspension was diluted with 2 mL of FACS buffer, followed by subjecting the resulting dilution to flow cytometry analysis.

Staining plots for CFSE by flow cytometry in mice to which the target cells labeled with CFSE were transferred are shown in FIG. **3**. The rightmost peak in each graph indicates the cell population pulsed with the peptide, and the peak in its left indicates the cells which were not pulsed with the peptide. It was revealed that, in each CTL epitope peptide, the mouse immunized with the peptide-bound liposomes (right graph) shows a decreased number of the cells pulsed with the peptide compared to the control immunized with the liposomes (left graph). The peptide-specific lysis rates (%) of the target cells by CTLs were: pp1a-2187, 79.2%; pp1a-2340, 68.5%; pp1a-2755, 70.5%; pp1a-2990, 73.4%; and pp1a-3709, 96.3%; showing that all of these peptides have high CTL response activities. Among the peptides, pp1a-3709 was revealed to have an especially high CTL response activity. Therefore, it was revealed that these CTL epitope peptides (pp1a-2187, pp1a-2340, pp1a-2755, pp1a-2990 and pp1a-3709; SEQ ID NOs: 10, 12, 15, 17 and 24) are dominant peptides having high CTL response activities.

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Gly Thr Cys Gly Leu Val Glu Leu Glu Lys Gly Val Leu Pro Gln Leu
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Glu Gln Pro Tyr Val Phe Ile Lys Arg Ser Asp Ala Leu Ser Thr Asn
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His Gly His Lys Val Val Glu Leu Val Ala Glu Met Asp Gly Ile Gln
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Lys Ser Phe Lys Thr Ile Val Glu Ser Cys Gly Asn Tyr Lys Val Thr
            500                 505                 510

Lys Gly Lys Pro Val Lys Gly Ala Trp Asn Ile Gly Gln Gln Arg Ser
        515                 520                 525

Val Leu Thr Pro Leu Cys Gly Phe Pro Ser Gln Ala Ala Gly Val Ile
    530                 535                 540

Arg Ser Ile Phe Ala Arg Thr Leu Asp Ala Ala Asn His Ser Ile Pro
545                 550                 555                 560

Asp Leu Gln Arg Ala Ala Val Thr Ile Leu Asp Gly Ile Ser Glu Gln
                565                 570                 575

Ser Leu Arg Leu Val Asp Ala Met Val Tyr Thr Ser Asp Leu Leu Thr
            580                 585                 590

Asn Ser Val Ile Ile Met Ala Tyr Val Thr Gly Gly Leu Val Gln Gln
        595                 600                 605

Thr Ser Gln Trp Leu Ser Asn Leu Leu Gly Thr Thr Val Glu Lys Leu
    610                 615                 620

Arg Pro Ile Phe Glu Trp Ile Glu Ala Lys Leu Ser Ala Gly Val Glu
625                 630                 635                 640

Phe Leu Lys Asp Ala Trp Glu Ile Leu Lys Phe Leu Ile Thr Gly Val
                645                 650                 655

Phe Asp Ile Val Lys Gly Gln Ile Gln Val Ala Ser Asp Asn Ile Lys
            660                 665                 670

Asp Cys Val Lys Cys Phe Ile Asp Val Val Asn Lys Ala Leu Glu Met
        675                 680                 685

Cys Ile Asp Gln Val Thr Ile Ala Gly Ala Lys Leu Arg Ser Leu Asn
    690                 695                 700

Leu Gly Glu Val Phe Ile Ala Gln Ser Lys Gly Leu Tyr Arg Gln Cys
705                 710                 715                 720

Ile Arg Gly Lys Glu Gln Leu Gln Leu Leu Met Pro Leu Lys Ala Pro
                725                 730                 735

Lys Glu Val Thr Phe Leu Glu Gly Asp Ser His Asp Thr Val Leu Thr
            740                 745                 750

Ser Glu Glu Val Val Leu Lys Asn Gly Glu Leu Glu Ala Leu Glu Thr
        755                 760                 765

Pro Val Asp Ser Phe Thr Asn Gly Ala Ile Val Gly Thr Pro Val Cys
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    770                 775                 780

Val Asn Gly Leu Met Leu Leu Glu Ile Lys Asp Lys Glu Gln Tyr Cys
785                 790                 795                 800

Ala Leu Ser Pro Gly Leu Leu Ala Thr Asn Asn Val Phe Arg Leu Lys
                805                 810                 815

Gly Gly Ala Pro Ile Lys Gly Val Thr Phe Gly Glu Asp Thr Val Trp
            820                 825                 830

Glu Val Gln Gly Tyr Lys Asn Val Arg Ile Thr Phe Glu Leu Asp Glu
        835                 840                 845

Arg Val Asp Lys Val Leu Asn Glu Lys Cys Ser Val Tyr Thr Val Glu
    850                 855                 860

Ser Gly Thr Glu Val Thr Glu Phe Ala Cys Val Val Ala Glu Ala Val
865                 870                 875                 880

Val Lys Thr Leu Gln Pro Val Ser Asp Leu Leu Thr Asn Met Gly Ile
                885                 890                 895

Asp Leu Asp Glu Trp Ser Val Ala Thr Phe Tyr Leu Phe Asp Asp Ala
            900                 905                 910

Gly Glu Glu Asn Phe Ser Ser Arg Met Tyr Cys Ser Phe Tyr Pro Pro
        915                 920                 925

Asp Glu Glu Glu Glu Asp Asp Ala Glu Cys Glu Glu Glu Glu Ile Asp
    930                 935                 940

Glu Thr Cys Glu His Glu Tyr Gly Thr Glu Asp Asp Tyr Gln Gly Leu
945                 950                 955                 960

Pro Leu Glu Phe Gly Ala Ser Ala Glu Thr Val Arg Val Glu Glu Glu
                965                 970                 975

Glu Glu Glu Asp Trp Leu Asp Asp Thr Thr Glu Gln Ser Glu Ile Glu
            980                 985                 990

Pro Glu Pro Glu Pro Thr Pro Glu  Glu Pro Val Asn Gln  Phe Thr Gly
        995                 1000                1005

Tyr Leu  Lys Leu Thr Asp Asn  Val Ala Ile Lys Cys  Val Asp Ile
    1010                1015                1020

Val Lys  Glu Ala Gln Ser Ala  Asn Pro Met Val Ile  Val Asn Ala
    1025                1030                1035

Ala Asn  Ile His Leu Lys His  Gly Gly Gly Val Ala  Gly Ala Leu
    1040                1045                1050

Asn Lys  Ala Thr Asn Gly Ala  Met Gln Lys Glu Ser  Asp Asp Tyr
    1055                1060                1065

Ile Lys  Leu Asn Gly Pro Leu  Thr Val Gly Gly Ser  Cys Leu Leu
    1070                1075                1080

Ser Gly  His Asn Leu Ala Lys  Lys Cys Leu His Val  Val Gly Pro
    1085                1090                1095

Asn Leu  Asn Ala Gly Glu Asp  Ile Gln Leu Leu Lys  Ala Ala Tyr
    1100                1105                1110

Glu Asn  Phe Asn Ser Gln Asp  Ile Leu Leu Ala Pro  Leu Leu Ser
    1115                1120                1125

Ala Gly  Ile Phe Gly Ala Lys  Pro Leu Gln Ser Leu  Gln Val Cys
    1130                1135                1140

Val Gln  Thr Val Arg Thr Gln  Val Tyr Ile Ala Val  Asn Asp Lys
    1145                1150                1155

Ala Leu  Tyr Glu Gln Val Val  Met Asp Tyr Leu Asp  Asn Leu Lys
    1160                1165                1170

Pro Arg  Val Glu Ala Pro Lys  Gln Glu Glu Pro Pro  Asn Thr Glu
    1175                1180                1185
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Asp Ser  Lys Thr Glu Glu Lys  Ser Val Val Gln Lys  Pro Val Asp
    1190                 1195                 1200

Val Lys  Pro Lys Ile Lys Ala  Cys Ile Asp Glu Val  Thr Thr Thr
    1205                 1210                 1215

Leu Glu  Glu Thr Lys Phe Leu  Thr Asn Lys Leu Leu  Leu Phe Ala
    1220                 1225                 1230

Asp Ile  Asn Gly Lys Leu Tyr  His Asp Ser Gln Asn  Met Leu Arg
    1235                 1240                 1245

Gly Glu  Asp Met Ser Phe Leu  Glu Lys Asp Ala Pro  Tyr Met Val
    1250                 1255                 1260

Gly Asp  Val Ile Thr Ser Gly  Asp Ile Thr Cys Val  Val Ile Pro
    1265                 1270                 1275

Ser Lys  Lys Ala Gly Gly Thr  Thr Glu Met Leu Ser  Arg Ala Leu
    1280                 1285                 1290

Lys Lys  Val Pro Val Asp Glu  Tyr Ile Thr Thr Tyr  Pro Gly Gln
    1295                 1300                 1305

Gly Cys  Ala Gly Tyr Thr Leu  Glu Glu Ala Lys Thr  Ala Leu Lys
    1310                 1315                 1320

Lys Cys  Lys Ser Ala Phe Tyr  Val Leu Pro Ser Glu  Ala Pro Asn
    1325                 1330                 1335

Ala Lys  Glu Glu Ile Leu Gly  Thr Val Ser Trp Asn  Leu Arg Glu
    1340                 1345                 1350

Met Leu  Ala His Ala Glu Glu  Thr Arg Lys Leu Met  Pro Ile Cys
    1355                 1360                 1365

Met Asp  Val Arg Ala Ile Met  Ala Thr Ile Gln Arg  Lys Tyr Lys
    1370                 1375                 1380

Gly Ile  Lys Ile Gln Glu Gly  Ile Val Asp Tyr Gly  Val Arg Phe
    1385                 1390                 1395

Phe Phe  Tyr Thr Ser Lys Glu  Pro Val Ala Ser Ile  Ile Thr Lys
    1400                 1405                 1410

Leu Asn  Ser Leu Asn Glu Pro  Leu Val Thr Met Pro  Ile Gly Tyr
    1415                 1420                 1425

Val Thr  His Gly Phe Asn Leu  Glu Glu Ala Ala Arg  Cys Met Arg
    1430                 1435                 1440

Ser Leu  Lys Ala Pro Ala Val  Val Ser Val Ser Ser  Pro Asp Ala
    1445                 1450                 1455

Val Thr  Thr Tyr Asn Gly Tyr  Leu Thr Ser Ser Ser  Lys Thr Ser
    1460                 1465                 1470

Glu Glu  His Phe Val Glu Thr  Val Ser Leu Ala Gly  Ser Tyr Arg
    1475                 1480                 1485

Asp Trp  Ser Tyr Ser Gly Gln  Arg Thr Glu Leu Gly  Val Glu Phe
    1490                 1495                 1500

Leu Lys  Arg Gly Asp Lys Ile  Val Tyr His Thr Leu  Glu Ser Pro
    1505                 1510                 1515

Val Glu  Phe His Leu Asp Gly  Glu Val Leu Ser Leu  Asp Lys Leu
    1520                 1525                 1530

Lys Ser  Leu Leu Ser Leu Arg  Glu Val Lys Thr Ile  Lys Val Phe
    1535                 1540                 1545

Thr Thr  Val Asp Asn Thr Asn  Leu His Thr Gln Leu  Val Asp Met
    1550                 1555                 1560

Ser Met  Thr Tyr Gly Gln Gln  Phe Gly Pro Thr Tyr  Leu Asp Gly
    1565                 1570                 1575
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Ala Asp  Val Thr Lys Ile Lys  Pro His Val Asn His  Glu Gly Lys
    1580                1585                1590

Thr Phe  Phe Val Leu Pro Ser  Asp Asp Thr Leu Arg  Ser Glu Ala
    1595                1600                1605

Phe Glu  Tyr Tyr His Thr Leu  Asp Glu Ser Phe Leu  Gly Arg Tyr
    1610                1615                1620

Met Ser  Ala Leu Asn His Thr  Lys Lys Trp Lys Phe  Pro Gln Val
    1625                1630                1635

Gly Gly  Leu Thr Ser Ile Lys  Trp Ala Asp Asn Asn  Cys Tyr Leu
    1640                1645                1650

Ser Ser  Val Leu Leu Ala Leu  Gln Gln Leu Glu Val  Lys Phe Asn
    1655                1660                1665

Ala Pro  Ala Leu Gln Glu Ala  Tyr Tyr Arg Ala Arg  Ala Gly Asp
    1670                1675                1680

Ala Ala  Asn Phe Cys Ala Leu  Ile Leu Ala Tyr Ser  Asn Lys Thr
    1685                1690                1695

Val Gly  Glu Leu Gly Asp Val  Arg Glu Thr Met Thr  His Leu Leu
    1700                1705                1710

Gln His  Ala Asn Leu Glu Ser  Ala Lys Arg Val Leu  Asn Val Val
    1715                1720                1725

Cys Lys  His Cys Gly Gln Lys  Thr Thr Thr Leu Thr  Gly Val Glu
    1730                1735                1740

Ala Val  Met Tyr Met Gly Thr  Leu Ser Tyr Asp Asn  Leu Lys Thr
    1745                1750                1755

Gly Val  Ser Ile Pro Cys Val  Cys Gly Arg Asp Ala  Thr Gln Tyr
    1760                1765                1770

Leu Val  Gln Gln Glu Ser Ser  Phe Val Met Met Ser  Ala Pro Pro
    1775                1780                1785

Ala Glu  Tyr Lys Leu Gln Gln  Gly Thr Phe Leu Cys  Ala Asn Glu
    1790                1795                1800

Tyr Thr  Gly Asn Tyr Gln Cys  Gly His Tyr Thr His  Ile Thr Ala
    1805                1810                1815

Lys Glu  Thr Leu Tyr Arg Ile  Asp Gly Ala His Leu  Thr Lys Met
    1820                1825                1830

Ser Glu  Tyr Lys Gly Pro Val  Thr Asp Val Phe Tyr  Lys Glu Thr
    1835                1840                1845

Ser Tyr  Thr Thr Thr Ile Lys  Pro Val Ser Tyr Lys  Leu Asp Gly
    1850                1855                1860

Val Thr  Tyr Thr Glu Ile Glu  Pro Lys Leu Asp Gly  Tyr Tyr Lys
    1865                1870                1875

Lys Asp  Asn Ala Tyr Tyr Thr  Glu Gln Pro Ile Asp  Leu Val Pro
    1880                1885                1890

Thr Gln  Pro Leu Pro Asn Ala  Ser Phe Asp Asn Phe  Lys Leu Thr
    1895                1900                1905

Cys Ser  Asn Thr Lys Phe Ala  Asp Asp Leu Asn Gln  Met Thr Gly
    1910                1915                1920

Phe Thr  Lys Pro Ala Ser Arg  Glu Leu Ser Val Thr  Phe Phe Pro
    1925                1930                1935

Asp Leu  Asn Gly Asp Val Val  Ala Ile Asp Tyr Arg  His Tyr Ser
    1940                1945                1950

Ala Ser  Phe Lys Lys Gly Ala  Lys Leu Leu His Lys  Pro Ile Val
    1955                1960                1965

Trp His  Ile Asn Gln Ala Thr  Thr Lys Thr Thr Phe  Lys Pro Asn
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    1970                1975                1980

Thr Trp  Cys Leu Arg Cys Leu  Trp Ser Thr Lys Pro  Val Asp Thr
    1985                1990                1995

Ser Asn  Ser Phe Glu Val Leu  Ala Val Glu Asp Thr  Gln Gly Met
    2000                2005                2010

Asp Asn  Leu Ala Cys Glu Ser  Gln Gln Pro Thr Ser  Glu Glu Val
    2015                2020                2025

Val Glu  Asn Pro Thr Ile Gln  Lys Glu Val Ile Glu  Cys Asp Val
    2030                2035                2040

Lys Thr  Thr Glu Val Val Gly  Asn Val Ile Leu Lys  Pro Ser Asp
    2045                2050                2055

Glu Gly  Val Lys Val Thr Gln  Glu Leu Gly His Glu  Asp Leu Met
    2060                2065                2070

Ala Ala  Tyr Val Glu Asn Thr  Ser Ile Thr Ile Lys  Lys Pro Asn
    2075                2080                2085

Glu Leu  Ser Leu Ala Leu Gly  Leu Lys Thr Ile Ala  Thr His Gly
    2090                2095                2100

Ile Ala  Ala Ile Asn Ser Val  Pro Trp Ser Lys Ile  Leu Ala Tyr
    2105                2110                2115

Val Lys  Pro Phe Leu Gly Gln  Ala Ala Ile Thr Thr  Ser Asn Cys
    2120                2125                2130

Ala Lys  Arg Leu Ala Gln Arg  Val Phe Asn Asn Tyr  Met Pro Tyr
    2135                2140                2145

Val Phe  Thr Leu Leu Phe Gln  Leu Cys Thr Phe Thr  Lys Ser Thr
    2150                2155                2160

Asn Ser  Arg Ile Arg Ala Ser  Leu Pro Thr Thr Ile  Ala Lys Asn
    2165                2170                2175

Ser Val  Lys Ser Val Ala Lys  Leu Cys Leu Asp Ala  Gly Ile Asn
    2180                2185                2190

Tyr Val  Lys Ser Pro Lys Phe  Ser Lys Leu Phe Thr  Ile Ala Met
    2195                2200                2205

Trp Leu  Leu Leu Leu Ser Ile  Cys Leu Gly Ser Leu  Ile Cys Val
    2210                2215                2220

Thr Ala  Ala Phe Gly Val Leu  Leu Ser Asn Phe Gly  Ala Pro Ser
    2225                2230                2235

Tyr Cys  Asn Gly Val Arg Glu  Leu Tyr Leu Asn Ser  Ser Asn Val
    2240                2245                2250

Thr Thr  Met Asp Phe Cys Glu  Gly Ser Phe Pro Cys  Ser Ile Cys
    2255                2260                2265

Leu Ser  Gly Leu Asp Ser Leu  Asp Ser Tyr Pro Ala  Leu Glu Thr
    2270                2275                2280

Ile Gln  Val Thr Ile Ser Ser  Tyr Lys Leu Asp Leu  Thr Ile Leu
    2285                2290                2295

Gly Leu  Ala Ala Glu Trp Val  Leu Ala Tyr Met Leu  Phe Thr Lys
    2300                2305                2310

Phe Phe  Tyr Leu Leu Gly Leu  Ser Ala Ile Met Gln  Val Phe Phe
    2315                2320                2325

Gly Tyr  Phe Ala Ser His Phe  Ile Ser Asn Ser Trp  Leu Met Trp
    2330                2335                2340

Phe Ile  Ile Ser Ile Val Gln  Met Ala Pro Val Ser  Ala Met Val
    2345                2350                2355

Arg Met  Tyr Ile Phe Phe Ala  Ser Phe Tyr Tyr Ile  Trp Lys Ser
    2360                2365                2370
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Tyr Val  His Ile Met Asp Gly  Cys Thr Ser Ser Thr  Cys Met Met
    2375                2380                2385

Cys Tyr  Lys Arg Asn Arg Ala  Thr Arg Val Glu Cys  Thr Thr Ile
    2390                2395                2400

Val Asn  Gly Met Lys Arg Ser  Phe Tyr Val Tyr Ala  Asn Gly Gly
    2405                2410                2415

Arg Gly  Phe Cys Lys Thr His  Asn Trp Asn Cys Leu  Asn Cys Asp
    2420                2425                2430

Thr Phe  Cys Thr Gly Ser Thr  Phe Ile Ser Asp Glu  Val Ala Arg
    2435                2440                2445

Asp Leu  Ser Leu Gln Phe Lys  Arg Pro Ile Asn Pro  Thr Asp Gln
    2450                2455                2460

Ser Ser  Tyr Ile Val Asp Ser  Val Ala Val Lys Asn  Gly Ala Leu
    2465                2470                2475

His Leu  Tyr Phe Asp Lys Ala  Gly Gln Lys Thr Tyr  Glu Arg His
    2480                2485                2490

Pro Leu  Ser His Phe Val Asn  Leu Asp Asn Leu Arg  Ala Asn Asn
    2495                2500                2505

Thr Lys  Gly Ser Leu Pro Ile  Asn Val Ile Val Phe  Asp Gly Lys
    2510                2515                2520

Ser Lys  Cys Asp Glu Ser Ala  Ser Lys Ser Ala Ser  Val Tyr Tyr
    2525                2530                2535

Ser Gln  Leu Met Cys Gln Pro  Ile Leu Leu Leu Asp  Gln Val Leu
    2540                2545                2550

Val Ser  Asp Val Gly Asp Ser  Thr Glu Val Ser Val  Lys Met Phe
    2555                2560                2565

Asp Ala  Tyr Val Asp Thr Phe  Ser Ala Thr Phe Ser  Val Pro Met
    2570                2575                2580

Glu Lys  Leu Lys Ala Leu Val  Ala Thr Ala His Ser  Glu Leu Ala
    2585                2590                2595

Lys Gly  Val Ala Leu Asp Gly  Val Leu Ser Thr Phe  Val Ser Ala
    2600                2605                2610

Ala Arg  Gln Gly Val Val Asp  Thr Asp Val Asp Thr  Lys Asp Val
    2615                2620                2625

Ile Glu  Cys Leu Lys Leu Ser  His His Ser Asp Leu  Glu Val Thr
    2630                2635                2640

Gly Asp  Ser Cys Asn Asn Phe  Met Leu Thr Tyr Asn  Lys Val Glu
    2645                2650                2655

Asn Met  Thr Pro Arg Asp Leu  Gly Ala Cys Ile Asp  Cys Asn Ala
    2660                2665                2670

Arg His  Ile Asn Ala Gln Val  Ala Lys Ser His Asn  Val Ser Leu
    2675                2680                2685

Ile Trp  Asn Val Lys Asp Tyr  Met Ser Leu Ser Glu  Gln Leu Arg
    2690                2695                2700

Lys Gln  Ile Arg Ser Ala Ala  Lys Lys Asn Asn Ile  Pro Phe Arg
    2705                2710                2715

Leu Thr  Cys Ala Thr Thr Arg  Gln Val Val Asn Val  Ile Thr Thr
    2720                2725                2730

Lys Ile  Ser Leu Lys Gly Gly  Lys Ile Val Ser Thr  Cys Phe Lys
    2735                2740                2745

Leu Met  Leu Lys Ala Thr Leu  Leu Cys Val Leu Ala  Ala Leu Val
    2750                2755                2760
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Cys Tyr  Ile Val Met Pro Val  His Thr Leu Ser Ile  His Asp Gly
    2765                2770                2775

Tyr Thr  Asn Glu Ile Ile Gly  Tyr Lys Ala Ile Gln  Asp Gly Val
    2780                2785                2790

Thr Arg  Asp Ile Ile Ser Thr  Asp Asp Cys Phe Ala  Asn Lys His
    2795                2800                2805

Ala Gly  Phe Asp Ala Trp Phe  Ser Gln Arg Gly Gly  Ser Tyr Lys
    2810                2815                2820

Asn Asp  Lys Ser Cys Pro Val  Val Ala Ala Ile Ile  Thr Arg Glu
    2825                2830                2835

Ile Gly  Phe Ile Val Pro Gly  Leu Pro Gly Thr Val  Leu Arg Ala
    2840                2845                2850

Ile Asn  Gly Asp Phe Leu His  Phe Leu Pro Arg Val  Phe Ser Ala
    2855                2860                2865

Val Gly  Asn Ile Cys Tyr Thr  Pro Ser Lys Leu Ile  Glu Tyr Ser
    2870                2875                2880

Asp Phe  Ala Thr Ser Ala Cys  Val Leu Ala Ala Glu  Cys Thr Ile
    2885                2890                2895

Phe Lys  Asp Ala Met Gly Lys  Pro Val Pro Tyr Cys  Tyr Asp Thr
    2900                2905                2910

Asn Leu  Leu Glu Gly Ser Ile  Ser Tyr Ser Glu Leu  Arg Pro Asp
    2915                2920                2925

Thr Arg  Tyr Val Leu Met Asp  Gly Ser Ile Ile Gln  Phe Pro Asn
    2930                2935                2940

Thr Tyr  Leu Glu Gly Ser Val  Arg Val Val Thr Thr  Phe Asp Ala
    2945                2950                2955

Glu Tyr  Cys Arg His Gly Thr  Cys Glu Arg Ser Glu  Val Gly Ile
    2960                2965                2970

Cys Leu  Ser Thr Ser Gly Arg  Trp Val Leu Asn Asn  Glu His Tyr
    2975                2980                2985

Arg Ala  Leu Ser Gly Val Phe  Cys Gly Val Asp Ala  Met Asn Leu
    2990                2995                3000

Ile Ala  Asn Ile Phe Thr Pro  Leu Val Gln Pro Val  Gly Ala Leu
    3005                3010                3015

Asp Val  Ser Ala Ser Val Val  Ala Gly Gly Ile Ile  Ala Ile Leu
    3020                3025                3030

Val Thr  Cys Ala Ala Tyr Tyr  Phe Met Lys Phe Arg  Arg Val Phe
    3035                3040                3045

Gly Glu  Tyr Asn His Val Val  Ala Ala Asn Ala Leu  Leu Phe Leu
    3050                3055                3060

Met Ser  Phe Thr Ile Leu Cys  Leu Val Pro Ala Tyr  Ser Phe Leu
    3065                3070                3075

Pro Gly  Val Tyr Ser Val Phe  Tyr Leu Tyr Leu Thr  Phe Tyr Phe
    3080                3085                3090

Thr Asn  Asp Val Ser Phe Leu  Ala His Leu Gln Trp  Phe Ala Met
    3095                3100                3105

Phe Ser  Pro Ile Val Pro Phe  Trp Ile Thr Ala Ile  Tyr Val Phe
    3110                3115                3120

Cys Ile  Ser Leu Lys His Cys  His Trp Phe Phe Asn  Asn Tyr Leu
    3125                3130                3135

Arg Lys  Arg Val Met Phe Asn  Gly Val Thr Phe Ser  Thr Phe Glu
    3140                3145                3150

Glu Ala  Ala Leu Cys Thr Phe  Leu Leu Asn Lys Glu  Met Tyr Leu
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    3155                3160                3165

Lys Leu  Arg Ser Glu Thr Leu  Leu Pro Leu Thr Gln  Tyr Asn Arg
    3170                3175                3180

Tyr Leu  Ala Leu Tyr Asn Lys  Tyr Lys Tyr Phe Ser  Gly Ala Leu
    3185                3190                3195

Asp Thr  Thr Ser Tyr Arg Glu  Ala Ala Cys Cys His  Leu Ala Lys
    3200                3205                3210

Ala Leu  Asn Asp Phe Ser Asn  Ser Gly Ala Asp Val  Leu Tyr Gln
    3215                3220                3225

Pro Pro  Gln Thr Ser Ile Thr  Ser Ala Val Leu Gln  Ser Gly Phe
    3230                3235                3240

Arg Lys  Met Ala Phe Pro Ser  Gly Lys Val Glu Gly  Cys Met Val
    3245                3250                3255

Gln Val  Thr Cys Gly Thr Thr  Thr Leu Asn Gly Leu  Trp Leu Asp
    3260                3265                3270

Asp Thr  Val Tyr Cys Pro Arg  His Val Ile Cys Thr  Ala Glu Asp
    3275                3280                3285

Met Leu  Asn Pro Asn Tyr Glu  Asp Leu Leu Ile Arg  Lys Ser Asn
    3290                3295                3300

His Ser  Phe Leu Val Gln Ala  Gly Asn Val Gln Leu  Arg Val Ile
    3305                3310                3315

Gly His  Ser Met Gln Asn Cys  Leu Leu Arg Leu Lys  Val Asp Thr
    3320                3325                3330

Ser Asn  Pro Lys Thr Pro Lys  Tyr Lys Phe Val Arg  Ile Gln Pro
    3335                3340                3345

Gly Gln  Thr Phe Ser Val Leu  Ala Cys Tyr Asn Gly  Ser Pro Ser
    3350                3355                3360

Gly Val  Tyr Gln Cys Ala Met  Arg Pro Asn His Thr  Ile Lys Gly
    3365                3370                3375

Ser Phe  Leu Asn Gly Ser Cys  Gly Ser Val Gly Phe  Asn Ile Asp
    3380                3385                3390

Tyr Asp  Cys Val Ser Phe Cys  Tyr Met His His Met  Glu Leu Pro
    3395                3400                3405

Thr Gly  Val His Ala Gly Thr  Asp Leu Glu Gly Lys  Phe Tyr Gly
    3410                3415                3420

Pro Phe  Val Asp Arg Gln Thr  Ala Gln Ala Ala Gly  Thr Asp Thr
    3425                3430                3435

Thr Ile  Thr Leu Asn Val Leu  Ala Trp Leu Tyr Ala  Ala Val Ile
    3440                3445                3450

Asn Gly  Asp Arg Trp Phe Leu  Asn Arg Phe Thr Thr  Thr Leu Asn
    3455                3460                3465

Asp Phe  Asn Leu Val Ala Met  Lys Tyr Asn Tyr Glu  Pro Leu Thr
    3470                3475                3480

Gln Asp  His Val Asp Ile Leu  Gly Pro Leu Ser Ala  Gln Thr Gly
    3485                3490                3495

Ile Ala  Val Leu Asp Met Cys  Ala Ala Leu Lys Glu  Leu Leu Gln
    3500                3505                3510

Asn Gly  Met Asn Gly Arg Thr  Ile Leu Gly Ser Thr  Ile Leu Glu
    3515                3520                3525

Asp Glu  Phe Thr Pro Phe Asp  Val Val Arg Gln Cys  Ser Gly Val
    3530                3535                3540

Thr Phe  Gln Gly Lys Phe Lys  Lys Ile Val Lys Gly  Thr His His
    3545                3550                3555
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Trp Met  Leu Leu Thr Phe Leu  Thr Ser Leu Leu Ile  Leu Val Gln
    3560                3565                3570

Ser Thr  Gln Trp Ser Leu Phe  Phe Phe Val Tyr Glu  Asn Ala Phe
    3575                3580                3585

Leu Pro  Phe Thr Leu Gly Ile  Met Ala Ile Ala Ala  Cys Ala Met
    3590                3595                3600

Leu Leu  Val Lys His Lys His  Ala Phe Leu Cys Leu  Phe Leu Leu
    3605                3610                3615

Pro Ser  Leu Ala Thr Val Ala  Tyr Phe Asn Met Val  Tyr Met Pro
    3620                3625                3630

Ala Ser  Trp Val Met Arg Ile  Met Thr Trp Leu Glu  Leu Ala Asp
    3635                3640                3645

Thr Ser  Leu Ser Gly Tyr Arg  Leu Lys Asp Cys Val  Met Tyr Ala
    3650                3655                3660

Ser Ala  Leu Val Leu Leu Ile  Leu Met Thr Ala Arg  Thr Val Tyr
    3665                3670                3675

Asp Asp  Ala Ala Arg Arg Val  Trp Thr Leu Met Asn  Val Ile Thr
    3680                3685                3690

Leu Val  Tyr Lys Val Tyr Tyr  Gly Asn Ala Leu Asp  Gln Ala Ile
    3695                3700                3705

Ser Met  Trp Ala Leu Val Ile  Ser Val Thr Ser Asn  Tyr Ser Gly
    3710                3715                3720

Val Val  Thr Thr Ile Met Phe  Leu Ala Arg Ala Ile  Val Phe Val
    3725                3730                3735

Cys Val  Glu Tyr Tyr Pro Leu  Leu Phe Ile Thr Gly  Asn Thr Leu
    3740                3745                3750

Gln Cys  Ile Met Leu Val Tyr  Cys Phe Leu Gly Tyr  Cys Cys Cys
    3755                3760                3765

Cys Tyr  Phe Gly Leu Phe Cys  Leu Leu Asn Arg Tyr  Phe Arg Leu
    3770                3775                3780

Thr Leu  Gly Val Tyr Asp Tyr  Leu Val Ser Thr Gln  Glu Phe Arg
    3785                3790                3795

Tyr Met  Asn Ser Gln Gly Leu  Leu Pro Pro Lys Ser  Ser Ile Asp
    3800                3805                3810

Ala Phe  Lys Leu Asn Ile Lys  Leu Leu Gly Ile Gly  Gly Lys Pro
    3815                3820                3825

Cys Ile  Lys Val Ala Thr Val  Gln Ser Lys Met Ser  Asp Val Lys
    3830                3835                3840

Cys Thr  Ser Val Val Leu Leu  Ser Val Leu Gln Gln  Leu Arg Val
    3845                3850                3855

Glu Ser  Ser Ser Lys Leu Trp  Ala Gln Cys Val Gln  Leu His Asn
    3860                3865                3870

Asp Ile  Leu Leu Ala Lys Asp  Thr Thr Glu Ala Phe  Glu Lys Met
    3875                3880                3885

Val Ser  Leu Leu Ser Val Leu  Leu Ser Met Gln Gly  Ala Val Asp
    3890                3895                3900

Ile Asn  Arg Leu Cys Glu Glu  Met Leu Asp Asn Arg  Ala Thr Leu
    3905                3910                3915

Gln Ala  Ile Ala Ser Glu Phe  Ser Ser Leu Pro Ser  Tyr Ala Ala
    3920                3925                3930

Tyr Ala  Thr Ala Gln Glu Ala  Tyr Glu Gln Ala Val  Ala Asn Gly
    3935                3940                3945
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Asp Ser  Glu Val Val Leu Lys  Lys Leu Lys Lys Ser  Leu Asn Val
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Ala Lys  Ser Glu Phe Asp Arg  Asp Ala Ala Met Gln  Arg Lys Leu
    3965                 3970                 3975

Glu Lys  Met Ala Asp Gln Ala  Met Thr Gln Met Tyr  Lys Gln Ala
    3980                 3985                 3990

Arg Ser  Glu Asp Lys Arg Ala  Lys Val Thr Ser Ala  Met Gln Thr
    3995                 4000                 4005

Met Leu  Phe Thr Met Leu Arg  Lys Leu Asp Asn Asp  Ala Leu Asn
    4010                 4015                 4020

Asn Ile  Ile Asn Asn Ala Arg  Asp Gly Cys Val Pro  Leu Asn Ile
    4025                 4030                 4035

Ile Pro  Leu Thr Thr Ala Ala  Lys Leu Met Val Val  Val Pro Asp
    4040                 4045                 4050

Tyr Gly  Thr Tyr Lys Asn Thr  Cys Asp Gly Asn Thr  Phe Thr Tyr
    4055                 4060                 4065

Ala Ser  Ala Leu Trp Glu Ile  Gln Gln Val Val Asp  Ala Asp Ser
    4070                 4075                 4080

Lys Ile  Val Gln Leu Ser Glu  Ile Asn Met Asp Asn  Ser Pro Asn
    4085                 4090                 4095

Leu Ala  Trp Pro Leu Ile Val  Thr Ala Leu Arg Ala  Asn Ser Ala
    4100                 4105                 4110

Val Lys  Leu Gln Asn Asn Glu  Leu Ser Pro Val Ala  Leu Arg Gln
    4115                 4120                 4125

Met Ser  Cys Ala Ala Gly Thr  Thr Gln Thr Ala Cys  Thr Asp Asp
    4130                 4135                 4140

Asn Ala  Leu Ala Tyr Tyr Asn  Asn Ser Lys Gly Gly  Arg Phe Val
    4145                 4150                 4155

Leu Ala  Leu Leu Ser Asp His  Gln Asp Leu Lys Trp  Ala Arg Phe
    4160                 4165                 4170

Pro Lys  Ser Asp Gly Thr Gly  Thr Ile Tyr Thr Glu  Leu Glu Pro
    4175                 4180                 4185

Pro Cys  Arg Phe Val Thr Asp  Thr Pro Lys Gly Pro  Lys Val Lys
    4190                 4195                 4200

Tyr Leu  Tyr Phe Ile Lys Gly  Leu Asn Asn Leu Asn  Arg Gly Met
    4205                 4210                 4215

Val Leu  Gly Ser Leu Ala Ala  Thr Val Arg Leu Gln  Ala Gly Asn
    4220                 4225                 4230

Ala Thr  Glu Val Pro Ala Asn  Ser Thr Val Leu Ser  Phe Cys Ala
    4235                 4240                 4245

Phe Ala  Val Asp Pro Ala Lys  Ala Tyr Lys Asp Tyr  Leu Ala Ser
    4250                 4255                 4260

Gly Gly  Gln Pro Ile Thr Asn  Cys Val Lys Met Leu  Cys Thr His
    4265                 4270                 4275

Thr Gly  Thr Gly Gln Ala Ile  Thr Val Thr Pro Glu  Ala Asn Met
    4280                 4285                 4290

Asp Gln  Glu Ser Phe Gly Gly  Ala Ser Cys Cys Leu  Tyr Cys Arg
    4295                 4300                 4305

Cys His  Ile Asp His Pro Asn  Pro Lys Gly Phe Cys  Asp Leu Lys
    4310                 4315                 4320

Gly Lys  Tyr Val Gln Ile Pro  Thr Thr Cys Ala Asn  Asp Pro Val
    4325                 4330                 4335

Gly Phe  Thr Leu Arg Asn Thr  Val Cys Thr Val Cys  Gly Met Trp
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    4340                4345                4350

Lys Gly  Tyr Gly Cys Ser Cys  Asp Gln Leu Arg Glu  Pro Leu Met
    4355                4360                4365

Gln Ser  Ala Asp Ala Ser Thr  Phe Leu Asn Gly Phe  Ala Val
    4370                4375                4380


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<223> OTHER INFORMATION: HBV core 128

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The invention claimed is:

1. A peptide-bound liposome, wherein the peptide is covalently bound to the surface of the liposome; the liposome comprises: a phospholipid comprising a C_{14}-C_{24} acyl group containing one unsaturated bond, or a C_{14}-C_{24} hydrocarbon group containing one unsaturated bond, and a stabilizer; and the peptide comprises the amino acid sequence of SEQ ID NO: 24, wherein the peptide-bound liposome induces an immune response that produces human leukocyte antigen type A2 (HLA-A2)-restricted cytotoxic T lymphocytes (CTLs) specific to severe acute respiratory syndrome (SARS) coronavirus.

2. The peptide-bound liposome according to claim **1**, wherein the phospholipid comprises a C_{14}-C_{24} acyl group containing one unsaturated bond.

3. The peptide-bound liposome according to claim **1**, wherein the phospholipid comprises an oleoyl group.

4. The peptide-bound liposome according to claim **1**, wherein the phospholipid is at least one selected from the group consisting of diacylphosphatidylserine, diacylphosphatidylglycerol, diacylphosphatidic acid, diacylphosphatidylcholine, diacylphosphatidylethanolamine, succinimidyl-diacylphosphatidylethanolamine and maleimide-diacylphosphatidylethanolamine.

5. The peptide-bound liposome according to claim **1**, wherein the stabilizer is cholesterol.

6. The peptide-bound liposome according to claim **1**, wherein the peptide is covalently bound to the phospholipid on the surface of the liposome.

7. The peptide-bound liposome according to claim **1**, wherein the liposome comprises:

(A) 1 to 99.8 mol % of a phospholipid comprising:

a C_{14}-C_{24} acyl group containing one unsaturated bond, or a C_{14}-C_{24} hydrocarbon group containing one unsaturated bond; and

(B) 0.2 to 75 mol % of a stabilizer.

8. A peptide-bound liposome according to claim **1** comprising:

(I) 1 to 85 mol % of an acidic phospholipid comprising a C_{14}-C_{24} acyl group containing one unsaturated bond, or a C_{14}-C_{24} hydrocarbon group containing one unsaturated bond;

(II) 0.01 to 80 mol % of a neutral phospholipid comprising a C_{14}-C_{24} acyl group containing one unsaturated bond, or a C_{14}-C_{24} hydrocarbon group containing one unsaturated bond;

(III) 0.2 to 80 mol % of a phospholipid comprising a C_{14}-C_{24} acyl group containing one unsaturated bond, or a C_{14}-C_{24} hydrocarbon group containing one unsaturated bond, wherein the phospholipid is bound to the at least one peptide; and

(IV) 0.2 to 75 mol % of a stabilizer.

9. An inducing agent for human leukocyte antigen type A2 (HLA-A2)-restricted cytotoxic T lymphocytes (CTLs) specific to severe acute respiratory syndrome (SARS) coronavirus comprising: the peptide-bound liposome according to claim **1** as an active ingredient, and a pharmaceutically

acceptable carrier, wherein the agent induces an immune response that produces HLA-A2-restricted CTLs specific to SARS coronavirus.

10. A vaccine comprising: the peptide-bound liposome according to claim **1**, and a pharmaceutically acceptable carrier, wherein the vaccine induces an immune response that produces HLA-A2-restricted CTLs specific to SARS coronavirus.

11. The inducing agent for HLA-A2-restricted CTLs specific to the SARS coronavirus according to claim **9**, further comprising CpG-DNA.

12. The vaccine according to claim **10**, further comprising CpG-DNA.

13. A method of producing an immune response to severe acute respiratory syndrome (SARS) coronavirus in a subject, comprising administering to the subject an effective amount of the vaccine composition of claim **10**, thereby inducing the immune response to SARS coronavirus in the subject.

14. The method of claim **13**, wherein the subject is human.

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