infected. Because of the fact that, 17 years after the epidemic, CTLs against SARS-CoV were still detected in the blood of people who had recovered (NPL 10: Le Bert N *et al.*, Nature 2020, 584(7821): 457-462), CTLs against SARS-CoV-2 may also remain in the body for a long time after being induced.

Accordingly, a vaccine aimed at eliciting cellular immunity can be an effective preventive measure against COVID-19. It is considered that CTLs induced by a vaccine comprising an epitope peptide derived from a SARS-CoV-2 protein remain in the body as memory T cells and show cytotoxic activity against virus-infected cells rapidly after SARS-CoV-2 infection, leading to suppression of aggravation of COVID-19. Thus, it is desired to identify an epitope peptide derived from a SARS-CoV-2 protein that can induce CTLs in the human body.

[0006]

Candidate epitope peptides determined based on bioinformatics have been reported; however, it has not been verified whether or not they actually bind to an HLA and have the ability to induce CTLs, and no epitope peptide has been identified (NPL 11: Grifoni A *et al.*, Cell Host Microbe 2020, 27(4): 671-680; NPL12: Crooke SN *et al.*, Sci Rep 2020, 10(1): 14179).

[Citation List]

[Non-Patent Literature]

[0007]

[NPL 1] Giamarellos-Bourboulis EJ *et al.*, Cell 2020, Online ahead of print
[NPL 2] Toyoshima Y *et al.*, J Hum Genet 2020, Online ahead of print
[NPL 3] Berg MK *et al.*, Science Advances 2020, Online ahead of print
[NPL 4] Netea MG *et al.*, Cell 2020, 181(5):969-977
[NPL 5] Hoffmann M *et al.*, Cell 2020, 181(2):271-280
[NPL 6] Jeyanathan M *et al.*, Nat Rev Immunol 2020, Online ahead of print
[NPL 7] Ibarrondo FJ *et al.*, N Engl J Med 2020, 383(11):1085-1087
[NPL 8] Long QX *et al.*, Nat Med 2020, 26(8):1200-1204
[NPL 9] Zheng M *et al.*, Cell Mol Immunol 2020, 17(5):533-535
[NPL 10] Le Bert N *et al.*, Nature 2020, 584(7821):457-462
[NPL 11] Grifoni A *et al.*, Cell Host Microbe 2020, 27(4):671-680
[NPL 12] Crooke SN *et al.*, Sci Rep 2020, 10(1):14179

[Summary ~~of Invention~~]

[0007a]

In one aspect, the present disclosure provides a peptide of less than 15 amino acids having cytotoxic T cell (CTL)-inducing ability, which comprises the amino acid sequence

selected from the group below: (a) the amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 1, 3, 4, 5, 7, 9, 10, 11, 12, 13 and 15; and (b) the amino acid sequence in which one, two or several amino acids are substituted, deleted, inserted and/or added to the amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 1, 3, 4, 5, 7, 9, 10, 11, 12, 13 and 15.

In another aspect, the present disclosure provides a polynucleotide, which encodes the peptide as described herein.

In another aspect, the present disclosure provides a composition comprising a pharmaceutically acceptable carrier and at least one active ingredient selected from the group consisting of (a) to (e) below: (a) one or more types of peptides as described herein; (b) one or more types of polynucleotides encoding the peptide(s) as described herein in an expressible form; (c) an antigen-presenting cell (APC) that presents on its cell surface a complex of the peptide as described herein and an HLA antigen; (d) an exosome that presents on its cell surface a complex of the peptide as described herein and an HLA antigen; and (e) a CTL that targets the peptide as described herein.

In another aspect, the present disclosure provides an *in vitro* or *ex vivo* method of inducing an APC(s) having CTL-inducing ability, which comprises a step selected from the group consisting of (a) and (b) below: (a) contacting an APC(s) with the peptide as described herein *in vitro* or *ex vivo*; and (b) introducing a polynucleotide encoding the peptide as described herein into an APC(s).

In another aspect, the present disclosure provides an *in vitro* method of inducing a CTL(s), which comprises a step selected from the group consisting of (a) to (c) below: (a) co-culturing a CD8-positive T cell(s) with an APC(s) that presents on its surface a complex of an HLA antigen and the peptide of any one of claims 1 to 4; (b) co-culturing a CD8-positive T cell(s) with an exosome(s) that presents on its surface a complex of an HLA antigen and the peptide as described herein; and (c) introducing into a CD8-positive T cell(s) a polynucleotide encoding each subunit of a T cell receptor (TCR) capable of binding to the peptide as described herein presented by an HLA antigen on a cell surface.

In another aspect, the present disclosure provides an APC that presents on its surface a complex of an HLA antigen and the peptide as described herein.

In another aspect, the present disclosure provides a CTL that targets the peptide as described herein.

In another aspect, the present disclosure provides the use of at least one ingredient selected from the group consisting of (a) to (e) below: (a) one or more types of peptides as described herein; (b) one or more types of polynucleotides encoding the peptide(s) as described herein in an expressible form; (c) an antigen-presenting cell (APC) that presents on its cell

surface a complex of the peptide as described herein and an HLA antigen; (d) an exosome that presents on its cell surface a complex of the peptide as described herein and an HLA antigen; and (e) a CTL that targets the peptide as described herein in the manufacture of a medicament for inducing an immune response against coronavirus infection.

In another aspect, the present disclosure provides the use of at least one active ingredient selected from the group consisting of (a) to (e) below: (a) one or more types of peptides as described herein; (b) one or more types of polynucleotides encoding the peptide(s) as described herein in an expressible form; (c) an antigen-presenting cell (APC) that presents on its cell surface a complex of the peptide as described herein and an HLA antigen; (d) an exosome that presents on its cell surface a complex of the peptide as described herein and an HLA antigen; and (e) a CTL that targets the peptide as described herein in the manufacture of a medicament for treating and/or preventing coronavirus infectious disease, and/or suppressing aggravation of coronavirus infectious disease.

In another aspect, the present disclosure provides a method of screening for a peptide having CTL-inducing ability, which comprises the steps of: (a) generating candidate sequences consisting of an amino acid sequence in which one, two or several amino acid residues are substituted, deleted, inserted and/or added to an original amino acid sequence consisting of the amino acid sequence selected from among SEQ ID NOs: 2, 1, 3, 4, 5, 7, 9, 10, 11, 12, 13 and 15; (b) selecting from among the candidate sequences generated in (a), a candidate sequence that does not have significant homology (sequence identity) with any known human gene product; (c) contacting an APC(s) with a peptide consisting of the candidate sequence selected in (b); (d) contacting the APC(s) of (c) with a CD8-positive T cell(s); and (e) selecting a peptide having an equal to or higher CTL-inducing ability than that of a peptide consisting of the original amino acid sequence.

In another aspect, the present disclosure provides an emulsion comprising one or more types of peptides as described herein, a water-soluble carrier and an oil adjuvant.

In another aspect, the present disclosure provides a kit comprising a container that houses the composition as described herein and a container that houses an adjuvant.

[0008]

The present ~~invention~~disclosure provides epitope peptides that bind to HLA-A*24:02 or HLA-A*02:01 and have the ability to induce CTLs, which are selected from peptides derived from

four structural proteins and six non-structural proteins that SARS-CoV-2 (reference sequence: GenBank accession number MN908947 (SEQ ID NO: 16)) has.

The four structural proteins specifically refer to the following proteins:

spike protein

(reference sequence: GenBank accession number QHD43416 (SEQ ID NO: 17));

envelope protein

(reference sequence: GenBank accession number QHD43418 (SEQ ID NO: 18));

matrix protein

(reference sequence: GenBank accession number QHD43419 (SEQ ID NO: 19)); and

nucleoprotein

(reference sequence: GenBank accession number QHD43423 (SEQ ID NO: 20)).

On the other hand, the six non-structural proteins specifically refer to the following proteins:

ORF1ab

(reference sequence: GenBank accession number QHD43415 (SEQ ID NO: 21));

ORF3a

(reference sequence: GenBank accession number QHD43417 (SEQ ID NO: 22));

ORF6

(reference sequence: GenBank accession number QHD43420 (SEQ ID NO: 23));

ORF7a

(reference sequence: GenBank accession number QHD43421 (SEQ ID NO: 24));

ORF8

(reference sequence: GenBank accession number QHD43422 (SEQ ID NO: 25)); and

ORF10

(reference sequence: GenBank accession number QHI42199 (SEQ ID NO: 26)).

The epitope peptides of the present ~~invention~~disclosure are demonstrated to be able to induce specific and potent immune responses against COVID-19 targeting HLA-A*24:02-positive individuals, who constitute a large population in Asian countries including Japan, or targeting HLA-A*02:01-positive individuals, who constitute a large population in Europe and the United States (Cao K *et al.*, Hum Immunol 2001, 62(9): 1009-1030; Gonzalez-Galarza FF *et al.*, Nucleic Acids Res 2020, 48(D1): D783-D788).

Since the amino acid sequences of the epitope peptides provided in the present ~~invention~~disclosure have low similarity to those derived from human proteins, creation of highly safe vaccines that are less likely to cause unexpected side reactions can be expected. On the other hand, since those amino acid sequences are commonly found in SARS-CoV and MERS-CoV, which were epidemic in the past, they may also be found in proteins of new types of coronaviruses that will

emerge in the future. Accordingly, vaccines comprising such epitope peptide can be effective against not only the current SARS-CoV-2 but also coronavirus infectious diseases that will be prevalent in the future.

[0009]

The present ~~invention~~disclosure also provides compositions comprising one or more types of peptides of the present ~~invention~~disclosure, one or more types of polynucleotides encoding one or more types of peptides of the present ~~invention~~disclosure, APCs of the present ~~invention~~disclosure, exosomes presenting peptides of the present ~~invention~~disclosure, and/or CTLs of the present ~~invention~~disclosure. The compositions of the present ~~invention~~disclosure are preferably pharmaceutical compositions. The pharmaceutical compositions of the present ~~invention~~disclosure can be used for treating and/or preventing coronavirus infectious diseases, as well as suppressing aggravation. They can also be used for inducing an immune response against coronavirus infection. When administered to a subject, a peptide of the present ~~invention~~disclosure is presented on the surface of an APC, and as a result CTLs targeting the peptide are induced. Therefore, another objective of the present ~~invention~~disclosure is to provide compositions inducing CTLs, wherein the compositions comprise one or more types of peptides of the present ~~invention~~disclosure, one or more types of polynucleotides encoding one or more types of peptides of the present ~~invention~~disclosure, APCs of the present ~~invention~~disclosure, and/or exosomes presenting peptides of the present ~~invention~~disclosure.

[0010]

A further objective of the present ~~invention~~disclosure is to provide methods of inducing APCs having CTL-inducing ability, wherein the methods comprise a step of contacting one or more types of peptides of the present ~~invention~~disclosure with an APC, or a step of introducing a polynucleotide encoding any one peptide of the present ~~invention~~disclosure into an APC.

[0011]

The present ~~invention~~disclosure further provides a method of inducing CTLs, comprising a step of co-culturing a CD8-positive T cell with an APC that presents on its surface a complex of an HLA antigen and a peptide of the present ~~invention~~disclosure, a step of co-culturing a CD8-positive T cell with an exosome that presents on its surface a complex of an HLA antigen and a peptide of the present ~~invention~~disclosure, or a step of introducing into a CD8-positive T cell a vector comprising a polynucleotide encoding each subunit of a T cell receptor (TCR) capable of binding to a peptide of the present ~~invention~~disclosure presented by an HLA antigen on a cell surface.

[0012]

A further objective of the present ~~invention~~disclosure is to provide isolated APCs that present on their surface a complex of an HLA antigen and a peptide of the present ~~invention~~disclosure. The present ~~invention~~disclosure further provides isolated CTLs targeting a peptide of the present ~~invention~~disclosure. These APCs and CTLs can be used in immunotherapy for coronavirus infectious diseases.

[0013]

Another objective of the present ~~invention~~disclosure is to provide methods of inducing an immune response against coronavirus infection in a subject, wherein the methods comprise a step of administering to the subject a peptide(s) of the present ~~invention~~disclosure, a polynucleotide(s) encoding the peptide(s), an APC(s) of the present ~~invention~~disclosure, an exosome(s) presenting a peptide(s) of the present ~~invention~~disclosure, and/or a CTL(s) of the present ~~invention~~disclosure. Another objective of the present ~~invention~~disclosure is to provide methods of treating and/or preventing coronavirus infectious disease, as well as suppressing aggravation in a subject, wherein the methods comprise a step of administering to the subject a peptide(s) of the present ~~invention~~disclosure, a polynucleotide(s) encoding the peptide(s), an APC(s) of the present ~~invention~~disclosure, an exosome(s) presenting a peptide(s) of the present ~~invention~~disclosure, and/or a CTL(s) of the present ~~invention~~disclosure.

[0014]

In addition to the above, other objects and features of the present ~~invention~~disclosure will become more fully apparent when the following detailed description is read in conjunction with the accompanying figures and examples. However, it is to be understood that both the foregoing summary of the present ~~invention~~disclosure and the following detailed description are of exemplified ~~embodiment~~examples, and not restrictive of the present ~~invention~~disclosure or other alternate ~~embodiment~~examples of the present ~~invention~~disclosure. In particular, while the present ~~invention~~disclosure is described herein with reference to a number of specific ~~embodiment~~examples, it will be appreciated that the description is illustrative of the present ~~invention~~disclosure and is not constructed as limiting of the present ~~invention~~disclosure. Various modifications and applications may occur to those who are skilled in the art, without departing from the spirit and the scope of the present ~~invention~~disclosure, as described by the appended claims. Likewise, other objects, features, benefits and advantages of the present ~~invention~~disclosure will be apparent from this summary and certain ~~embodiment~~examples described below, and will be readily apparent to those skilled in the art. Such objects, features, benefits and advantages will be apparent from the above in conjunction with the accompanying examples, data, figures and all reasonable inferences to be drawn therefrom, alone or with consideration of the references incorporated herein.

[Brief Description of Drawings]

[0015]

Fig. 1 is composed of images showing the results of IFN-γ enzyme-linked immunospot (ELISPOT) assays performed using cells induced with peptides derived from SARS-CoV-2 proteins. In the figure, "(+)" indicates IFN-γ production for HLA-A*24:02-expressing target cells (TISI cells) pulsed with a peptide of interest, and "(-)" indicates IFN-γ production for TISI cells not pulsed with any peptide (negative control). It can be seen by comparison with the

(a) one or more types of peptides of any one of [1] to [4];

(b) one or more types of polynucleotides encoding the peptide(s) of any one of [1] to [4] in an expressible form;

(c) an antigen-presenting cell (APC) that presents on its cell surface a complex of the peptide of any one of [1] to [4] and an HLA antigen;

(d) an exosome that presents on its cell surface a complex of the peptide of any one of [1] to [4] and an HLA antigen; and

(e) a CTL that targets the peptide of any one of [1] to [4].

[7] The composition of [~~5~~6], which is a composition for inducing a CTL(s), wherein the active ingredient is at least one ingredient selected from the group consisting of (a) to (d) below:

(a) one or more types of peptides of any one of [1] to [4];

(b) one or more types of polynucleotides encoding the peptide(s) of any one of [1] to [4] in an expressible form;

(c) an antigen-presenting cell (APC) that presents on its cell surface a complex of the peptide of any one of [1] to [4] and an HLA antigen; and

(d) an exosome that presents on its cell surface a complex of the peptide of any one of [1] to [4] and an HLA antigen.

[8] The composition of [6], which is a pharmaceutical composition.

[9] The composition of [8], which is a pharmaceutical composition for one or more uses selected from the group consisting of (i) treatment of coronavirus infectious disease, (ii) prevention (prophylaxis) of coronavirus infectious disease and (iii) suppression of aggravation of coronavirus infectious disease.

[10] The composition of [8], which is for inducing an immune response against coronavirus infection.

[11] The composition of [9] or [10], wherein coronavirus of the coronavirus infectious disease is selected from the group consisting of SARS-CoV-2, MERS-CoV and SARS-CoV.

[12] The composition of any one of [6] to [11], which is formulated for administration to a subject positive for HLA-A24 or HLA-A02.

[13] A method of inducing an APC(s) having CTL-inducing ability, which comprises a step selected from the group consisting of (a) and (b) below:

(a) contacting an APC(s) with the peptide of any one of [1] to [4] *in vitro*, *ex vivo* or *in vivo*; and

(b) introducing a polynucleotide encoding the peptide of any one of [1] to [4] into an APC(s).

[14] A method of inducing a CTL(s), which comprises a step selected from the group consisting of (a) to (c) below:

[CLAIMS]

1. A peptide of less than 15 amino acids having cytotoxic T cell (CTL)-inducing ability, which comprises the amino acid sequence selected from the group below:

(a) the amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 1, ~~2,~~ 3, 4, 5, 7, 9, 10, 11, 12, 13 and 15; and

(b) the amino acid sequence in which one, two or several amino acids are substituted, deleted, inserted and/or added to the amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 1, ~~2,~~ 3, 4, 5, 7, 9, 10, 11, 12, 13 and 15.

2. The peptide of claim 1, which has either or both of features below to the amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 1, ~~2,~~ 3, 4, 5, 7, 9, 10, 11, 12, 13 and 15:

(a) the second amino acid from the N terminus is substituted with an amino acid selected from the group consisting of phenylalanine, tyrosine, methionine and tryptophan; and

(b) the C-terminal amino acid is substituted with an amino acid selected from the group consisting of phenylalanine, leucine, isoleucine, tryptophan and methionine.

3. The peptide of claim 1, which has either or both of features below to the amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 1, ~~2,~~ 4, 7, 10, 12 and 13:

(a) the second amino acid from the N terminus is substituted with an amino acid selected from the group consisting of leucine and methionine; and

(b) the C-terminal amino acid is substituted with an amino acid selected from the group consisting of valine and leucine.

4. The peptide of claim 1, which consists of the amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 1, ~~2,~~ 3, 4, 5, 7, 9, 10, 11, 12, 13 and 15.

5. A polynucleotide, which encodes the peptide of any one of claims 1 to 4.

6. A composition comprising a pharmaceutically acceptable carrier and at least one active ingredient selected from the group consisting of (a) to (e) below:

(a) one or more types of peptides of any one of claims 1 to 4;

(b) one or more types of polynucleotides encoding the peptide(s) of any one of claims 1 to 4 in an expressible form;

(c) an antigen-presenting cell (APC) that presents on its cell surface a complex of the peptide of any one of claims 1 to 4 and an HLA antigen;
(d) an exosome that presents on its cell surface a complex of the peptide of any one of claims 1 to 4 and an HLA antigen; and
(e) a CTL that targets the peptide of any one of claims 1 to 4.

7. The composition of claim ~~5~~6, which is a composition for inducing a CTL(s), wherein the active ingredient is at least one ingredient selected from the group consisting of (a) to (d) below:
(a) one or more types of peptides of any one of claims 1 to 4;
(b) one or more types of polynucleotides encoding the peptide(s) of any one of claims 1 to 4 in an expressible form;
(c) an antigen-presenting cell (APC) that presents on its cell surface a complex of the peptide of any one of claims 1 to 4 and an HLA antigen; and
(d) an exosome that presents on its cell surface a complex of the peptide of any one of claims 1 to 4 and an HLA antigen.

8. The composition of claim 6, which is a pharmaceutical composition.

9. The composition of claim 8, which is for one or more uses selected from the group consisting of (i) treatment of coronavirus infectious disease, (ii) prevention (prophylaxis) of coronavirus infectious disease and (iii) suppression of aggravation of coronavirus infectious disease.

10. The composition of claim 8, which is for inducing an immune response against coronavirus infection.

11. The composition of claim 9 or 10, wherein coronavirus is selected from the group consisting of SARS-CoV-2, MERS-CoV and SARS-CoV.

12. The composition of any one of claims 6 to 11, which is formulated for administration to a subject positive for HLA-A24 or HLA-A02.

13. An *in vitro* or *ex vivo* method of inducing an APC(s) having CTL-inducing ability, which comprises a step selected from the group consisting of (a) and (b) below:
(a) contacting an APC(s) with the peptide of any one of claims 1 to 4 *in vitro*~~,~~ or *ex vivo* ~~or *in vivo*~~; and

(b) introducing a polynucleotide encoding the peptide of any one of claims 1 to 4 into an APC(s).

14. An *in vitro* method of inducing a CTL(s), which comprises a step selected from the group consisting of (a) to (c) below:

(a) co-culturing a CD8-positive T cell(s) with an APC(s) that presents on its surface a complex of an HLA antigen and the peptide of any one of claims 1 to 4;

(b) co-culturing a CD8-positive T cell(s) with an exosome(s) that presents on its surface a complex of an HLA antigen and the peptide of any one of claims 1 to 4; and

(c) introducing into a CD8-positive T cell(s) a polynucleotide encoding each subunit of a T cell receptor (TCR) capable of binding to the peptide of any one of claims 1 to 4 presented by an HLA antigen on a cell surface.

15. An APC that presents on its surface a complex of an HLA antigen and the peptide of any one of claims 1 to 4.

16. The APC of claim 15, which is induced by the method of claim 13.

17. A CTL that targets the peptide of any one of claims 1 to 4.

18. The CTL of claim 17, which is induced by the method of claim 14.

19. ~~A method of inducing an immune response against coronavirus infection, which comprises administering to a subject~~Use of at least one ingredient selected from the group consisting of (a) to (e) below:

(a) one or more types of peptides of any one of claims 1 to 4;

(b) one or more types of polynucleotides encoding the peptide(s) of any one of claims 1 to 4 in an expressible form;

(c) an antigen-presenting cell (APC) that presents on its cell surface a complex of the peptide of any one of claims 1 to 4 and an HLA antigen;

(d) an exosome that presents on its cell surface a complex of the peptide of any one of claims 1 to 4 and an HLA antigen; and

(e) a CTL that targets the peptide of any one of claims 1 to 4

in the manufacture of a medicament for inducing an immune response against coronavirus infection.

20. ~~A method of treating and/or preventing coronavirus infectious disease, and/or suppressing aggravation of coronavirus infectious disease, which comprises administering to a subject a composition comprising~~Use of at least one active ingredient selected from the group consisting of (a) to (e) below:

(a) one or more types of peptides of any one of claims 1 to 4;

(b) one or more types of polynucleotides encoding the peptide(s) of any one of claims 1 to 4 in an expressible form;

(c) an antigen-presenting cell (APC) that presents on its cell surface a complex of the peptide of any one of claims 1 to 4 and an HLA antigen;

(d) an exosome that presents on its cell surface a complex of the peptide of any one of claims 1 to 4 and an HLA antigen; and

(e) a CTL that targets the peptide of any one of claims 1 to 4

in the manufacture of a medicament for treating and/or preventing coronavirus infectious disease, and/or suppressing aggravation of coronavirus infectious disease.

~~21. An antibody that binds to the peptide of any one of claims 1 to 4.~~

~~22~~21. A method of screening for a peptide having CTL-inducing ability, which comprises the steps of:

(a) generating candidate sequences consisting of an amino acid sequence in which one, two or several amino acid residues are substituted, deleted, inserted and/or added to an original amino acid sequence consisting of the amino acid sequence selected from among SEQ ID NOs: 2, 1, ~~2,~~ 3, 4, 5, 7, 9, 10, 11, 12, 13 and 15;

(b) selecting from among the candidate sequences generated in (a), a candidate sequence that does not have significant homology (sequence identity) with any known human gene product;

(c) contacting an APC(s) with a peptide consisting of the candidate sequence selected in (b);

(d) contacting the APC(s) of (c) with a CD8-positive T cell(s); and

(e) selecting a peptide having an equal to or higher CTL-inducing ability than that of a peptide consisting of the original amino acid sequence.

~~23~~22. An emulsion comprising one or more types of peptides of any one of claims 1 to 4, a water-soluble carrier and an oil adjuvant.

~~24~~23. A kit comprising a container that houses the composition of any one of claims 6 to 12 and a container that houses an adjuvant.