

DESCRIPTION

VACCINE ANTIGEN CAPABLE OF INDUCING CROSS-REACTING AND
NEUTRALIZING ANTIBODY AGAINST HIGH-RISK-TYPE HUMAN
PAPILLOMAVIRUS

Cross-Reference to Related Applications

[0001]

This application claims priority from Japanese Patent Application No. 2007-167154 filed on June 26, 2007, and all disclosure therein is incorporated herein by reference.

TECHNICAL FIELD

[0002]

The present invention relates to a vaccine antigen inducing cross-reactive neutralizing antibodies to high-risk group human papillomaviruses. In particular, the present invention relates to a chimeric protein composed of human papillomavirus (HPV) 16 L1 protein and a particular human papillomavirus 16 L2 epitope inserted to a particular site thereof and capsid which is an aggregate formed by assembly of the chimeric protein. The capsid according to the present invention is useful as an antigen for use in a vaccine for prevention of infection by human papillomaviruses which may lead to cervical cancer.

[0003]

BACKGROUND ART

Human papillomavirus (HPV) (Figure 1) is a small DNA virus, and there are 100 or more genotypes. Fifteen genotypes (high-risk genotypes: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 73) cause cervical cancer. HPV16 is detected in 50 to 60% of cervical cancer. HPV18 is next frequently found in Europe and the United States, while HPV52 and HPV58 are next frequently found in Japan. Although mass examination was carried out for earlier diagnosis in Japan, there are still onset thereof in 15,000 patients and death of 2,500 patients every year.

[0004]

The HPV capsid has a regular icosahedral skeleton consisting of 72 L1 protein pentamers (capsomeres) and 12 L2 protein molecules bound thereto. The both terminals of the L2 protein are located in the capsid, but part of the N-terminal region is located on the surface of the capsid (L2 surface region) (Figure 2). Expression of the L1 protein in a great amount by recombinant DNA technique gives virus-like particles (VLPs). Inoculation of the VLP or the L2 protein of bovine or cottontail rabbit papillomavirus makes the inoculated animals resistant to viral challenge. Figure 2 is a schematic crosssectional view illustrating the HPV and the VLP.

[0005]

There is currently no cultured cell line allowing proliferation of the HPV. Pseudoviruses are prepared for monitoring HPV infection. Introduction of a secretory alkali phosphatase (SEAP)-expressing plasmid having the replication origin of SV40, an L1 protein-expressing plasmid, and an L2 protein-expressing plasmid into SV40T antigen-expressing human 293 cell leads to incorporation of the replicated SEAP-expressing plasmid into the L1/L2 capsid, giving an infectious pseudovirus (Figure 3). The activity of neutralization antibodies is determined by measuring the activity of inhibiting the pseudoviral infection (Nonpatent Document 1).

[0006]

Each of the antisera obtained by inoculation of the VLP of HPV into animals has a type-specific neutralization activity. Merck developed a vaccine in combination of the VLPs of HPV16 and HPV18 and the VLPs of HPV6 and HPV11, which are possible causes of condyloma acuminatum (benign), while GlaxoSmithKline developed a vaccine in combination of the VLPs of HPV16 and HPV18 (Nonpatent Documents 2 and 3).

[0007]

These vaccines were shown in large-scale clinical tests to have type-specific infection-preventing action, and the vaccine of Merck was approved in 2006 by FDA and Commission of the European Communities and sold in the U.S and EC countries.

[0008]

As described above, immunization of the HPV L1 capsid into animals leads to induction of extremely type-specific immune response. Preliminary results in a clinical test by using the HPV16 L1 capsid vaccine showed that the vaccine was

effective in preventing HPV16 infection, but almost not effective in preventing other HPV genotypes. Accordingly for prevention of onset of cervical cancer with vaccines, there is a need for development of a vaccine antigen that is effective at least to all high-risk HPVs.

[0009]

The inventors had earlier developed a vaccine antigen, by using a common neutralization epitope to high-risk HPVs that is present in the amino acid 108-120 region of HPV16 L2 protein. However, the epitope has an amino acid sequence having a homology of about 60 to 75% with high-risk L2 proteins, and the antibody induced thereby bound to multiple high-risk HPVs but was lower in binding efficiency than to HPV16. Thus, there has been a demand for an antigen having higher type-commonality.

[0010]

The inventors had found that it was possible to prepare a vaccine antigen capable of inducing a more potent type-common neutralizing antibody by producing a chimeric protein having the HPV16 L1 protein and the amino acid 64-81 region of HPV16 L2 protein inserted thereto and that the vaccine antigen was an antigen having higher type-commonality that was compatible at least with all high-risk HPVs, and filed an patent application (WO2007/018049, Patent Document 1) earlier. Further as described above, in view of the fact that the particles formed of the chimeric protein composed of the HPV 16 L1 protein and an amino acid 108-120 region of HPV16 L2 protein inserted in the HPV16 L1 protein, have strong immunogenicity and potential to induce neutralizing antibody, which is in common to high-risk HPVs, a chimeric protein consisting of the chimeric protein having the additional amino acid 64-81 region inserted by an amino acid 109-117 region of HPV16 L2 protein was provided in Patent Document 1.

[0011]

Nonpatent Document 1: Pastrana, D.V., Buck, C.B., Pang, Y.Y., Thompson, C.D., Castle, P.E., FitzGerald, D.C., Kruger, Kjaer, S., Lowy, D.R., Schiller, J.T., 2004. Reactivity of human sera in a sensitive, high throughput pseudovirus-based papillomavirus neutralization assay for HPV 16 and HPV 18. *Virology*. 321: 205-216.
Nonpatent Document 2: Villa, L.L., et al.: Prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in young

women: a randomised double-blind placebo-controlled multicentre phase II efficacy trial. *Lancet Oncology* 6, 271-278, 2005

Nonpatent Document 3: Harper, D.M., et al.: Sustained efficacy up to 4.5 years of a bivalent L1 virus-like particle vaccine against human papillomavirus type 16 and 18: follow up from a randomized control trial. *Lancet* 367 (9518), 1247-1255.

Patent Document 1: WO2007/018049

All descriptions in the Nonpatent Documents 1 to 3 and Patent Document 1 above are incorporated herein by reference.

[0012]

HPV vaccines currently commercially available are effective only to HPV16 and HPV18 among 15 high-risk HPVs. Each VLP induces a type-specific neutralizing antibody, and thus, a cocktail of 15 kinds of VLPs are needed for induction of antibodies to all high-risk HPVs, making it difficult to prepare a practical vaccine antigen. Therefore, there is a demand for development of a vaccine antigen that induces a cross-reactive neutralizing antibody.

[0013]

The antigen described in Patent Document 1 is an antigen compatible at least with all high-risk HPVs that have higher type-commonality. However, it had the following problem. The infectious pseudoviruses used in the study of Patent Document 1 were only HPV16 and HPV18, and the antigen described in Patent Document 1 showed favorable neutralizing activity to these infectious pseudoviruses. However, in the subsequent neutralizing experiment by using HPV31, HPV52 and HPV58 infectious pseudoviruses newly developed by the inventors, the antigen described in Patent Document 1 was lower in neutralizing activity to HPV31, HPV52, and HPV58.

[0014]

Thus, an object of the present invention is to provide a vaccine antigen capable of inducing a cross-reactive neutralizing antibody to high-risk group human papillomaviruses.

[0015]

SUMMARY OF THE INVENTION

A first aspect of the present invention is a capsid which is an aggregate formed by assembly of chimeric protein composed of human papillomavirus (HPV) 16 L1 protein

and an HPV16 L2 epitope inserted in the loop region of the HPV16 L1 protein, wherein

the region for insertion of the L2 epitope is a region of amino acids 430 to 433 and the L2 epitope has an amino acid sequence represented by:

LYKTCKQAGTCPPDIIPKVEG (hereinafter, referred to as 18-38 L2 epitope),

GGLGIGTGSGTGGRTGYIPL (hereinafter, referred to as 56-75 L2 epitope) or

DPVGPLDPSIVSLVEESSFI (hereinafter, referred to as 96-115 L2 epitope).

[0016]

The capsid according to the present invention includes the following typical embodiments.

- 1) In the capsid above, the L2 epitope comprises amino acids having said amino acid sequence of which one or more amino acids are deleted or substituted or to which one or more amino acids are added, wherein the amino acids provide the capsid with capability inducing a cross-neutralizing antibody similar to that of the HPV having the original L2 epitope.
- 2) The aggregate is an aggregate formed by assembly of multiple pentamer capsomeres, each of which composed of five chimeric protein molecules.
- 3) The an aggregate formed by assembly of multiple pentamer capsomeres has a particle structure.
- 4) The number of the pentamer capsomeres constituting the aggregate is in the range of 65 to 80.
- 5) The number of the pentamer capsomeres constituting the aggregate is 72.
- 6) The capsid is used for production of an L1 capsid vaccine.

[0017]

A second aspect of the present invention is a capsid mixture comprising two or more kinds of capsids according to the present invention.

[0018]

The capsid mixtures according to the present invention include the following typical embodiments.

- 1) The capsid mixture comprises a capsid which is an aggregate formed by assembly of the 18-38 L2 epitope-inserted chimeric protein and a capsid which is an aggregate formed by assembly of the 56-75 L2 epitope-inserted chimeric protein.
- 2) The capsid mixture comprises a capsid which is an aggregate formed by assembly of

the 18-38 L2 epitope-inserted chimeric protein and a capsid which is an aggregate formed by assembly of the 96-115 L2 epitope-inserted chimeric protein.

3) The capsid mixture comprises a capsid which is an aggregate formed by assembly of the 56-75 L2 epitope-inserted chimeric protein and a capsid which is an aggregate formed by assembly of the 96-115 L2 epitope-inserted chimeric protein.

4) The capsid mixture comprises a capsid which is an aggregate formed by assembly of the 18-38 L2 epitope-inserted chimeric protein, a capsid which is an aggregate formed by assembly of the 56-75 L2 epitope-inserted chimeric protein, and a capsid which is an aggregate formed by assembly of the 96-115 L2 epitope-inserted chimeric protein.

5) The capsid mixture is used for production of an L1 capsid vaccine.

6) The capsid mixture is used for production of an L1 capsid vaccine that can induce the neutralizing antibodies at least to human papillomaviruses 16, 18, 31, 52, and 58.

[0019]

A third aspect of the present invention is

a chimeric protein composed of human papillomavirus (HPV) 16 L1 protein and an HPV16 L2 epitope inserted in the loop region of the HPV16 L1 protein, wherein the loop region for insertion of the L2 epitope is a region of amino acids 430 to 433, and the L2 epitope has an amino acid sequence represented by:

LYKTCKQAGTCPPDIIPKVEG (hereinafter, referred to as 18-38 L2 epitope),

GGLGIGTGSGTGGRTGYIPL (hereinafter, referred to as 56-75 L2 epitope) or

DPVGPLDPSIVSLVEESSFI (hereinafter, referred to as 96-115 L2 epitope).

[0020]

In a typical embodiment of the chimeric protein according to the present invention, the L2 epitope is amino acids having said amino acid sequence of which one or more amino acids are deleted or substituted or to which one or more amino acids are added, wherein the amino acids provide the capsid constituted by the chimeric protein with capability inducing a cross-neutralizing antibody similar to that of the HPV having the L2 epitope.

[0021]

A fourth aspect of the present invention is a method of producing the capsid according to the present invention, wherein the capsid is formed by assembly of the chimeric protein above.

[0022]

A fifth aspect of the present invention is the method of producing the capsid mixture according to the present invention, comprising preparing two or more kinds of capsids by the method according to the method of producing the capsid of the present invention and mixing the resulting capsids.

[0023]

A sixth aspect of the present invention is a complex of keyhole limpet hemocyanin and human papillomavirus 16 L2 epitope, the L2 epitope having an amino acid sequence represented by:

LYKTCKQAGTCPPDIIPKVEG (hereinafter, referred to as 18-38 L2 epitope),

GGLGIGTGSGTGGRTGYIPL (hereinafter, referred to as 56-75 L2 epitope) or

DPVGPLDPSIVSLVEESSFI (hereinafter, referred to as 96-115 L2 epitope).

[0024]

In a typical embodiment of the complex according to the present invention, the keyhole limpet hemocyanin and the L2 epitope are bound to each other via cysteine.

[0025]

The present invention provides an L1 capsid vaccine antigen in which a particle structure has a strong immunoinducing activity, and the L1 capsid vaccine antigen is capable of strong antigen-presenting of the HPV L2 epitope, and which is also capable of inducing a neutralizing antibody, in particular, inducing a neutralizing antibody to HPV16, 18, 31, 52, and 58. Considering the homology of the L2 amino acid sequences in the 15 kinds of high-risk human papillomaviruses described above, the L1 capsid vaccine antigen capable of inducing a neutralizing antibody to HPV16, HPV18, HPV31, HPV52 and HPV58 according to the present invention is quite likely an antigen which can cover almost all of the 15 kinds of high-risk HPVs.

[0026]

VLP vaccines were already commercialized, and shown to have type-specific infection-preventing action. The chimeric VLP developed by the inventors, which is an antigen consisting of the current vaccine antigen and an additional cross-neutralization L2 epitope, retains the neutralizing epitope of the current vaccine, and thus, induces an HPV16-specific neutralizing antibody and also a cross-reactive neutralizing antibody to HPV18, HPV31, HPV52, and HPV58. The VLP is

composed of 360 L1 protein molecules, and thus, the chimeric VLP has 360 cross-neutralization L2 epitopes. It is a new vaccine antigen in a particle structure having a strong immunoinducing activity and presenting the cross-neutralization L2 epitope strongly.

[0027]

BEST MODE FOR CARRYING OUT THE INVENTION

(L1 capsid)

The L1 capsid according to the present invention is a capsid which is an aggregate formed by assembly of chimeric protein composed of human papillomavirus (HPV) 16 L1 protein and a human papillomavirus 16 L2 epitope that is inserted in a loop region of the HPV16 L1 protein.

[0028]

As described above, HPV particle is a regular icosahedral capsid consisting of 72 capsomeres, each of which contains five L1 protein molecules. High level expression of the L1 protein in cell results in accumulation of the L1 proteins in the nucleus, autonomously forming capsids. The capsid formed only with the L1 proteins is called L1 capsid or virus-like particle (VLP). Simultaneous expression of L1 and L2 proteins gives an L1/L2 capsid (or L1/L2 VLP) containing 12 L2 protein molecules in the L1 capsid. However, the L1 capsid and the L1/L2 capsid are not differentiated from each other by electron microscopy.

[0029]

The L1 capsid according to the present invention is a capsid based on the HPV16 L1 capsid. Because there are many HPV genotypes, each capsid is in principle indicated with a genotype such as HPV16 or HPV58. Thus, for example, the L1 capsid of HPV16 is designated as 16 L1 capsid.

[0030]

The L1 capsid according to the present invention is an aggregate formed by assembly of chimeric protein, having HPV16 L2 epitopes inserted into the HPV16 L1 protein. Hereinafter, the chimeric protein means a "chimeric protein having an HPV16 L2 epitope inserted into the loop region of the human papillomavirus (HPV) 16 L1 protein," and the present invention includes such chimeric proteins.

[0031]

The HPV16 L2 epitope inserted into the L1 protein for production of the chimeric protein according to the present invention has an amino acid sequence represented by: LYKTCKQAGTCPPDIIPKVEG (18-38 L2 epitope), GGLGIGTGSGTGGRTGYIPL (56-75 L2 epitope) or DPVGPLDPSIVSLVEESSFI (96-115 L2 epitope).

[0032]

The 18-38 L2 epitope has 21 amino acids in the 18-38 amino acid region of the HPV16 L2 protein. The 56-75 L2 epitope has 20 amino acids in the 56-75 amino acid region of the HPV16 L2 protein. The 96-115 L2 epitope has 20 amino acids in the 96-115 amino acid region of the HPV16 L2 protein (wherein, the 101st and 112th amino acids are replaced respectively with leucine and serine).

[0033]

In the present invention, the amino acid residues in protein are numbered from the amino terminal and, for example, the 50th amino acid is designated as amino acid 50.

[0034]

The L2 surface region binds to multiple cellular proteins. Introduction of amino acid substitution mutation results in loss of infectivity, indicating that the L2 surface region has a function essential for HPV infection (Figure 4). The amino acid sequence of this region is preserved quite well in all high-risk HPV L2 proteins (Figure 5). The inventors studied antisera obtained by immunizing rabbits with synthetic peptides having the amino acid sequence in the HPV16 L2 surface region. The results will be described below in Example (Table 1). The results in Table 1 showed that there are cross-neutralization epitopes in the regions of amino acids 18-38, 49-75, and 96-115.

[0035]

Specifically, the results shown in Table 1 indicate that there are cross-neutralization epitopes to HPV16, HPV18, HPV31, and HPV58 in the regions of amino acid 18-38, 56-75, and 96-115. However, the cross reactivity to HPV52 was not studied in this stage.

[0036]

Here, in the 96-115 region, the 101st S was replaced with L, and the 112th T with S.

It is because the mutant epitope having L replacing 101st S and S replacing 112th T is superior in inducing cross-reactive antibody to the epitope having the naturally occurring amino acid sequence 96-115.

[0037]

The results in Table 1 show that there are cross-neutralization epitopes to HPV16, HPV18, HPV31 and HPV58 also in the 49-68 region. However, because the chimeric protein with a 49-75 region including the 49-68 region did not form a particle structure and the homology of L2 amino acids in the 49-55 region was not high in 15 kinds of HPVs as shown in figure 5, the 49-68 region was not studied in the present invention.

[0038]

Thus, chimeric proteins were prepared by inserting the cross-neutralization epitope in the region of amino acids 430 to 433 of L1 protein and chimeric VLPs were produced with the chimeric proteins.

[0039]

Therefore, the present invention relates to a chimeric protein composed of human papillomavirus (HPV) 16 L1 protein and an HPV16 L2 epitope inserted in the loop region of the HPV16 L1 protein, wherein the loop region for insertion of the L2 epitope is a region of amino acids 430 to 433, and

The L2 epitope has an amino acid sequence presented by:

LYKTCKQAGTCPPDIIPKVEG (hereinafter, referred to as 18-38 L2 epitope),
GGLGIGTGSGTGGRTGYIPL (hereinafter, referred to as 56-75 L2 epitope) or
DPVGPLDPSIVSLVEESSFI (hereinafter, referred to as 96-115 L2 epitope).

[0040]

The present invention also relates to a capsid which is an aggregate formed by assembly of the chimeric protein. The aggregate is composed of multiple pentamer capsomeres each of which composed of five chimeric protein molecules, and the aggregate composed of the multiple pentamer capsomeres preferably has a particle structure for greater antigenicity. The number of the pentamer capsomeres constituting the particle is, for example, in the range of 65 to 80, preferably 72, which is identical with the number thereof in the naturally occurring viruses.

[0041]

The naturally occurring VLP generally contains 360 L1 protein molecules and, in such a case, has 360 cross-neutralization epitopes in the particle (Figure 6). A chimeric protein having a sequence of amino acids 18 to 38, 56 to 75, or 96 to 115 (101st and 112th amino acids replaced respectively with leucine and serine) of HPV16 L2 protein inserted in the region of 430 to 433 of HPV16 L1 protein was expressed in insect sf9 cells by using a recombinant baculovirus, to give a chimeric VLP, which was designated as Ch18/38, Ch56/75, or Ch96/115 (101L, 112S), respectively (Figure 7). The electron micrograph in Figure 7 shows the particle structure of respective chimeric VLPs. ELISA by using these chimeric VLPs as antigen showed specific-binding of antibodies to the inserted epitopes to the chimeric VLP (as described below in Table 2 of Example), indicating that the epitopes were exposed on the surface of VLP.

[0042]

Antisera were prepared by immunizing rabbits with these chimeric VLPs. In ELISA by using a synthetic peptide having the sequence identical with that of the cross-neutralization epitope as an antigen, each antiserum reacted specifically with the inserted epitope. The peptide 96/115 (101L, 112S) aggregated easily, resulting in decrease in the amount bound to the ELISA plate, and thus, the titer obtained was slightly lower (as shown below in Table 3 of Example).

[0043]

Analysis of the neutralizing activity against HPV16, HPV18, HPV31, HPV52, and HPV58 pseudoviruses showed that: the antibody to Ch18/38 neutralized the HPV16, HPV18 and HPV31; the antibody to Ch56/75, all HPV types; and the antibody to Ch96/115 (101L, 112S), HPV16, HPV18, HPV31 and HPV58 (no neutralization of HPV58 observed with antisera having a low titer) (as shown below in Tables 4 and 5 of Example).

[0044]

All antisera were found to react intensely with HPV16, the skeleton for the chimeric VLP, indicating that the neutralization epitope inherent to the VLP was conserved in the chimeric VLPs. Because the infectious pseudoviruses consist of 360 L1 protein molecules and 12 L2 protein molecules, the L2 protein excessively produced in the sample is present as liberated in cell. For that reason, the neutralization titer of anti-L2 antibody was obtained rather low.

[0045]

Animal papillomavirus experiments showed that VLP vaccines and L2 protein vaccines are almost similar in efficacy, indicating that the chimeric VLPs according to the present invention could be practical vaccine antigens inducing generation of antibodies to the cross-neutralization epitopes.

[0046]

The L2 epitope may be the amino acids having the amino acid sequence above, i.e., that represented by one of the SEQ ID Nos. 2 to 4, of which one or more amino acids are deleted or substituted or to which one or more amino acids are added, wherein the amino acids provide the capsid according to the present invention with capability inducing a cross-neutralizing antibody similar to that of the VLP having the original L2 epitope (having the amino acid sequence represented by any one of the SEQ ID Nos. 2 to 4).

[0047]

Thus, the 18-38 L2 epitope may include the mutant L2 epitopes of the amino acids having the amino acid sequence represented by LYKTCKQAGTCTPPDIIPKVEG (SEQ ID No. 2) of which one or more amino acids are deleted or substituted or to which one or more amino acids are added that provides the capsid according to the present invention with capability inducing a cross-neutralizing antibody similar to that of the HPV16 VLP having the 18-38 L2 epitope. The capabilities inducing a cross-neutralizing antibody of the HPV16 VLPs having the 18-38 L2 epitope are shown in Table 4.

[0048]

The 56-75 L2 epitope may include the mutant L2 epitopes of the amino acids having the amino acid sequence represented by GGLGIGTGSGTGGRGTGYIPL (SEQ ID No. 3) of which one or more amino acids are deleted or substituted or to which one or more amino acids are added that provides the capsid according to the present invention with capability inducing a cross-neutralizing antibody similar to that of the HPV16 VLP having the 56-75 L2 epitope. The capabilities inducing a cross-neutralizing antibody of the HPV16 VLPs having the 56-75 L2 epitope are shown in Table 4.

[0049]

Similarly, the 96-115 L2 epitope may include the mutant L2 epitopes of the amino acids having the amino acid sequence represented by DPVGPLDPSIVSLVEESSFI (SEQ ID No. 4) of which one or more amino acids are deleted or substituted or to which one or more amino acids are added that provides the capsid according to the present invention with capability inducing a cross-neutralizing antibody similar to that of the HPV16 VLP having the 96-115 L2 epitope. The capabilities inducing a cross-neutralizing antibody of the HPV16 VLPs having the 96-115 L2 epitope are shown in Table 4. In the present description, the number of the more amino acids in deletion, substitution, or addition is 2, 3, 4, 5, 6, 7, 8, 9 or 10.

[0050]

The site in the L1 protein of the chimeric protein according to the present invention where the L2 epitope is inserted is in the region of amino acids 430 to 433 of the loop region of L1 protein. Specifically, an L2 epitope of 20 to 21 amino acids is inserted, replacing the four amino acids 430 to 433 in the L1 protein. The amino acid sequence of the HPV16 L1 protein is shown as SEQ ID No. 1. The 426-446 region including amino acids 430 to 433 is estimated to be a region recognizable as the antigen that is located outside the L1 protein, from the viewpoints of structural analysis of HPV16 L1 protein, distribution of neutralizing antibody epitopes, and the position of the cysteine residue essential for capsid formation etc.

[0051]

The number of the L2 epitopes inserted into the L1 protein is one for a single L1 protein. However, at least two different kinds of L2 epitopes may be inserted into different loop regions in a single L1 protein. For example, a peptide except for the three cross-neutralization epitopes may be inserted to a site except for the amino acids 430 to 433 of L1 protein. Alternatively, one of the three cross-neutralization epitopes may be inserted to a site except for the amino acids 430 to 433 of L1 protein.

[0052]

(Preparation of L2 epitope-containing peptides)

The L2 peptide (amino acids 18-38, 56-75 or 96-115) can be prepared by a common Fmoc solid-phase method in a 96-column automatic peptide synthesizer. The peptide, which is aimed at preparing an antibody, is connected to KLH (keyhole limpet hemocyanin), and a cysteine residue is added to the N terminal for prevention of masking of the peptide region by KLH.

[0053]

The present invention includes the complex of keyhole limpet hemocyanin and an HPV16 L2 epitope. The L2 epitope has an amino acid sequence represented by:

LYKTCKQAGTCPPDIIPKVEG (18-38 L2 epitope),

GGLGIGTSGTGGRTGYIPL (56-75 L2 epitope) or

DPVGPLDPSIVSLVEESSFI (96-115 L2 epitope).

The keyhole limpet hemocyanin and the L2 epitope are bound to each other preferably via cysteine.

[0054]

(Preparation of L2 epitope-inserted L1 proteins (chimeric proteins))

A chimeric L1 gene was prepared, and the chimeric L1 gene prepared was expressed by using a baculovirus vector, to give a chimeric L1 protein and a chimeric capsid. The chimeric L1 gene is prepared by PCR. Specifically, it is prepared in the following manner:

[0055]

(A) A primer pair having a region desirably inserted is prepared.

(B) Primer set composed of the primer having an insertion region and the primer 1 and the set composed of the other primer having an insertion region and primer 2 are used for amplification by PCR.

(C) Two amplified DNA fragments having an inserted fragment in one side are prepared. The respective complementary regions are annealed for extension reaction.

(D) A chimeric gene containing the inserted DNA is prepared.

(E) PCR is performed by using the inserted DNA as template and the primers 1 and 2.

(F) Thus, the DNA containing the inserted gene is amplified.

[0056]

The present invention includes a method of producing a capsid including forming it by an aggregate formed by assembly of the chimeric protein according to the present invention. As described above, a chimeric L1 gene is prepared and expressed by using a baculovirus vector, to give a chimeric L1 protein. The chimeric L1 proteins, once produced, form autonomously a chimeric capsid (the capsid according to the present invention). Expression thereof by using a baculovirus vector may be carried out by a common method, but the condition is preferably optimized for acceleration of

the autonomous chimeric capsid formation.

[0057]

The capsid according to the present invention is used for production of an L1 capsid vaccine. The L1 capsid vaccine may be produced, for example, by using a baculovirus expression system.

[0058]

The present invention includes capsid mixtures containing two or more kinds of capsids according to the present invention. The three kinds of capsids according to the present invention are different from each other in capability inducing a cross-neutralizing antibody, and use of two or more of these capsids different in capability inducing a cross-neutralizing antibody in combination advantageously gives higher neutralizing activity.

[0059]

The capsid mixture according to the present invention may be, for example, a mixture of a capsid which is an aggregate formed by assembly of the 18-38 L2 epitope-inserted chimeric protein and a capsid which is an aggregate formed by assembly of the 56-75 L2 epitope-inserted chimeric protein. The two kinds of capsids may be mixed, for example, at a weight ratio in the range of 1:100 to 100:1. However, the mixing ratio of the two kinds of capsids is not limited thereto, and may be modified according to the desired immunogenicity and neutralizing activity of induced antibodies.

[0060]

The capsid mixture according to the present invention may be, for example, a mixture of a capsid which is an aggregate formed by assembly of the 18-38 L2 epitope-inserted chimeric protein and a capsid which is an aggregate formed by assembly of the 96-115 L2 epitope-inserted chimeric protein. The two kinds of capsids may be mixed, for example, at a weight ratio in the range of 1:100 to 100:1. However, the mixing ratio of the two kinds of capsids is not limited thereto, and may be modified according to the desired immunogenicity and neutralizing activity of induced antibodies.

[0061]

The capsid mixture according to the present invention may be, for example, a mixture of a capsid which is an aggregate formed by assembly of the 56-75 L2 epitope-inserted

chimeric protein and a capsid which is an aggregate formed by assembly of the 96-115 L2 epitope-inserted chimeric protein. The two kinds of capsids may be mixed, for example, at a weight ratio in the range of 1:100 to 100:1. However, the mixing ratio of the two kinds of capsids is not limited thereto, and may be modified according to the desired immunogenicity and neutralizing activity of induced antibodies.

[0062]

The capsid mixture according to the present invention may be, for example, a mixture of a capsid which is an aggregate formed by assembly of the 18-38 L2 epitope-inserted chimeric protein, a capsid which is an aggregate formed by assembly of the 56-75 L2 epitope-inserted chimeric protein, and a capsid which is an aggregate formed by assembly of the 96-115 L2 epitope-inserted chimeric protein. The three kinds of capsids may be mixed, for example, at a weight ratio between respective two kinds of capsids in the range of 1:100 to 100:1. However, the mixing ratio of the three kinds of capsids is not limited thereto, and may be modified according to the desired immunogenicity and neutralizing activity of induced antibodies.

[0063]

The capsid mixture according to the present invention is also used for production of the L1 capsid vaccine. The method of producing a vaccine by using the capsid mixture is the same as that described above.

[0064]

The capsid mixture according to the present invention is used for production of an L1 capsid vaccine that can induce neutralizing antibodies to HPV16, HPV18, HPV31, HPV52 and HPV58.

[0065]

EXAMPLES

Hereinafter, the present invention will be described in more detail with reference to Examples.

[0066]

Example 1

Preparation of rabbit peptide antisera

Two Japanese white rabbits (2.5 kg to 3.0 kg) were immunized. The antigen used

was a conjugate of a chemically prepared peptide and KLH (keyhole limpet hemocyanin). In the first sensitization, 0.5 mg of the antigen was administered subcutaneously with complete Freund's adjuvant. After two weeks from the first administration, 0.25 mg of the antigen was administered subcutaneously with the complete Freund's adjuvant. The rabbits were sensitized similarly (0.25mg) additionally after 2, 4, and 6 weeks, cumulatively five times. After one week from the final sensitization, the total blood was collected, to give a serum sample. (The preparation was performed by Scrum Inc. under contract.)

[0067]

A rabbit was immunized with a synthetic peptide having the amino acid sequence of the HPV16 L2 surface region and the resulting antiserum was analyzed in the neutralization experiment shown below. Results are summarized in Table 1. The results shown in Table 1 show that there are cross-neutralization epitopes in the regions of amino acids 18-38, 49-75, and 96-115.

[0068]

Neutralization experiment

1. 293FT cells (purchased from Invitrogen) were inoculated on a 96-well cell culture plate at a concentration of 10,000 cells/well, a day before neutralization experiment.
2. The serum was diluted with a neutralization buffer (DMEM medium without phenol red, containing 10% FCS, 1% non-essential amino acids, 1% L-glutamine acid, and 10 mM HEPES) and mixed with a stock of an infectious pseudovirus, and the mixture was allowed to react at 4°C for one hour and then added to the 293FT cells inoculated on the previous day.
3. After culture for about 72 hours, 20 µl of the supernatant was collected, and the alkali phosphatase activity thereof was determined according to the method by Roden et al., (see <http://home.ccr.cancer.gov/lco/ColorimetricSEAP.htm>).

[0069]

[Table 1]

Neutralizing activity of anti-L2 peptide antibodies

anti-peptide antibody	HPV16	HPV18	HPV31	HPV58
anti-14/27	<50	<50	<50	<50
	<50	<50	<50	<50
anti-18/38	800	50	<50	<50
	400	100	<50	<50
anti-28/42	<50	<50	<50	<50
	800	<50	<50	50
anti-49/68	800	50	800	800
anti-56/75	400	200	200	400
	200	50	100	200
anti-61/75	400	100	<50	50
	800	200	<50	100
anti-64/81	3200	400	<50	100
	800	200	<50	50
anti-90/111	200	<50	50	<50
	200	<50	<50	<50
anti-96/115	200	<50	50	<50
	400	<50	400	200
anti-96/115m	100	<50	200	200
(101L,112S)	100	50	100	100
anti-107/122	100	<50	<50	50
anti-131/144	<50	<50	<50	<50
	200	<50	<50	<50

[0070]

Example 2

Preparative method of capsid antigen

16 L1 and 16L1/L2 genes were expressed in a recombinant baculovirus system. A recombinant virus was prepared in a Bac-to-Bac baculovirus expression system (GIBCO-BRL Inc., New York, NY), and expressed in Sf9 cells (Mamestra brassicae-derived cells). The 16L1 gene was cloned into pFastbac1 vector, to give pFastbac1/16L1. The 16L1/L2 gene was cloned into pFastbac dual vector, to give pFastbac dual/16L1/L2. Then, each cloned pFastbac vector was introduced into DH10BAC E. coli (Max efficiency competent cell containing baculovirus DNA and helper plasmid, GIBCO BRL), to give Bacmid. The Bacmid DNA was introduced into the sf-9 cells by using an Effectene Transfection Reagent (QIAGEN GmbH, Hilden, Germany), to give a capsid protein-expressing recombinant baculovirus.

[0071]

The recombinant baculovirus was infected to sf-9 cells, and the cells were collected after incubation for 72 hours. The infected cells were suspended in 0.5% NP40 solution; the mixture was left still for 10 minutes at room temperature and centrifuged (9000 rpm, 15 minutes, 4°C), for separation of its nuclear fraction (precipitate) from the cytoplasmic fraction. The nuclear fraction was resuspended in 1.28 g/ml cesium chloride-PBS solution, ultrasonicated for cell destruction (in Sonifier 250, Branson) and ultracentrifuged (34,000 rpm, 20 hours, 20°C) by using a SW50.1 rotor (Beckman Coulter Inc., Fullerton, CA). The protein at a specific density of about 1.28 g/ml in cesium chloride gradient were collected and dialyzed against 0.5 M NaCl-PBS, to give a capsid protein solution.

[0072]

The sequences of the HPV16 L2 protein amino acids 18 to 38, 56 to 75, or 96 to 115 (101st and 112th amino acids replaced respectively with leucine and serine) were inserted in the region of amino acids 430 to 433 of HPV16 L1 protein by using the method of preparing a capsid antigen, and the resulting chimeric protein was expressed in insect sf9 cells by using a recombinant baculovirus, to give a chimeric VLPs, which were designated respectively as Ch18/38, Ch56/75, and Ch96/115 (101L, 112S). The electron micrograph in Figure 7 shows the particles of the chimeric VLPs. ELISA by using these chimeric VLPs as antigen showed specific binding of the antibodies to the inserted epitopes, indicating that the epitopes were exposed on the surface of the VLPs.

[0073]

Measurement method of capsid ELISA

1. Each capsid antigen was added in an amount of 1 $\mu\text{g}/100 \mu\text{l}/\text{well}$ and the mixture was left at 4°C overnight.
2. After removal of the antigen solution, 350 μl of blocking solution (5% skim milk in PBS) was added, and the well plate was left still at 37°C for 2 hours.
3. After removal of the blocking solution, each well was washed with a washing solution (0.05% Tween20/0.05% NP40 in PBS) three times.
4. Fifty μl of the antiserum diluted 500 times with the blocking solution was added to each well, and the solution was left still at room temperature for 1 hour.
5. After removal of the serum sample, the well was washed nine times, and a HRP-bound anti-rabbit IgG antibody diluted 2000 times was added to each well in an amount of 50 μl . The solution is left still at room temperature for 30 minutes, and the well was washed with the washing solution six times after removal of the solution.
6. Fifty μl of a substrate solution (24 mg of o-phenylenediamine dissolved in 12 ml of a citrate-phosphate buffer solution at pH5.0, containing 1.2 μl of H_2O_2) was added thereto, and, after color development for 15 minutes, the light intensity at a wavelength of 450 nm was determined by using an Immuno reader.

[0074]

By using the preparative method for capsid antigen (Example 2), the chimeric proteins, each having the sequences of the amino acids 18 to 38, 56 to 75, or 96 to 115 (101st and 112th amino acids replaced respectively with leucine and serine) of HPV16 L2 protein inserted in the region of the amino acids 430 to 433 of HPV16 L1 protein were expressed in insect sf9 cells by using a recombinant baculovirus, to give chimeric VLPs, which were designated respectively as Ch18/38, Ch56/75, and Ch96/115 (101L, 112S). The electron micrographs in Figure 7 show the particles of the chimeric VLPs. ELISA by using these chimeric VLPs as antigen showed specific binding of the antibodies to the inserted epitopes to the chimeric VLPs, indicating that the epitopes were exposed on the surface of the VLPs. Results are summarized in Table 2.

[0075]

[Table 2]

Binding of anti L2-peptide antibodies to chimeric VLP

antigen antiserum	HPV16 L1VLP	Ch18/38	Ch56/75	Ch96/115(101L,112S)
anti-P18/38	0.042	0.872	0.037	0.042
anti-P56/75	0.041	0.039	0.803	0.041
anti-P96/115 (101L, 112S)	0.040	0.043	0.045	0.821

(Absorbancy in ELISA with serum diluted at 1 to 500 for the peptide antigens and at 1:2,000 for HPV16 L1VLP)

[0076]

Antisera were prepared by immunizing rabbits with these chimeric VLPs. The antisera were prepared in a manner similar to Example 1. (However, the dosage of the chimeric capsid was 50 µg, and the adjuvant used was TiterMax (manufactured by TiterMax, U.S.). In ELISA by using a synthetic peptide having the sequence identical with that of the cross-neutralization epitope as an antigen, each antiserum reacted specifically with the inserted epitope. Results are summarized in Table 3. The peptide 96/115 (101L, 112S) aggregated easily, resulting in decrease in the amount bound to the ELISA plate, and thus, the titer obtained was slightly lower than those obtained with other two peptides.

[0077]

[Table 3]

Binding of anti-chimeric VLP antibodies (serum) to L2-peptide

Antigen Serum		Peptide			
		HPV16VLP	P18/38	P56/75	P96/115 (101L, 112S)
anti-Ch18/38	#1	0.711	0.543	0.041	0.042
	#2	0.633	0.402	0.039	0.039
anti-Ch56/75	#1	0.844	0.040	0.965	0.039
	#2	0.354	0.037	0.135	0.042
	#3	0.923	0.043	1.034	0.045
	#4	0.854	0.042	0.972	0.041
anti-Ch96/115 (101L, 112S)	#1	0.765	0.041	0.045	0.421(0.170)*
	#2	0.590	0.043	0.042	0.380(0.158)*

(Absorbancy in ELISA with serum diluted at 1 to 500 for the peptide antigens and at 1 to 2000 for HPV16VLP)

* indicates the second test result.

Prarenthesis indicate the first test result.

[0078]

Example 3

Preparation of infectious pseudovirus

1. An HPV16 L1 protein-expressing plasmid, an HPV16 L2 protein-expressing plasmid, and a secretory alkali phosphatase-expressing plasmid were transfected to 293TT cells by using Fugene HD. The cells were collected 72 hours after transfection.
2. The collected cells were suspended in a detergent buffer (0.5% Brij58, 0.5% Benzodase and 1% ATP-dependent plasmid safe exonuclease C in D-PBS (CaCl₂: 1 mM, MgCl₂: 10 mM)) and the suspension was left still at 37°C overnight.
3. Then, the suspension was left still at 4°C for 10 minutes, and 5 M NaCl was added thereto to a final NaCl concentration of 850 mM.
4. The cell suspension was centrifuged at 1,500g for 10 minutes, and the supernatant was collected.
5. The collected supernatant was layered on Optiprep (manufactured by AXIS-SHIELD PoC AS) solution at 27%, 33%, or 39% (diluted with PBS), and the solution was ultracentrifuged at 50,000rpm for 3 hours at 16°C.
6. After ultracentrifugation, each fraction from the bottom face in an amount of about 300 µl was collected, and the fraction having the highest titer was used as the infectious pseudovirus fraction in infection experiment.

[0079]

Neutralization experiment

1. 293FT cells (purchased from Invitrogen) were inoculated on a 96-well cell culture plate at a concentration of 10,000 cells/well, a day before neutralization experiment.
2. The serum was diluted with a neutralization buffer (DMEM medium without phenol red, containing 10% FCS, 1% non-essential amino acids, 1% L-glutamine acid, and 10 mM HEPES) and mixed with a stock of an infectious pseudovirus, and the mixture was allowed to react at 4°C for one hour and then added to the 293FT cells inoculated on the previous day.
3. After culture for about 72 hours, 20 µl of the supernatant was collected, and the alkali phosphatase activity thereof was determined according to the method by Roden et al., (see <http://home.ccr.cancer.gov/lco/ColorimetricSEAP.htm>).

[0080]

The neutralizing activities of anti-chimeric VLP antibodies against the HPV16, HPV18, HPV31, HPV35, HPV52, and HPV58 pseudoviruses were studied by using the method

above. Results are summarized in Table 4. The antibody to Ch18/38 neutralized the HPV16, HPV18 and HPV31 pseudoviruses. The antibody to Ch56/75 neutralized all six kinds of pseudoviruses. The antibody to Ch96/115 (101L, 112S) neutralized the HPV16, HPV18, HPV31 and HPV58 pseudoviruses (neutralization of low-titer antisera was not observed with the HPV58 pseudovirus). The anti- HPV16 VLP for comparison neutralized the HPV16, HPV31 and HPV35 pseudoviruses.

[0081]

[Table 4]

Neutralization of HPV16, HPV18, HPV31, HPV52, and HPV58 pseudoviruses by anti-chimeric VLP serum

		Neutralization titer					
Serum		HPV16	HPV18	HPV31	HPV35	HPV52	HPV58
anti-Ch18/38	#1	204,800	400	3,200	NT	<50	<50
	#2	102,400	200	50	NT	<50	<50
anti-Ch56/75	#1	51,200	200	3,200	6,400	6,400	1,600
	#2	1,600	<50	50	400	400	50
	#3	51,200	800	50	100	50	400
	#4	409,600	100	800	50	6,400	400
	#5	204,800	100	3200	NT	3,200	800
anti-Ch96/115 (101L, 112S)	#1	204,800	100	800	NT	<50	50
	#2	102,400	50	50	NT	<50	<50
anti-HPV16VLP		204,800	<50	800	50	<50	<50

[0082]

In addition, the neutralizing activities of the antibody mixtures (weight ratio: 1:1) to the HPV16, HPV18, HPV31, HPV52, and HPV58 pseudoviruses were studied. Results are summarized in Table 5. The mixture of the antibodies to Ch18/38 and Ch56/75 and the mixture of the antibodies to Ch56/75 and Ch96/115 (101L, 112S)

neutralized all five types of pseudoviruses. The mixture of the antibodies to Ch18/38 and Ch96/115 (101L, 112S) neutralized HPV16, HPV18, HPV31 and HPV58 pseudoviruses.

[0083]

[Table 5]

Neutralizing activity of anti chimeric VLP antibodies

Mixture of antisera	Neutralizing titer				
	HPV16	HPV18	HPV31	HPV52	HPV58
anti-Ch18/38 #1 anti-Ch56/78 #1	204,800	800	3,200	3,200	400
anti-Ch56/78 #1 anti-Ch96/115 (101L, 112S) #1	204,800	200	3,200	3,200	400
anti-Ch18/38 #1 anti-Ch96/115 (101L, 112S) #1	204,800	200	1,600	<50	50

[0084]

The following literatures may be referred to, in performing the experiments in the Examples of the present description. The following literatures and all disclosures

therein are incorporated herein by reference.

Reference literatures

- 1) Matsumoto. K, et al.: Antibodies to human papillomavirus 16, 18, 58, and 6b major capsid proteins among Japanese females. *Jpn. J. Cancer Res.*, 88, 369-375, 1997.
- 2) Xiaojiang S. Chen, et al.: Structure of small virus-like particles assembled from the L1 protein of human papillomavirus 16. *Mol. Cell.*, 5, 557-567, 2000.
- 3) Jean-Remy Sadeyen, et al.: Insertion of a foreign sequence on capsid surface loops of human papillomavirus type 16 virus-like particles reduces their capacity to induce neutralizing antibodies and delineates a conformational neutralizing epitope. *Virology.*, 309, 32-40, 2003.
- 4) Ishii. Y, et al.: Mutational analysis of human papillomavirus type 16 major capsid protein L1: the cysteines affecting the intermolecular bonding and structure of L1 capsids. *Virology.*, 308, 128-136, 2003.

[0085]

Industrial applicability

The capsid according to the present invention is an antigen of HPV16 VLP having an additional L2 type-common epitope. The capsid contains additional 360 new epitopes in a particle, while retaining its antigenicity of current vaccines. After verification tests of the infection-preventing activity with the anti-L2 antibody, it can be used as a vaccine antigen that may possibly prevent infection of all carcinogenic HPVs. It is possibly a second-generation HPV vaccine antigen that can prevent infection of high-risk HPV-related diseases, such as cervical cancer, which accounts for about 11% of the world female malignant tumors (450 thousand patients).

BRIEF DESCRIPTION OF THE DRAWINGS

[0086]

Figure 1 is an illustration for explaining human papillomavirus particles. Genome: double-stranded circular DNA, Particle: regular icosahedral (55 nm), Various mammals are persistently infected with papillomaviruses specific to each species. There are 100 or more HPV types, and infection of 15 types thereof (in high-risk group) causes onset of cervical cancer.

Figure 2 are views explaining the virus-like particle (VLP). There is practically no cultured cell line allowing proliferation of the HPV. Expression of the L1 protein for

example with a recombinant baculovirus results in assembly thereof in the nucleus, forming VLPs. First-generation vaccines contain a type 16 or 18 VLP as an antigen. The antigenicity of VLPs is highly type-specific.

Figure 3 is a drawing explaining the preparative method for an infectious pseudovirus.

Figure 4 is a drawing explaining the L2 surface region. The amino acid sequence in the L2 surface region of carcinogenic HPV, highly homologous in amino acid sequences, plays an essential role in infectivity of HPVs.

Figure 5 shows the amino acid sequence of the L2 surface region of HPV16.

Figure 6 is a drawing explaining the chimeric VLP prepared by inserting cross-neutralization epitope into the L1 protein. The Chimeric VLP has 360 cross-neutralization L2 epitopes. The antigenicity and the stability of the VLP were verified.

Figure 7 includes drawings explaining chimeric VLPs, Ch18/38, Ch56/75 and Ch96/115 (101L, 112S) (including amino acid sequences) and photographs of the particles. 16 L1 WT-VLP is a photograph of non-chimeric VLP.

CLAIMS

What is claimed is:

1. A capsid which is an aggregate formed by assembly of chimeric protein composed of human papillomavirus (HPV) 16 L1 protein and an HPV16 L2 epitope inserted in the loop region of the HPV16 L1 protein, wherein the region for insertion of the L2 epitope is a region of amino acids 430 to 433 and the L2 epitope has an amino acid sequence represented by:
LYKTCKQAGTCPPDIIPKVEG (hereinafter, referred to as 18-38 L2 epitope),
GGLGIGTGSGTGGRTGYIPL (hereinafter, referred to as 56-75 L2 epitope) or
DPVGPLDPSIVSLVEESSFI (hereinafter, referred to as 96-115 L2 epitope).
2. The capsid according to Claim 1, wherein the L2 epitope has an amino acid sequence represented by GGLGIGTGSGTGGRTGYIPL (hereinafter, referred to as 56-75 L2 epitope).
3. The capsid according to Claim 1 or 2, wherein the L2 epitope comprises amino acids having said amino acid sequence of which one or more amino acids are deleted or substituted or to which one or more amino acids are added, wherein the amino acids provide the capsid with capability inducing a cross-neutralizing antibody similar to that of the HPV having the original L2 epitope.
4. The capsid according to any one of Claims 1 to 3, wherein the aggregate is an aggregate formed by assembly of multiple pentamer capsomeres, each of which composed of five chimeric protein molecules.
5. The capsid according to Claim 4, wherein the aggregate formed by assembly of multiple pentamer capsomeres has a particle structure.
6. The capsid according to Claim 5, wherein the number of the pentamer capsomeres constituting the aggregate is in the range of 65 to 80.
7. The capsid according to Claim 5, wherein the number of the pentamer capsomeres constituting the aggregate is 72.
8. The capsid according to any one of Claims 1 to 7, which is used for production of an L1 capsid vaccine.

9. A capsid mixture comprising two or more kinds of capsids according to any one of Claims 1 to 7.

10. The capsid mixture according to Claim 9, comprising a capsid which is an aggregate formed by assembly of the 18-38 L2 epitope-inserted chimeric protein and a capsid which is an aggregate formed by assembly of the 56-75 L2 epitope-inserted chimeric protein.

11. The capsid mixture according to Claim 9, comprising a capsid which is an aggregate formed by assembly of the 18-38 L2 epitope-inserted chimeric protein and a capsid which is an aggregate formed by assembly of the 96-115 L2 epitope-inserted chimeric protein.

12. The capsid mixture according to Claim 9, comprising a capsid which is an aggregate formed by assembly of the 56-75 L2 epitope-inserted chimeric protein and a capsid which is an aggregate formed by assembly of the 96-115 L2 epitope-inserted chimeric protein.

13. The capsid mixture according to Claim 9, comprising a capsid which is an aggregate formed by assembly of the 18-38 L2 epitope-inserted chimeric protein, a capsid which is an aggregate formed by assembly of the 56-75 L2 epitope-inserted chimeric protein, and a capsid which is an aggregate formed by assembly of the 96-115 L2 epitope-inserted chimeric protein.

14. The capsid mixture according to any one of Claims 9 to 13, which is used for production of an L1 capsid vaccine.

15. The capsid mixture according to any one of Claims 9 to 13, which is used for production of an L1 capsid vaccine that can induce the neutralizing antibodies at least to human papillomaviruses 16, 18, 31, 52, and 58.

16. A chimeric protein composed of human papillomavirus (HPV) 16 L1 protein and an HPV16 L2 epitope inserted in the loop region of the HPV16 L1 protein, wherein the loop region for insertion of the L2 epitope is a region of amino acids 430 to 433, and

the L2 epitope has an amino acid sequence represented by:

LYKTCCKQAGTCPPDIIPKVEG (hereinafter, referred to as 18-38 L2 epitope),

GGLGIGTGSGTGGRTGYIPL (hereinafter, referred to as 56-75 L2 epitope) or

DPVGPLDPSIVSLVEESSFI (hereinafter, referred to as 96-115 L2 epitope).

17. The chimeric protein according to Claim 16, wherein the L2 epitope has an amino acid sequence represented by GGLGIGTGSGTGGRTGYIPL (hereinafter, referred to as 56-75 L2 epitope).

18. The chimeric protein according to Claim 16 or 17, wherein the L2 epitope is amino acids having said amino acid sequence of which one or more amino acids are deleted or substituted or to which one or more amino acids are added, wherein the amino acids provide the capsid constituted by the chimeric protein with capability inducing a cross-neutralizing antibody similar to that of the HPV having the L2 epitope.

19. A method of producing the capsid according any one of Claims 1 to 7, wherein the capsid is formed by assembly of the chimeric protein according to any one of Claims 16 to 18.

20. A method of producing the capsid mixture according to any one of Claims 9 to 15, comprising preparing two or more kinds of capsids by the method according to Claim 19 and mixing the resulting capsids.