

Description

Title of Invention:

RECOMBINANT LIVE ATTENUATED HERPES SIMPLEX
VIRUS TYPE 2 VACCINE

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Technical Field

[0001]

The present invention relates to recombinant live
attenuated herpes simplex virus type 2 and a recombinant live
attenuated herpes simplex virus type 2 vaccine.

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Background Art

[0002]

Human herpes simplex virus (HSV) is a pathogen that is
widely spread in humans. HSV that is a dsDNA virus belongs
to Alphaherpesvirinae and has two serotypes HSV-1 and HSV-
2. HSV causes various diseases such as encephalitis,
meningitis, herpes labialis, genital herpes, skin diseases,
corneal herpes, and neonatal systemic herpes in humans.
Thus, HSV is a virus of critical medical health importance,
and an antiviral agent such as acyclovir or valacyclovir
actually has been developed.

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[0003]

However, anti-HSV agents that have been developed to
date inhibit replication of viral DNA, and thus an effect

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thereof on HSV in a DNA state with which the interior of the ganglion is latently infected is limited. In a case where a timing of administration of an anti-HSV agent is missed due to, for example, cessation of medication, medication reduction, or forgetting to take medicine, a therapeutic effect of the anti-HSV agent may be reduced. This prevents achievement of fundamental disease control. In order to overcome such a situation, it is necessary to develop a vaccine that is effective in preventing primary infection and recurrence and that is highly safe.

[0004]

A pathogen causing an infectious disease is roughly divided into a class I pathogen that makes it possible to obtain a sufficient effect with an existing vaccine and a class II pathogen that makes it impossible to acquire sufficient protective immunity with an existing vaccine or pathogen infection history. An ingenious immune evasion mechanism possessed by class II pathogens is pointed out as a reason why it is difficult to defend against the class II pathogens. HSV is classified as a class II pathogen. This is considered to be because HSV has an immune evasion mechanism and ingeniously evades a host immune response. Regarding HSV vaccine development, a study mainly on subunit vaccines has been attempted until now, and the subunit vaccines are all insufficiently effective (Non-patent Literatures 1 to 3).

[0005]

It is pointed out that induction of CD4-positive and CD8-positive T cells is important for pathological control, and induction of tissue-resident memory T cells is also important (Non-patent Literatures 4 to 7). It is also known that a live attenuated vaccine can strongly induce humoral immunity and cellular immunity (Non-patent Literatures 8 to 11).

[0006]

A gene Us3 of HSV-1 encodes protein kinase Us3 and plays an important role in pathogenicity expression. A Us3 mutant is shown to have reduced pathogenicity (Non-patent Literatures 12 to 15) and is shown not to be essential for viral propagation in cultured cells (Non-patent Literatures 16 and 17). Furthermore, Us3 of HSV-1 is shown to evade cytotoxic T cells (CTLs) by suppressing cell surface expression of major histocompatibility antigen class I (MHC-I) (Non-patent Literature 18).

[0007]

A gene UL41 of HSV-1 encodes a ribonuclease virion host shut-off (VHS) and plays an important role in pathogenicity expression. A UL41 mutant is shown to have reduced pathogenicity (Non-patent Literatures 19 to 23) and is shown not to be essential for propagation of cultured cells (Non-patent Literatures 24 to 27). Furthermore, UL41 of HSV-1 is shown to evade host immunity by, for example, inhibiting

protein kinase R activation, inhibiting dendritic cell activation, inhibiting cGAS/STING pathway activation, or suppressing inflammatory cytokine production (Non-patent Literatures 28 to 32).

5 [0008]

A gene UL35 of HSV-1 encodes a capsid protein V26 and plays an important role in pathogenicity expression. A VP26 mutant is shown to have reduced pathogenicity (Non-patent Literature 33) and is shown not to be essential for propagation
10 of cultured cells (Non-patent Literature 33).

[0009]

A gene UL50 of HSV-1 encodes vdUTPase serving as a homolog of a host nucleic acid metabolic enzyme dUTPase and plays an important role in pathogenicity expression. A
15 vdUTPase mutant is shown to have reduced pathogenicity (Non-patent Literature 34) and is shown not to be essential for propagation of cultured cells (Non-patent Literature 15).

[0010]

A gene UL27 of HSV-1 encodes an envelope glycoprotein
20 B (gB) and plays an important role in pathogenicity expression. A gB mutant is shown to have reduced pathogenicity (Non-patent Literature 35). gB is shown to be essential for propagation of cultured cells, and, although a gB deletion variant lacks proliferative potential, a gB point
25 mutant can obtain certain proliferative potential (Non-patent

Literatures 36 to 39 of a deletion variant and Non-patent
Literatures 13 and 35 of a point mutant). gB of HSV-1 is
shown to inhibit the function of an NKT cell by suppressing
cell surface expression of CD1d (Non-patent Literature 40).

5 [0011]

It is not guaranteed that the functions of HSV-1 and
HSV-2 are preserved. For example, the function of Us3 is
shown to differ between HSV-1 and HSV-2 (Non-patent
Literature 12).

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Citation List

[Non-patent Literature]

[0012]

[Non-patent Literature 1]

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Imai, T. et al., Us3 kinase encoded by herpes simplex virus 1 mediates downregulation of cell surface major histocompatibility complex class I and evasion of CD8+ T
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[Non-patent Literature 34]

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20 Summary of Invention

Technical Problem

[0013]

As described earlier, antiviral drugs such as acyclovir are used for treatment of HSV. However, these antiviral drugs cannot completely eliminate a virus and have a risk of

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reactivation of the virus due to, for example, a reduction in adherence. Thus, development of a prophylactic vaccine to prevent HSV infection itself or a therapeutic vaccine to alleviate relapse symptoms is desired. However, currently,
5 there is no effective vaccine, and unmet needs for effective vaccines are high.

[0014]

The present invention has an object to provide a recombinant live attenuated vaccine that can be used for
10 prevention and treatment of an HSV-2 infection, that has enhanced immune inducibility of HSV-2 through mutation introduction, and that has high safety.

Solution to Problem

[0015]

15 Since it is not guaranteed that the functions of HSV-1 and HSV-2 are preserved, it is important also to evaluate HSV-2 in detail with reference to the above-described information pertaining to HSV-1. The inventors of the present invention comprehensively produced and evaluated a multiple mutant of
20 HSV-2 with the aim of developing a live attenuated vaccine that has both high effectiveness and high safety.

[0016]

On the basis of the hypothesis that a recombinant live attenuated vaccine which has high safety and more enhanced
25 immune inducibility can be constructed by introducing a

similar mutation in HSV-2 into a virulence factor and an immune evasion factor which are identified in HSV-1, the inventors of the present invention produced a variety of mutated viruses and carried out animal experiments for evaluation. As a result, the inventors of the present invention found the following: In a mouse primary infection model infected with genital herpes, the following double mutant virus obtained by further introducing a mutation (inactivation) into another gene with respect to a virus into which a mutation (inactivation) is introduced into a gene Us3 or UL41 of herpes simplex virus type 2 exhibits a superior primary infection prevention effect (virus shedding suppression effect or disease prevention effect) as compared with Δ Tk (thymidine kinase-deficient)-inoculated group inoculated with Δ Tk which is a traditional vaccine prototype live attenuated virus having the same origin as that of the double mutant virus. Note that Δ Tk is focused as a comparison target because Non-patent Literatures (Non-Patent Literatures 41 to 43) suggest safety and effectiveness of Δ Tk. Note here that, for example, "Us3/UL2" denotes a double mutant virus of Us3 and UL2.

*Double mutant virus:

Us3/UL2, Us3/UL3, Us3/UL4, Us3/UL13, Us3/UL27, Us3/UL35, Us3/UL41, Us3/UL44, Us3/UL45, Us3/UL46, Us3/UL50, Us3/UL56, Us3/Us4, Us3/Us5, Us3/Us8A,

Us3/Us9, Us3/Us11

UL41/UL4, UL41/UL12, UL41/UL12.5, UL41/UL24,
UL41/UL27, UL41/ICP34.5, UL41/UL35, UL41/UL43,
UL41/UL56, UL41/Us2, UL41/Us4, UL41/Us5, UL41/Us8A,
5 UL41/Us9

[0017]

Furthermore, among the above, Us3/UL3, Us3/UL4,
Us3/UL27, Us3/UL35, Us3/UL44, Us3/UL46, Us3/UL56,
Us3/Us4, Us3/Us5, Us3/Us8A, UL41/UL4, and UL41/Us5
10 had a higher survival rate than a Us3 single mutant live
attenuated virus-inoculated group or a UL41 single mutant
live attenuated virus-inoculated group and had attenuated
pathogenicity.

[0018]

Moreover, among the above, Us3/UL27, Us3/UL35,
15 Us3/Us4, and UL41/Us5 exhibited a recurrence prevention
effect of 40% or more in a guinea pig recurrence model, and
among these, Us3/UL35 exhibited a recurrence prevention
effect of 80% or more.

20 [0019]

Thus, the inventors of the present invention completed
the present invention by finding that it is possible obtain a
recombinant live attenuated vaccine which has enhanced
immune inducibility by introducing a mutation (inactivation)
25 into two or more types of genes of herpes simplex virus type 2

and which has enhanced safety by attenuating pathogenicity.

[0020]

That is, a virus in accordance with an aspect of the present invention is a multiple mutant virus in which: two or
5 more genes of herpes simplex virus type 2 (HSV-2) are modified; and a modification of the two or more genes involves a loss or reduction in gene function.

Advantageous Effects of Invention

[0021]

10 In accordance with an aspect of the present invention, in a case where immunity is induced with recombinant live attenuated herpes simplex virus type 2 and a vaccine containing the recombinant live attenuated herpes simplex virus type 2, a superior primary infection prevention effect
15 can be exhibited as compared with a case where immunity is induced with a traditional live attenuated virus Δ Tk which has the same origin as that of the recombinant live attenuated herpes simplex virus type 2. Thus, a high prevention effect on an HSV infection can be expected.

Brief Description of Drawings

[0022]

Fig. 1 is a schematic view of a mouse primary infection prevention test in Example 4.

25 Fig. 2 is a diagram showing a virus shedding

suppression effect of the mouse primary infection prevention test in Example 4.

Fig. 3 is a diagram showing a virus shedding suppression effect of the mouse primary infection prevention test in Example 4.

Fig. 4 is a diagram showing a virus shedding suppression effect of the mouse primary infection prevention test in Example 4.

Fig. 5 is a diagram showing a virus shedding suppression effect of the mouse primary infection prevention test in Example 4.

Fig. 6 is a diagram showing a virus shedding suppression effect of the mouse primary infection prevention test in Example 4.

Fig. 7 is a diagram showing a virus shedding suppression effect of the mouse primary infection prevention test in Example 4.

Fig. 8 is a diagram showing a virus shedding suppression effect of the mouse primary infection prevention test in Example 4.

Fig. 9 is a diagram showing a virus shedding suppression effect of the mouse primary infection prevention test in Example 4.

Fig. 10 is a diagram showing a virus shedding suppression effect of the mouse primary infection prevention

test in Example 4.

Fig. 11 is a diagram showing a virus shedding suppression effect of the mouse primary infection prevention test in Example 4.

5 Fig. 12 is a diagram showing a virus shedding suppression effect of the mouse primary infection prevention test in Example 4.

10 Fig. 13 is a diagram showing a virus shedding suppression effect of the mouse primary infection prevention test in Example 4.

Fig. 14 is a diagram showing a virus shedding suppression effect of the mouse primary infection prevention test in Example 4.

15 Fig. 15 is a diagram showing a virus shedding suppression effect of the mouse primary infection prevention test in Example 4.

Fig. 16 is a diagram showing a virus shedding suppression effect of the mouse primary infection prevention test in Example 4.

20 Fig. 17 is a diagram showing a virus shedding suppression effect of the mouse primary infection prevention test in Example 4.

25 Fig. 18 is a diagram showing a virus shedding suppression effect of the mouse primary infection prevention test in Example 4.

Fig. 19 is a diagram showing a virus shedding suppression effect of the mouse primary infection prevention test in Example 4.

Fig. 20 is a diagram showing a virus shedding suppression effect of the mouse primary infection prevention test in Example 4.

Fig. 21 is a diagram showing a virus shedding suppression effect of the mouse primary infection prevention test in Example 4.

Fig. 22 is a diagram showing a virus shedding suppression effect of the mouse primary infection prevention test in Example 4.

Fig. 23 is a diagram showing a virus shedding suppression effect of the mouse primary infection prevention test in Example 4.

Fig. 24 is a diagram showing a virus shedding suppression effect of the mouse primary infection prevention test in Example 4.

Fig. 25 is a diagram showing a virus shedding suppression effect of the mouse primary infection prevention test in Example 4.

Fig. 26 is a diagram showing a virus shedding suppression effect of the mouse primary infection prevention test in Example 4.

Fig. 27 is a diagram showing a virus shedding

suppression effect of the mouse primary infection prevention test in Example 4.

Fig. 28 is a diagram showing a virus shedding suppression effect of the mouse primary infection prevention test in Example 4.

Fig. 29 is a diagram showing a virus shedding suppression effect of the mouse primary infection prevention test in Example 4.

Fig. 30 is a diagram showing a virus shedding suppression effect of the mouse primary infection prevention test in Example 4.

Fig. 31 is a diagram showing a virus shedding suppression effect of the mouse primary infection prevention test in Example 4.

Fig. 32 is a diagram showing a virus shedding suppression effect of the mouse primary infection prevention test in Example 4.

Fig. 33 is a diagram showing a pathological score in the mouse primary infection prevention test in Example 4.

Fig. 34 is a diagram showing a pathological score in the mouse primary infection prevention test in Example 4.

Fig. 35 is a diagram showing a pathological score in the mouse primary infection prevention test in Example 4.

Fig. 36 is a diagram showing a pathological score in the mouse primary infection prevention test in Example 4.

Fig. 37 is a diagram showing a pathological score in the mouse primary infection prevention test in Example 4.

Fig. 38 is a diagram showing a pathological score in the mouse primary infection prevention test in Example 4.

5 Fig. 39 is a diagram showing a pathological score in the mouse primary infection prevention test in Example 4.

Fig. 40 is a diagram showing a pathological score in the mouse primary infection prevention test in Example 4.

10 Fig. 41 is a diagram showing a pathological score in the mouse primary infection prevention test in Example 4.

Fig. 42 is a diagram showing a pathological score in the mouse primary infection prevention test in Example 4.

Fig. 43 is a diagram showing a pathological score in the mouse primary infection prevention test in Example 4.

15 Fig. 44 is a diagram showing a pathological score in the mouse primary infection prevention test in Example 4.

Fig. 45 is a diagram showing a pathological score in the mouse primary infection prevention test in Example 4.

20 Fig. 46 is a diagram showing a pathological score in the mouse primary infection prevention test in Example 4.

Fig. 47 is a diagram showing a pathological score in the mouse primary infection prevention test in Example 4.

Fig. 48 is a diagram showing a pathological score in the mouse primary infection prevention test in Example 4.

25 Fig. 49 is a diagram showing a pathological score

in the mouse primary infection prevention test in Example 4.

Fig. 50 is a diagram showing a pathological score
in the mouse primary infection prevention test in Example 4.

Fig. 51 is a diagram showing a pathological score
5 in the mouse primary infection prevention test in Example 4.

Fig. 52 is a diagram showing a pathological score
in the mouse primary infection prevention test in Example 4.

Fig. 53 is a diagram showing a pathological score
in the mouse primary infection prevention test in Example 4.

10 Fig. 54 is a diagram showing a pathological score
in the mouse primary infection prevention test in Example 4.

Fig. 55 is a diagram showing a pathological score
in the mouse primary infection prevention test in Example 4.

15 Fig. 56 is a diagram showing a pathological score
in the mouse primary infection prevention test in Example 4.

Fig. 57 is a diagram showing a pathological score
in the mouse primary infection prevention test in Example 4.

Fig. 58 is a diagram showing a pathological score
in the mouse primary infection prevention test in Example 4.

20 Fig. 59 is a diagram showing a pathological score
in the mouse primary infection prevention test in Example 4.

Fig. 60 is a diagram showing a pathological score
in the mouse primary infection prevention test in Example 4.

25 Fig. 61 is a diagram showing a pathological score
in the mouse primary infection prevention test in Example 4.

Fig. 62 is a schematic view of a mouse intracerebral infection test in Example 5.

Fig. 63 is a diagram showing a survival rate in the mouse intracerebral infection test in Example 5.

5 Fig. 64 is a diagram showing a survival rate in the mouse intracerebral infection test in Example 5.

Fig. 65 is a diagram showing a survival rate in the mouse intracerebral infection test in Example 5.

10 Fig. 66 is a diagram showing a survival rate in the mouse intracerebral infection test in Example 5.

Fig. 67 is a diagram showing a survival rate in the mouse intracerebral infection test in Example 5.

Fig. 68 is a diagram showing a survival rate in the mouse intracerebral infection test in Example 5.

15 Fig. 69 is a diagram showing a survival rate in the mouse intracerebral infection test in Example 5.

Fig. 70 is a diagram showing a survival rate in the mouse intracerebral infection test in Example 5.

20 Fig. 71 is a diagram showing a survival rate in the mouse intracerebral infection test in Example 5.

Fig. 72 is a diagram showing a survival rate in the mouse intracerebral infection test in Example 5.

Fig. 73 is a schematic view of a mouse nasal infection test in Example 6.

25 Fig. 74 is a diagram showing a survival rate in the

mouse nasal infection test in Example 6.

Fig. 75 is a diagram showing a survival rate in the mouse nasal infection test in Example 6.

5 Fig. 76 is a diagram showing a survival rate in the mouse nasal infection test in Example 6.

Fig. 77 is a diagram showing a survival rate in the mouse nasal infection test in Example 6.

Fig. 78 is a diagram showing a survival rate in the mouse nasal infection test in Example 6.

10 Fig. 79 is a schematic view of a guinea pig recurrence suppression test in Example 7.

Fig. 80 is a diagram showing a recurrence suppression effect of the guinea pig recurrence suppression test in Example 7.

15 Fig. 81 is a diagram showing a recurrence suppression effect of the guinea pig recurrence suppression test in Example 7.

20 Fig. 82 is a diagram showing a recurrence suppression effect of the guinea pig recurrence suppression test in Example 7.

Fig. 83 is a diagram showing a recurrence suppression effect of the guinea pig recurrence suppression test in Example 7.

[0023]

In the present specification, "A to B" means not less than A and not more than B unless otherwise specified.

[0024]

5 [Multiple mutant virus]

A multiple mutant virus in accordance with an aspect of the present invention (hereinafter sometimes referred to as "recombinant live attenuated herpes simplex virus type 2") is a virus in which two or more genes of herpes simplex virus
10 type 2 (HSV-2) are modified.

[0025]

In the present specification, a modification of a gene refers to a loss or reduction in gene function. Examples of a loss or reduction in gene function include: a loss or reduction
15 caused by the absence of expression of a protein encoded by a target gene, due to a gene deletion, a start codon substitution, or introduction of a stop codon directly below the start codon; and a loss or reduction in gene function caused by an amino acid substitution due to a change in base
20 sequence of a gene (e.g., a deletion, substitution, or insertion of one to several tens of bases in a coding region).

[0026]

HSV-2 may be a laboratory strain or a clinical isolate.

[0027]

25 Recombinant live attenuated herpes simplex virus type

2 is preferably configured such that a gene related to immunogenicity or immune evasion is modified in terms of high immune inducibility and high safety. Examples of the gene related to immunogenicity or immune evasion include

5 RL1 (Non-patent Literatures 44 and 45), RL2 (Non-patent Literatures 46 and 47), UL2 (Non-patent literature 48), UL3, UL4 (Non-patent literature 49), UL12 (Non-patent literature 50), UL12.5, UL13 (Non-patent literature 51), UL24 (Non-patent literature 52), UL27 (Non-patent Literatures 13 and 35

10 of a point mutant), UL35 (Non-patent literature 33 of a deletion variant and a point mutant), UL41 (Non-patent literature 53 of a deletion variant, Non-patent literature 54 reviewing a function, Non-patent Literature 55 of a point mutant), UL43, UL44 (Non-patent Literatures 56 and 57),

15 UL45 (Non-patent literature 58), UL46 (Non-patent Literatures 59 and 60), UL50 (Non-patent literature 61 reporting a function and Non-patent literature 62 of a point mutant), UL56 (Non-patent Literatures 63 and 64), Us2 (Non-patent literature 65), Us3 (Non-patent literature 66 of a deletion

20 variant and Non-patent literatures 12, 13, 14, 15, and 18 of a point mutant), Us4 (Non-patent Literatures 67 and 68), Us5 (Non-patent Literatures 69 and 70), Us8A (Non-patent literature 71), Us9 (Non-patent literature 72), Us11 (Non-patent Literatures 73 and 74), and Us12 (Non-patent

25 Literatures 75 to 77).

[0028]

The recombinant live attenuated herpes simplex virus type 2 is preferably configured such that two or more genes including Us3 are modified in terms of high immune inducibility and high safety. The recombinant live attenuated herpes simplex virus type 2 is more preferably configured such that at least Us3 and UL2, Us3 and UL3, Us3 and UL4, Us3 and UL13, Us3 and UL27, Us3 and UL35, Us3 and UL41, Us3 and UL44, Us3 and UL45, Us3 and UL46, Us3 and UL50, Us3 and UL56, Us3 and Us4, Us3 and Us5, Us3 and Us8A, Us3 and Us9, or Us3 and Us11 are modified.

[0029]

The recombinant live attenuated herpes simplex virus type 2 is preferably configured such that two or more genes including UL41 are modified in terms of high immune inducibility and high safety. The recombinant live attenuated herpes simplex virus type 2 is more preferably configured such that at least UL41 and UL2, UL41 and UL3, UL41 and UL4, UL41 and UL12, UL41 and UL12.5, UL41 and UL24, UL41 and UL27, UL41 and ICP34.5, UL41 and UL35, UL41 and UL43, UL41 and UL56, UL41 and Us2, UL41 and Us4, UL41 and Us5, UL41 and Us8A, or UL41 and Us9 are modified.

[0030]

Us3 is a gene encoding a protein kinase Us3. Examples of an amino acid sequence of a protein encoded by Us3 include

an amino acid sequence of SEQ ID NO: 1 (NCBI Reference Sequence: NC_001798.2).

[0031]

In terms of high immune inducibility and high safety, a
5 modification of Us3 is preferably a modification of a lysine
residue (K220) at position 220 in the amino acid sequence of
SEQ ID NO: 1.

[0032]

In the present specification, a modification of an amino
10 acid residue refers to a deletion or substitution of an amino
acid residue. Examples of the modification of K220 in SEQ ID
NO: 1 include a substitution of a lysine residue with a
methionine residue.

[0033]

15 RL1 is a gene encoding ICP34.5 (another name: γ 34.5).
Examples of an amino acid sequence of a protein encoded by
RL1 include an amino acid sequence registered as NCBI
Reference Sequence: NC_001798.2. Modified ICP34.5 is
preferably an expression deletion mutant of ICP34.5 in terms
20 of high immune inducibility and high safety.

[0034]

UL2 is a gene encoding an uracil-DNA glycosidase UL2.
Examples of an amino acid sequence of a protein encoded by
UL2 include an amino acid sequence registered as NCBI
25 Reference Sequence: NC_001798.2. Modified UL2 is preferably

an expression deletion mutant of UL2 in terms of high immune inducibility and high safety. In the present specification, the term "expression deletion mutant" refers to a mutant in which a protein encoded by each gene is not expressed by, for example, a gene deletion, a start codon substitution, or introduction of a stop codon directly below the start codon.

[0035]

UL3 is a gene encoding a phosphoprotein UL3 present mainly in a nucleus. Examples of an amino acid sequence of a protein encoded by UL3 include an amino acid sequence registered as NCBI Reference Sequence: NC_001798.2. Modified UL3 is preferably an expression deletion mutant of UL3 in terms of high immune inducibility and high safety.

[0036]

UL4 is a gene encoding a viral structural protein UL4 distributed in a nucleus. Examples of an amino acid sequence of a protein encoded by UL4 include an amino acid sequence registered as NCBI Reference Sequence: NC_001798.2. Modified UL4 is preferably an expression deletion mutant of UL4 in terms of high immune inducibility and high safety.

[0037]

UL12 is a gene encoding an alkaline nuclease UL12. Examples of an amino acid sequence of a protein encoded by UL12 include an amino acid sequence of SEQ ID NO: 2 (NCBI Reference Sequence: NC_001798.2). In terms of high immune

inducibility and high safety, a modification of UL12 is preferably a modification of a tyrosine residue (Y361) at position 361 in the amino acid sequence of SEQ ID NO: 2. Examples of the modification of Y361 in SEQ ID NO: 2 include
5 a substitution of a tyrosine residue with a phenylalanine residue.

[0038]

UL12.5 is a gene encoding an alkaline nuclease UL12.5. Examples of an amino acid sequence of a protein encoded by
10 UL12.5 include an amino acid sequence registered as NCBI Reference Sequence: NC_001798.2. Modified UL12.5 is preferably an expression deletion mutant of UL12.5 in terms of high immune inducibility and high safety.

[0039]

15 UL13 is a gene encoding a protein kinase UL13. Examples of an amino acid sequence of a protein encoded by UL13 include an amino acid sequence of SEQ ID NO: 3 (NCBI Reference Sequence: NC_001798.2). In terms of high immune inducibility and high safety, a modification of UL13 is
20 preferably a modification of a lysine residue (K176) at position 176 in the amino acid sequence of SEQ ID NO: 3. Examples of the modification of K176 in SEQ ID NO: 3 include a substitution of a lysine residue with a methionine residue.

[0040]

25 UL24 is a gene encoding a viral structural protein UL24

present in a membrane. Examples of an amino acid sequence of a protein encoded by UL24 include an amino acid sequence registered as NCBI Reference Sequence: NC_001798.2. Modified UL24 is preferably an expression deletion mutant of UL24 in terms of high immune inducibility and high safety.

[0041]

UL27 is a gene encoding a glycoprotein gB. Examples of an amino acid sequence of a protein encoded by UL27 include an amino acid sequence of SEQ ID NO: 4 (NCBI Reference Sequence: NC_001798.2). In terms of high immune inducibility and high safety, a modification of UL27 is preferably a modification of a tyrosine residue (Y889) at position 889 in the amino acid sequence of SEQ ID NO: 4. Examples of the modification of Y889 in SEQ ID NO: 4 include a substitution of a tyrosine residue with an alanine residue.

[0042]

UL35 is a gene encoding a capsid protein V26. Examples of an amino acid sequence of a protein encoded by UL35 include an amino acid sequence of SEQ ID NO: 5 (NCBI Reference Sequence: NC_001798.2). In terms of high immune inducibility and high safety, a modification of UL35 is preferably a modification of a threonine residue (T111) at position 111 in the amino acid sequence of SEQ ID NO: 5. Examples of the modification of T111 in SEQ ID NO: 5 include a substitution of a threonine residue with an alanine residue.

[0043]

UL41 is a gene encoding a ribonuclease (virion host shut-off (VHS)). Examples of an amino acid sequence of a protein encoded by UL41 include an amino acid sequence of
5 SEQ ID NO: 6 (NCBI Reference Sequence: NC_001798.2). In terms of high immune inducibility and high safety, a modification of UL41 is preferably a modification of an aspartic acid residue (D215) at position 215 in the amino acid sequence of SEQ ID NO: 6. Examples of the modification of
10 D215 in SEQ ID NO: 6 include a substitution of an aspartic acid residue with an asparagine residue.

[0044]

UL43 is a gene encoding a membrane protein UL43. Examples of an amino acid sequence of a protein encoded by
15 UL43 include an amino acid sequence registered as NCBI Reference Sequence: NC_001798.2. Modified UL43 is preferably an expression deletion mutant of UL43 in terms of high immune inducibility and high safety.

[0045]

20 UL44 is a gene encoding a glycoprotein gC. Examples of an amino acid sequence of a protein encoded by UL44 include an amino acid sequence registered as NCBI Reference Sequence: NC_001798.2. Modified UL44 is preferably an expression deletion mutant of UL44 in terms of high immune
25 inducibility and high safety.

[0046]

UL45 is a gene encoding a membrane protein UL45. Examples of an amino acid sequence of a protein encoded by UL45 include an amino acid sequence registered as NCBI Reference Sequence: NC_001798.2. Modified UL45 is preferably an expression deletion mutant of UL45 in terms of high immune inducibility and high safety.

[0047]

UL46 is a gene encoding VP11/12. Examples of an amino acid sequence of a protein encoded by UL46 include an amino acid sequence registered as NCBI Reference Sequence: NC_001798.2. Modified UL46 is preferably an expression deletion mutant of UL46 in terms of high immune inducibility and high safety.

[0048]

UL50 is a gene encoding vdUTPase serving as a homolog of a host nucleic acid metabolic enzyme dUTPase. Examples of an amino acid sequence of a protein encoded by UL50 include an amino acid sequence of SEQ ID NO: 7 (NCBI Reference Sequence: NC_001798.2). In terms of high immune inducibility and high safety, a modification of UL50 is preferably a modification of an aspartic acid residue (D97) at position 97 in the amino acid sequence of SEQ ID NO: 7. Examples of the modification of D97 in SEQ ID NO: 7 include a substitution of an aspartic acid residue with an alanine

residue.

[0049]

UL56 is a gene encoding a membrane protein UL56. Examples of an amino acid sequence of a protein encoded by
5 UL56 include an amino acid sequence registered as NCBI Reference Sequence: NC_001798.2. Modified UL56 is preferably an expression deletion mutant of UL56 in terms of high immune inducibility and high safety.

[0050]

10 Us2 is a gene encoding a tegument protein Us2. Examples of an amino acid sequence of a protein encoded by Us2 include an amino acid sequence registered as NCBI Reference Sequence: NC_001798.2. Modified Us2 is preferably an expression deletion mutant of Us2 in terms of high immune
15 inducibility and high safety.

[0051]

Us4 is a gene encoding a glycoprotein gG. Examples of an amino acid sequence of a protein encoded by Us4 include an amino acid sequence registered as NCBI Reference
20 Sequence: NC_001798.2. Modified Us4 is preferably an expression deletion mutant of Us4 in terms of high immune inducibility and high safety.

[0052]

Us5 is a gene encoding a glycoprotein gJ. Examples of
25 an amino acid sequence of a protein encoded by Us5 include

an amino acid sequence registered as NCBI Reference Sequence: NC_001798.2. Modified Us5 is preferably an expression deletion mutant of Us5 in terms of high immune inducibility and high safety.

5 [0053]

Us8A is a gene predicted to encode a membrane protein Us8A. Examples of an amino acid sequence of a protein encoded by Us8A include an amino acid sequence registered as NCBI Reference Sequence: NC_001798.2. Modified Us8A is preferably an expression deletion mutant of Us8A in terms of high immune inducibility and high safety.

[0054]

Us9 is a gene encoding a tegument protein Us9. Examples of an amino acid sequence of a protein encoded by Us9 include an amino acid sequence registered as NCBI Reference Sequence: NC_001798.2. Modified Us9 is preferably an expression deletion mutant of Us9 in terms of high immune inducibility and high safety.

[0055]

20 Us11 is a gene encoding a tegument protein Us11. Examples of an amino acid sequence of a protein encoded by Us11 include an amino acid sequence registered as NCBI Reference Sequence: NC_001798.2. Modified Us11 is preferably an expression deletion mutant of Us11 in terms of high immune inducibility and high safety.

25

[0056]

The recombinant live attenuated herpes simplex virus type 2 can be obtained by a known genetic modification method such as a genetic modification method carried out by using homologous recombination in cultured cells, a genetic modification method using a cosmid, or a genetic modification method using a bacterial artificial chromosome (BAC) system.

[0057]

[HSV-2 vaccine]

An HSV-2 vaccine in accordance with an aspect of the present invention is a vaccine containing the recombinant live attenuated herpes simplex virus type 2 as an antigen. The HSV-2 vaccine can be used for treatment or prevention of an HSV-2 infection. In the present specification, "treatment or prevention of an HSV-2 infection" includes, for example, alleviation or prevention of aggravation of one or more symptoms related to HSV-2 infection, suppression of onset of symptoms after HSV-2 infection, prevention, delay, or stopping of infection of cells with HSV-2 in vivo, and a reduction in number of HSV-2 in vivo.

[0058]

Examples of a symptom related to HSV-2 infection include: herpes labialis; corneal herpes; genital herpes; neonatal systemic herpes; stomatitis, skin disease, encephalitis, meningitis, and myelitis each caused by HSV-2;

and neurodegenerative diseases that may be caused by HSV-2.

[0059]

5 The HSV-2 vaccine in accordance with an aspect of the present invention is superior in safety or effectiveness to a vaccine containing, as an antigen, HSV-2 in which only Us3 is modified. The HSV-2 vaccine in accordance with an aspect of the present invention is superior in safety or effectiveness to a vaccine containing, as an antigen, HSV-2 in which only
10 UL41 is modified.

[0060]

The HSV-2 vaccine in accordance with an aspect of the present invention may contain one type or two or more types of the recombinant live attenuated herpes simplex virus type
15 2.

[0061]

The HSV-2 vaccine in accordance with an aspect of the present invention may contain a pharmaceutically acceptable carrier in accordance with a purpose, use, administration
20 route, and/or the like. The carrier can be a carrier that is normally used in production of a vaccine. Examples of the carrier include an adjuvant, a solvent, a thickener, a binder, a colorant, a stabilizer, a pH adjuster, a diluent, an excipient, a buffer, a tonicity agent, a soothing agent, a preservative,
25 and an antioxidant.

[0062]

Examples of a dosage form of the HSV-2 vaccine in accordance with an aspect of the present invention include powder formulations such as a freeze-dried formulation and a vacuum-dried formulation, liquid formulations, a capsule, an injection, an aerosolized agent, a spraying agent, a suppository, and a nasal spray. In terms of performance as a vaccine dosage form and a mechanism by which an effect is exhibited, a preferable dosage form of the HSV-2 vaccine in accordance with an aspect of the present invention is an injection, an aerosolized agent, or a nasal spray.

[0063]

The content of the recombinant live attenuated herpes simplex virus type 2 contained in the HSV-2 vaccine in accordance with an aspect of the present invention can be determined, as appropriate, by, for example, a type, age, body weight, a state, and a duration of treatment of an administration subject.

[0064]

A dose of the recombinant live attenuated herpes simplex virus type 2 contained in the HSV-2 vaccine in accordance with an aspect of the present invention is preferably 10^3 pfu to 10^9 pfu, more preferably 10^4 pfu to 10^8 pfu, and even more preferably 10^6 pfu to 10^7 pfu, in terms of safety and effectiveness.

[0065]

(HSV-2 vaccine administration method/administration route)

An administration method (administration route) for the HSV-2 vaccine in accordance with an aspect of the present invention can be determined, as appropriate, by, for example, age, a state, and a duration of treatment of an administration subject. Specifically, the administration route may be either oral administration or parenteral administration, but is preferably parenteral administration. Examples of a route of parenteral administration include intravenous administration, subcutaneous administration, intradermal administration, intramuscular administration, nasal administration, dermal administration, rectal administration, respiratory tract administration, vaginal administration, and ophthalmic administration. In terms of performance as a vaccine dosage form and a mechanism by which an effect is exhibited, a preferable administration route for the HSV-2 vaccine in accordance with an aspect of the present invention is nasal administration or intramuscular injection (intramuscular administration). A vaccine for nasal administration is administered via the nasal mucosa by, for example, spraying, application, or instillation of a vaccine into the nasal mucosa.

[0066]

A subject of administration of the HSV-2 vaccine in accordance with an aspect of the present invention is a subject that is infected with an HSV-2 virus or that is potentially infected with an HSV-2 virus. Examples of the
5 subject of administration of the HSV-2 vaccine include mammals, birds, reptiles, and amphibians, and among these, mammals are preferable. Examples of the mammals include humans and non-human animals. Examples of the non-human animals include domestic animals such as cattle, horses, pigs,
10 and sheep, and pet animals or laboratory animals such as dogs, cats, rats, mice, hamsters, monkeys, and rabbits. Preferable examples of the subject of administration of the HSV-2 vaccine include humans. Examples of the birds include poultry such as chickens, wild ducks, and domestic ducks.

15 [0067]

The number of times of administration and a timing of administration of the HSV-2 vaccine in accordance with an aspect of the present invention can be determined, as appropriate, by, for example, a type, a type, age, body weight,
20 and a state of an administration subject. In terms of effectiveness and safety, the HSV-2 vaccine is administered preferably once to five times, more preferably once to three times, and even more preferably twice.

[0068]

25 A method for treating or preventing an HSV-2 infection,

including a step of administering the HSV-2 vaccine in accordance with an aspect of the present invention to a subject is also encompassed in an aspect of the present invention.

5 [0069]

No vaccine that has both safety and effectiveness has been developed to date, and past knowledge suggests importance of a live attenuated vaccine that makes it possible to strongly induce humoral immunity and cellular immunity.

10 The present invention is innovative in that using a gene recombination technology has made it possible to develop a live attenuated vaccine which has high safety and high effectiveness.

[0070]

15 [Use as vaccine vector]

Recombinant live attenuated herpes simplex virus type 2 in accordance with an aspect of the present invention can be used also as a vaccine vector against another pathogen other than HSV-2. Examples of the another pathogen include an influenza virus and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

20 [0071]

Aspects of the present invention can also be expressed as follows:

25 A virus in accordance with Aspect 1 of the present

invention is a multiple mutant virus in which: two or more genes of herpes simplex virus type 2 (HSV-2) are modified; and a modification of the two or more genes involves a loss or reduction in gene function.

5 [0072]

In Aspect 2 of the present invention, a virus may be configured that, in Aspect 1 of the present invention, the modification of the two or more genes is a modification of two or more genes selected from genes related to immunogenicity or immune evasion.

10 [0073]

In Aspect 3 of the present invention, a virus may be configured that, in Aspect 2 of the present invention, the genes related to immunogenicity or immune evasion are genes selected from the group consisting of Us3, UL2, UL3, UL4, UL12, UL12.5, UL13, UL24, UL27, ICP34.5, UL35, UL41, UL43, UL44, UL45, UL46, UL50, UL56, Us2, Us4, Us5, Us8A, Us9, and Us11.

15 [0074]

20 In Aspect 4 of the present invention, a virus may be configured such that, in Aspect 2 or 3 of the present invention, the genes related to immunogenicity or immune evasion are two or more genes including Us3.

[0075]

25 In Aspect 5 of the present invention, a virus may be

configured such that, in any one of Aspects 2 to 4 of the present invention, the genes related to immunogenicity or immune evasion are two or more genes including UL41.

[0076]

5 In Aspect 6 of the present invention, a virus may be configured such that, in Aspect 4 of the present invention, the genes related to immunogenicity or immune evasion include a combination of genes selected from the group consisting of Us3 and UL2, Us3 and UL3, Us3 and UL4, Us3 and UL13, Us3
10 and UL27, Us3 and UL35, Us3 and UL41, Us3 and UL44, Us3 and UL45, Us3 and UL46, Us3 and UL50, Us3 and UL56, Us3 and Us4, Us3 and Us5, Us3 and Us8A, Us3 and Us9, and Us3 and Us11.

[0077]

15 In Aspect 7 of the present invention, a virus may be configured such that, in Aspect 5 of the present invention, the genes related to immunogenicity or immune evasion include a combination of genes selected from the group consisting of UL41 and UL2, UL41 and UL3, UL41 and UL4, UL41 and UL12,
20 UL41 and UL12.5, UL41 and UL24, UL41 and UL27, UL41 and ICP34.5, UL41 and UL35, UL41 and UL43, UL41 and UL56, UL41 and Us2, UL41 and Us4, UL41 and Us5, UL41 and Us8A, and UL41 and Us9.

[0078]

25 A vaccine in accordance with Aspect 8 of the present

invention is a herpes simplex virus type 2 (HSV-2) vaccine containing, as an antigen, a virus of any one of Aspects 1 to 7 of the present invention.

[0079]

5 In Aspect 9 of the present invention, a vaccine is configured such that, in Aspect 8 of the present invention, the vaccine is superior in safety or effectiveness to a vaccine containing, as an antigen, HSV-2 in which only Us3 is modified.

10 [0080]

 In Aspect 10 of the present invention, a vaccine is configured such that, in Aspect 8 or 9 of the present invention, the vaccine is superior in safety or effectiveness to a vaccine containing, as an antigen, HSV-2 in which only UL41
15 is modified.

[0081]

 In Aspect 11 of the present invention, a vaccine may be configured such that, in any one of Aspects 8 to 10 of the present invention, the vaccine is used for intramuscular
20 injection or nasal administration.

[0082]

 In Aspect 12 of the present invention, a vaccine may be configured such that, in any one of Aspects 8 to 11 of the present invention, the vaccine is administered twice to a
25 subject.

[0083]

In Aspect 13 of the present invention, a vaccine may be configured such that, in any one of Aspects 8 to 12 of the present invention, a dose of the virus is 10^6 pfu to 10^7 pfu.

5 [0084]

Use in accordance with Aspect 14 of the present invention is use of a virus of any one of Aspects 1 to 7 of the present invention as a vaccine vector against a pathogen other than herpes simplex virus type 2 (HSV-2).

10 [0085]

In Aspect 15 of the present invention, use may be configured such that, in Aspect 14 of the present invention, the pathogen is an influenza virus or SARS-CoV-2.

[0086]

15 The following description will more specifically discuss an embodiment of the present invention with reference to Examples. It is a matter of course that the present invention is not limited to the Examples below and that details of the present invention can have various aspects. Further, the
20 present invention is not limited to the embodiments, but can be altered by a skilled person in the art within the scope of the claims. An embodiment derived from a proper combination of technical means disclosed in respective different embodiments is also encompassed in the technical scope of
25 the present invention. Furthermore, all the documents listed

in the present specification are incorporated herein by reference.

Examples

[0087]

5 [Example 1]

Gene recombination in *E. coli*

A recombinant cassette having a kanamycin resistance gene was introduced, by an electroporation method, into HSV-BACmid-carrying *E. coli* (carrying a chloramphenicol resistance gene) including a gene sequence of strain 186, which is an HSV-2 wild-type virus. In 1 mL of an LB medium, shaking culture was carried out at 200 rpm at 32°C for 30 minutes, and bacteria were applied to a chloramphenicol and kanamycin-containing LB agar medium and were cultured overnight at 32°C.

[0088]

Grown clones were used to carry out PCR, and a clone with a desired sequence introduced therein was selected. The selected clone was subjected to shaking culture overnight at 32°C and 200 rpm in 1 mL of an LB medium. 100 µL of a culture solution was added to 2 mL of a chloramphenicol-containing LB medium to carry out shaking culture at 32°C and 200 rpm for 2.5 hours. 400 µL of 10% arabinose was added to carry out shaking culture at 32°C and 200 rpm for 1 hour. Shaking culture was carried out in a warm bath at 42°C

and 50 rpm for 30 minutes, and shaking culture was carried out at 32°C and 200 rpm for 1.5 hours. 10 µL of the culture solution was diluted in 1 mL of ultrapure water, and 10 µL of a diluent was diluted in 1 mL of ultrapure water. After that,
5 150 µL of a diluent was applied to a chloramphenicol-containing LB agar medium and cultured overnight at 32°C. The grown clones were streaked into the chloramphenicol-containing LB agar medium and the chloramphenicol and kanamycin-containing LB agar medium and cultured
10 overnight at 32°C. A clone growing only in the chloramphenicol-containing LB medium was selected by PCR.
[0089]

[Example 2]

Purification of BACmid

15 A selected clone was inoculated into 200 mL of a chloramphenicol-containing LB medium and cultured overnight at 32°C and 200 rpm. Centrifugation was carried out at 4°C and 7,000 rpm for 5 minutes to remove a supernatant. A pellet was suspended in 5 mL of Sol I (0.9% D-glucose, 25 mM Tris-HCl (pH 8.0), and 10 mM EDTA), and 10
20 mL of Sol II (0.1% SDS and 0.2 N NaOH) was added. After inversion mixing, incubation was carried out at room temperature for 10 minutes. 7.5 mL of Sol III (29.43% potassium acetate and 11.5% glacial acetic acid) was added to
25 carry out inversion mixing. Centrifugation was carried out at

4°C and 15,000 rpm for 20 minutes to filter a supernatant. A 0.6-fold amount of 2-propanol was added to carry out inversion mixing. Centrifugation was carried out at 4°C and 5,000 rpm for 5 minutes to remove a supernatant. A pellet was
5 suspended in 4 mL of Tris-EDTA (TE), and 5.4 g of cesium chloride was added. 180 µL of a 10 mg/mL ethidium bromide solution was added to carry out ultracentrifugation overnight at 20°C and 70,000 rpm.

[0090]

10 Among bands produced by ultracentrifugation, a desired band was harvested with a 1-mL syringe with an 18-G needle, and an equal amount of 99.5% ethanol was added. After inversion mixing, centrifugation was carried out at 4°C and 15,000 rpm for 2 minutes to remove a supernatant. A
15 pellet was suspended in 400 µL of TE, and 500 µL of a 1:1 mixed solution of phenol and chloroform was added. After inversion mixing, centrifugation was carried out at 4°C and 15,000 rpm for 3 minutes. An aqueous layer was transferred to a new tube to carry out a similar process, and the aqueous
20 layer was transferred to a new tube. An equal amount of diethyl ether was added to carry out inversion mixing. After that, centrifugation was carried out at 4°C and 15,000 rpm for 3 minutes. An ether layer was removed, and a similar process was carried out three times. The ether layer was
25 removed, and 40 µL of 3M sodium acetate (pH 5.2) and 1 mL

of 99.5% ethanol were added to carry out inversion mixing. Centrifugation was carried out at 4°C and 15,000 rpm for 10 minutes to remove a supernatant. 1 mL of 70% ethanol was added to carry out inversion mixing. After that, centrifugation
5 was carried out at 4°C and 15,000 rpm for 2 minutes to remove a supernatant. Centrifugation was carried out again under the same condition to remove a supernatant.

[0091]

[Example 3]

10 Virus reconstruction in rabbit skin cell

Suspension in 250 µL sterile ultrapure water was carried out, and 35 µL of a 2.2 M calcium chloride solution was added to carry out mixing by tapping. 250 µL of 2× HBS (1.6% sodium chloride, 0.074% potassium chloride, 0.027%
15 sodium hydrogen phosphate dihydrate, 0.2% dextrose, and 1% HEPES (pH 7.05)) was added to carry out mixing by tapping (a DNA solution). Ten minutes later, a medium was removed from rabbit skin cells cultured in a T-25 flask, and washing was carried out with 5 mL of PBS. 5 mL of 0.04% DEAE-dextran
20 was added to carry out incubation at 37°C under 5% CO₂ for 10 minutes. The DEAE-dextran was removed, and 5 mL of TEN (0.2 M sodium chloride, 20 mM Tris-HCl (pH8.0), and 2 mM EDTA) was added and removed. 5 mL of a 5% FCS-containing DMEM medium was added, and the DNA solution was added.
25 Four hours later, the medium was removed, and 5 mL of the

5% FCS-containing DMEM medium was added and removed. 5 mL of 5% FCS-containing DMEM was added to carry out incubation overnight at 37°C under 5% CO₂. The medium was removed, and 5 mL of a 199 V medium was added to carry out incubation at 37°C under 5% CO₂.

[0092]

As an indicator of virus production, it was observed whether there was any plaque. Upon successful confirmation of virus production, Vero cells were infected with a virus at an appropriate timing and subjected to expanded culture. A viral titer was measured by a plaque assay. A "recombinant live attenuated vaccine" (hereinafter referred to as an "HSV-2 vaccine") containing a resulting virus as an antigen was produced and used for an experiment.

[0093]

[Example 4]

Mouse primary infection prevention test

Mice were nasally inoculated with 1×10^4 pfu of an HSV-2 vaccine. Three weeks later, 8.35 mg/mL of Depo-Gestin was subcutaneously administered. One week later, 1×10^3 pfu of HSV-2 wild-type strain 186 was vaginally challenged. A vaginal lesion score was evaluated for 2 weeks, and a vaginal douche was collected to measure a viral titer by a plaque assay. The vaginal lesion score was evaluated as follows: 0: no symptom; 1: slight genital erythema and/or edema; 2:

genital alopecia, erythema, and/or edema; 3: marked genital alopecia, erythema, and/or edema; 4: hind-limb paralysis; and 5: death.

[0094]

5 Fig. 1 shows a schematic view of a mouse primary
infection prevention test. The HSV-2 vaccine containing the
following double mutant virus as an antigen further
suppressed virus shedding than a Δ Tk-inoculated group
inoculated with Δ Tk, which is a traditional vaccine prototype
10 live attenuated virus having the same origin as that of the
HSV-2 vaccine (Figs. 2 to 32). In the diagrams, "Us3KM"
means a virus in which a lysine at position 220 in Us3 is
substituted with a methionine, "UL13KM" means a virus in
which a lysine at position 173 in UL13 is substituted with a
15 methionine, "UL27YA" means a virus in which a tyrosine at
position 889 in UL27 is substituted with an alanine, "UL35TA"
means a virus in which a threonine at position 111 in UL35 is
substituted with an alanine, "UL41DN" means a virus in which
an aspartic acid at position 215 in UL41 is substituted with
20 an asparagine, "UL50DA" means a virus in which an aspartic
acid at position 97 in UL50 is substituted with an alanine,
and "UL12YF" means a virus in which a tyrosine at position
376 in UL12 is substituted with a phenylalanine. A notation
with Δ , such as " Δ UL2" means a virus in which such a gene is
25 deleted and in which a protein encoded by the gene is not

expressed.

*Double mutant virus:

Us3/UL2, Us3/UL3, Us3/UL4, Us3/UL13, Us3/UL27,
Us3/UL35, Us3/UL41, Us3/UL44, Us3/UL45, Us3/UL46,
5 Us3/UL50, Us3/UL56, Us3/Us4, Us3/Us8A, Us3/Us9,
Us3/Us11, UL41/UL2, UL41/UL3, UL41/UL4, UL41/UL12,
UL41/UL12.5, UL41/UL24, UL41/UL27, UL41/ICP34.5,
UL41/UL35, UL41/UL43, UL41/UL56, UL41/Us2, UL41/Us4,
UL41/Us5, UL41/Us8A, UL41/Us9

10 [0095]

The HSV-2 vaccine containing the following double
mutant virus as an antigen had significantly reduced
symptoms of a vaginal lesion as compared with the Δ Tk-
inoculated group and exhibited a favorable onset prevention
15 effect (Figs. 33 to 61).

*Double mutant virus: Us3/UL2, Us3/UL3, Us3/UL4,
Us3/UL13, Us3/UL27, Us3/UL35, Us3/UL41, Us3/UL45,
Us3/UL50, Us3/UL56, Us3/Us4, Us3/Us8A, Us3/Us9,
Us3/Us11, UL41/UL2, UL41/UL3, UL41/UL4, UL41/UL12,
20 UL41/UL12.5, UL41/UL24, UL41/UL27, UL41/ICP34.5,
UL41/UL35, UL41/UL43, UL41/UL56, UL41/Us2, UL41/Us4,
UL41/Us5, UL41/Us9

[0096]

[Example 5]

25 Mouse intracerebral infection test

Mice were intracerebrally inoculated with 1×10^4 pfu of an HSV-2 vaccine. A survival rate was observed for 2 weeks.
[0097]

Fig. 62 shows a schematic view of a mouse intracerebral
5 infection test. Survival rates after administration of HSV-2
vaccines containing Us3/UL3, Us3/UL4, Us3/UL27,
Us3/UL35, Us3/UL44, Us3/UL46, Us3/UL56, Us3/Us4,
Us3/Us5, and Us3/Us8A as antigens were higher than that in
a Us3 single mutant live attenuated virus-inoculated group,
10 and attenuation of pathogenicity was determined (Figs. 63 to
72).
[0098]

[Example 6]

Mouse nasal infection test

15 Mice were nasally inoculated with 1×10^5 pfu of an HSV-
2 vaccine. A survival rate was observed for 2 weeks.
[0099]

Fig. 73 shows a schematic view of a mouse nasal
infection test. Survival rates after administration of HSV-2
20 vaccines containing Us3/UL3, Us3/UL56, Us3/Us4,
UL41/UL4, and UL41/Us5 as antigens were higher than that
in a Us3 single mutant live attenuated virus or UL41 single
mutant live attenuated virus-inoculated group, and
attenuation of pathogenicity was determined (Figs. 74 to 78).
25 [0100]

[Example 7]

Guinea pig recurrence suppression test

A guinea pig was vaginally challenged with 1×10^5 pfu of HSV-2 wild-type virus strain MS. Two weeks later, 1×10^6 pfu
5 or 1×10^7 pfu of an HSV-2 vaccine was nasally inoculated or intramuscularly injected (prime vaccination). Two weeks later, the same amount of the HSV-2 vaccine was nasally inoculated or intramuscularly injected (boost vaccination). For up to 7 weeks after the prime vaccination, it was observed whether
10 there was any blister in the vagina. A case where a blister newly appeared in a different location was considered to be a recurrence.

[0101]

Fig. 79 shows a schematic view of a guinea pig
15 recurrence suppression test. By two-time vaccination (prime vaccination and boost vaccination), HSV-2 vaccines containing Us3/UL27, Us3/UL35, Us3/Us4, and UL41/Us5 as antigens exhibited a recurrence prevention effect of 40% or more, and an HSV-2 vaccine containing Us3/UL35 as an
20 antigen exhibited a recurrence prevention effect of 80% or more (Figs. 80 to 83).

Industrial Applicability

[0102]

25 An HSV-2 vaccine of the present invention obtained by

introducing a mutation into two types of genes of herpes simplex virus type 2 is expected to be industrially used as a vaccine that has both high safety and high effectiveness.

Claims

[Claim 1]

A multiple mutant virus wherein:

5 two or more genes of herpes simplex virus type 2 (HSV-2) are modified; and

 a modification of the two or more genes involves a loss or reduction in gene function.

10 [Claim 2]

 The virus as set forth in claim 1, wherein the modification of the two or more genes is a modification of two or more genes selected from genes related to immunogenicity or immune evasion.

15 [Claim 3]

 The virus as set forth in claim 2, wherein the genes related to immunogenicity or immune evasion are genes selected from the group consisting of Us3, UL2, UL3, UL4, UL12, UL12.5, UL13, UL24, UL27, ICP34.5, UL35, UL41, 20 UL43, UL44, UL45, UL46, UL50, UL56, Us2, Us4, Us5, Us8A, Us9, and Us11.

[Claim 4]

25 The virus as set forth in claim 3, wherein the genes

related to immunogenicity or immune evasion are two or more genes including Us3.

[Claim 5]

5 The virus as set forth in claim 3, wherein the genes related to immunogenicity or immune evasion are two or more genes including UL41.

[Claim 6]

10 The virus as set forth in claim 4, wherein the genes related to immunogenicity or immune evasion include a combination of genes selected from the group consisting of Us3 and UL2, Us3 and UL3, Us3 and UL4, Us3 and UL13, Us3 and UL27, Us3 and UL35, Us3 and UL41, Us3 and UL44, Us3
15 and UL45, Us3 and UL46, Us3 and UL50, Us3 and UL56, Us3 and Us4, Us3 and Us5, Us3 and Us8A, Us3 and Us9, and Us3 and Us11.

[Claim 7]

20 The virus as set forth in claim 5, wherein the genes related to immunogenicity or immune evasion include a combination of genes selected from the group consisting of UL41 and UL2, UL41 and UL3, UL41 and UL4, UL41 and UL12, UL41 and UL12.5, UL41 and UL24, UL41 and UL27, UL41 and
25 ICP34.5, UL41 and UL35, UL41 and UL43, UL41 and UL56,

UL41 and Us2, UL41 and Us4, UL41 and Us5, UL41 and Us8A,
and UL41 and Us9.

[Claim 8]

5 A vaccine against herpes simplex virus type 2 (HSV-2),
comprising, as an antigen, a virus recited in any one of claims
1 to 7.

[Claim 9]

10 The vaccine as set forth in claim 8, wherein the vaccine
is superior in safety or effectiveness to a vaccine containing,
as an antigen, HSV-2 in which only Us3 is modified.

[Claim 10]

15 The vaccine as set forth in claim 8, wherein the vaccine
is superior in safety or effectiveness to a vaccine containing,
as an antigen, HSV-2 in which only UL41 is modified.

[Claim 11]

20 The vaccine as set forth in claim 8, wherein the vaccine
is used for intramuscular injection or nasal administration.

[Claim 12]

25 The vaccine as set forth in claim 8, wherein the vaccine
is administered twice to a subject.

[Claim 13]

The vaccine as set forth in claim 8, wherein a dose of the virus is 10^6 pfu to 10^7 pfu.

5

[Claim 14]

Use of a virus recited in any one of claims 1 to 7 as a vaccine vector against a pathogen other than herpes simplex virus type 2 (HSV-2).

10

[Claim 15]

The use as a vaccine vector as set forth in claim 14, wherein the pathogen is an influenza virus or SARS-CoV-2.