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(56) Related Art

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(54) Title: CHIMERIC HUMAN PAPILLOMAVIRUS 16 L1 PROTEINS COMPRISING AN L2 PEPTIDE,VIRUS-LIKE PARTICLES PREPARED THEREFROM AND A METHOD FOR PREPARING THE PARTICLES.

(57) Abstract: The invention describes a method for producing a chimaeric human papillomavirus (HPV) L1 polypeptide containing a heterologous peptide, and in particular, a HPV L2 peptide. The method comprises the steps of introducing a DNA sequence coding for the heterologous peptide into a DNA sequence coding for the L1 polypeptide; introducing the DNA sequence including the sequences for the L1 polypeptide and heterologous peptide into a host cell in which the DNA sequence can be expressed; causing expression of the DNA sequence; and recovering the resulting chimaeric L1 polypeptide which includes the heterologous peptide. Typically, the nucleotides encoding the heterologous peptide replace the nucleotides of the L1 polypeptide at the point of insertion. The invention also describes a vector for use in the method, a host cell containing the vector, and a vaccine including the chimaeric HPV L1 polypeptide produced according to the method.

CHIMAERIC HUMAN PAPILLOMAVIRUS 16 L1 VIRUS-LIKE PARTICLES AND METHODS FOR PREPARING THE PARTICLES

BACKGROUND OF THE INVENTION

The invention relates to a method for preparing polypeptides, and in particular virus-like particles (VLPs) or capsomers of human papillomavirus.

The papillomaviruses are a group of small DNA viruses, which induce warts and other lesions in a variety of higher vertebrates including humans.

Papillomaviruses (PV) are members of the genus *Papillomavirus*, family *Papillomaviridae*, and contain a double stranded circular DNA genome with a typical size of 7 900 base pairs (Seedorf *et al.*, 1985). All PVs have a similar genomic organisation, with an early gene region encoding proteins involved in DNA replication and cellular transformation, and a late region encoding the viral capsid proteins (Figure 1). A non-coding region known as the long control region (LCR) contains control elements for transcription and replication.

Papillomaviruses encode two viral structural proteins, L1 and L2. The virion contains 360 L1 molecules arranged as 72 capsomers, each of which is a pentamer composed of five L1 molecules (Baker *et al.*, 1991). The ratio of L1 to L2 molecules has been estimated as approximately 30:1 (Doobar *et al.*, 1987), which suggests that each virion would contain approximately twelve L2 molecules. The greater number of L1 molecules per virion has led to L1 being referred to as the 'major' capsid protein and L2 being referred to as the 'minor' capsid protein.

HPV-16 L1 is encoded by a 1.518 kb gene, giving rise to a protein of 504 amino acids. L1 has a molecular weight of 55 to 58 kD (Browne *et al.*, 1988). Domains of L1 are likely to mediate cell binding and to contain antigenic determinants mediating antibody and T cell immune responses to the virus.

Among the genital human papillomaviruses (HPVs), there are low risk HPVs (for example, HPV 6 and HPV 11) that cause genital warts and cervical lesions that usually regress or do not progress to malignancy, and high risk (or oncogenic) genotypes (for example, HPV 16 and HPV 18), which are associated with high-grade cervical lesions and carcinomas. HPVs have also been implicated as the etiological agents in several other anogenital and upper aerodigestive tract cancers (Breitburd *et al.*, 1999). A compelling body of clinical, molecular, experimental and epidemiological evidence has established that certain HPV types are the main cause of cervical cancer (Lowy *et al.*, 1994; IARC, 1995).

HPV 16 is present in most cases of cervical cancer cases and an additional three types (HPV 18, 31 and 45) are present in approximately an additional 30% of cases (IARC, 1999).

Although the incidence of cervical cancer is decreasing in the US, it is the most common malignancy in women in developing countries, with about 500 000 new cases diagnosed each year.

Traditionally most prophylactic vaccines have consisted of live, attenuated virus or formalin inactivated virus. Papillomavirus virions are highly immunogenic, inducing high titres (>10 000) of neutralising antibodies when systemically inoculated (Doretzky *et al.*, 1980; Kirnbauer *et al.*,1991, 1992). However, due to the difficulties and risks involved in generating large quantities of these traditional vaccines there has been great emphasis on the development of viral protein subunit or virus-like particle (VLP) vaccines.

The best candidate protein for a prophylactic vaccine against HPV is the major capsid protein L1, which self-assembles into VLPs (Schiller and Lowy, 2001). These VLPs are very well characterised, and morphologically appear indistinguishable from whole virions (Chen *et al.*, 2001; Rose *et al.*, 1993). Injection of VLPs into experimental animals induces neutralising antibodies (Rose *et al.*, 1998); preliminary human trials of injected VLP vaccines have also shown that these are well tolerated and highly immunogenic, and in the former case, stimulated robust B and T cell responses (Evans *et al.*, 2001; Harro *et al.*, 2001).

An effective, cheap prophylactic vaccine against oncogenic types of mucotropic HPVs could potentially have an impact on the world cancer burden, especially against HPV 16.

A common-neutralizing epitope for HPV types 6 and 16 has been found in the region (aa) 108-120 of the HPV 16 minor capsid protein, L2 (Kawana *et al.*,1998, 1999). Balb/c mice that were nasally immunised with a synthetic peptide corresponding to the epitope elicited an immune response that resulted in IgA and IgG antibodies cross-reacting with L1/L2 capsids of HPV 6, 16 and 18 (Kawana *et al.*, 2001). Immunisation of rabbits with either of two overlapping peptides derived from the L2 sequence region 94-122 from either *Rabbit oral papillomavirus* (ROPV) or *Cottontail rabbit papillomavirus* (CRPV) resulted in sera which reacted to purified cognate L2, specifically recognised L2 in infected cells, and neutralised virus *in vitro*. Rabbits immunised with CRPV peptides were immune to CRPV challenge (Embers et al., 2002).

The inventors therefore decided to further investigate the presentation of this L2 epitope on chimaeric L1 VLPs as a vaccine in its own right, and as a model for the presentation of other immunogenic peptide sequences.

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed before the priority date of each claim of this application.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

SUMMARY OF THE INVENTION

According to a first embodiment of the invention, there is provided a method for producing a chimaeric human papillomavirus (HPV) L1 polypeptide containing a HPV L2 peptide, the method comprising the steps of:

introducing a DNA sequence coding for the L2 peptide into a DNA sequence coding for the L1 polypeptide;

introducing the DNA sequence including the sequences for the L1 polypeptide and L2 peptide into a host cell in which the DNA sequence can be expressed;

causing expression of the DNA sequence; and

recovering the resulting chimaeric L1 polypeptide which includes the L2 peptide.

The HPV L1 polypeptide and/or HPV L2 peptide may be a HPV-16 polypeptide or peptide.

The HPV L2 peptide may have the following amino acid sequence: LVEETSFIDAGAP (SEQ I.D. NO: 1), or a sequence which is modification or derivative thereof, with the proviso that the modified or derived sequence is a sequence which has at least 80% homology to the sequence of SEQ I.D. NO: 1 and codes for a peptide which elicits an immunogenic response against HPV.

One or more nucleotides of the L1 DNA sequence may be deleted at the point of introduction of the L2 DNA sequence, and typically the number of nucleotides deleted from the L1 sequence will correspond with the number of L2 nucleotides inserted.

The expression of the protein could either be in a prokaryotic or eukaryotic expression system.

The chimaeric polypeptide may have the amino acid sequence set out in any one of Figures 6, 8, 10, 12 and 14 (SEQ I.D. NOS: 5, 7, 9, 11 and 13), or a sequence which is at least 80% homologous to any one of the sequences set out in SEQ I.D. NOS: 5, 7, 9, 11 and 13 and which is capable of eliciting an immunogenic response against HPV. The DNA sequence coding for the chimaeric polypeptide may be the DNA sequence as set out in any one of Figures 5, 7, 9, 11 and 13 (SEQ I.D. NOS: 4, 6, 8, 10 and 12), or a sequence having at least 80% homology to any one of these sequences and which codes for a polypeptide capable of eliciting an immunogenic response against HPV.

The chimaeric L1 polypeptide may assemble into virus-like particles and/or capsomers. The virus-like particle or capsomer may be immunogenic.

According to a second embodiment of the invention, there is provided a chimaeric HPV LI DNA sequence into which the DNA sequence coding for the above HPV L2 peptide has been inserted, the resulting HPV L1 sequence being capable of expressing the HPV L2 peptide.

One or more nucleotides of the L1 DNA sequence at the point of introduction of the L2 DNA sequence may be deleted, and typically the number of nucleotides deleted from the L1 sequence will correspond with the number of L2 nucleotides inserted.

The chimaeric nucleic acid sequence may be a sequence as set out in any one of Figures 5, 7, 9, 11 and 13, or a DNA sequence which is a modification or derivative thereof, with the proviso that the modified or derived DNA sequence has at least 80% homology to any one of the sequences of SEQ I.D. NOS: 4, 6, 8, 10 and 12 and codes for a chimaeric L1 peptide which is capable of eliciting an immunogenic response against HPV.

The present invention further provides a human papillomavirus (HPV) DNA sequence having a DNA sequence set out in any one of SEQ I.D. NOS: 4, 6, 8, 10 and 12, or a DNA sequence which is a modification or derivative thereof, with the proviso that the modified or derived DNA sequence has at least 80% homology any one of SEQ I.D. NOS: 4, 6, 8, 10 and 12 and codes for a chimeric L1 peptide presenting an HPV L2 peptide, the chimeric L1 peptide being capable of eliciting an immunogenic response against HPV.

According to a third embodiment of the invention, there is provided a vector including the nucleic acid sequence described above.

According to a fourth embodiment of the invention, there is provided a host cell including the vector described above.

According to yet a further embodiment of the invention, there is provided a chimaeric HPV L1 polypeptide that includes the above HPV L2 peptide (SEQ I.D. NO: 1).

The chimaeric polypeptide may be a chimaeric HPV L1 virus-like particle or capsomer.

According to a further embodiment of the invention, there is provided an HPV polypeptide having the amino acid sequence set out in any one of Figures 6, 8, 10, 12 and 14 (SEQ I.D. NOS: 5, 7, 9, 11 and 13), or a sequence which is a modification or derivative thereof, the modification or derivative being a sequence which is at least 80% homologous to any one of the sequences set out in SEQ I.D. NOS: 5, 7, 9, 11 and 13 and which is capable of eliciting an immunogenic response against HPV.

The present invention further provides a human papilloma virus (HPV) polypeptide having an amino acid sequence set out in any one of SEQ I.D. NOS: 5, 7, 9, 11 and 13, or a sequence which is a modification or derivative thereof, with the proviso that the modified or derived amino acid sequence has at least 80% homology to any one of the sequences set out in SEQ I.D. NOS: 5, 7, 9, 11 and 13 and is a chimeric L1 peptide presenting an HPV L2 peptide, the chimeric L1 peptide being capable of eliciting an immunogenic response against HPV.

According to another embodiment of the invention, there is provided a method for producing a chimaeric human papillomavirus (HPV) L1 polypeptide containing a heterologous peptide, the method comprising the steps of:

introducing a DNA sequence coding for the heterologous peptide into a DNA sequence coding for the L1 polypeptide;

introducing the DNA sequence including the sequences for the L1 polypeptide and heterologous peptide into a host cell in which the DNA sequence can be expressed;

causing expression of the DNA sequence; and recovering the resulting chimaeric L1 polypeptide which includes the heterologous peptide.

The present invention further provides a method for producing a chimaeric human papillomavirus (HPV) L1 polypeptide containing a heterologous peptide, the method comprising the steps of:

introducing a DNA sequence coding for the heterologous peptide into a DNA sequence coding for the L1 polypeptide at any one of nucleotide positions 241-279, 391-429, 520-558, 1240-1278 or 1291-1329;

introducing the DNA sequence including the sequences for the L1 polypeptide and heterologous peptide into a host cell in which the DNA sequence can be expressed;

causing expression of the DNA sequence; and

recovering the resulting chimaeric L1 polypeptide which includes the heterologous peptide.

The heterologous peptide sequence may be any other HPV sequence, or may be derived from any antigenic epitope, B-cell or T-cell specific.

One or more nucleotides of the L1 DNA sequence at the point of introduction of the heterologous DNA sequence may be deleted, and typically the number of nucleotides deleted from the L1 sequence will correspond with the number of heterologous nucleotides inserted.

According to yet a further embodiment of the invention, there is provided a vaccine including the chimaeric HPV L1 polypeptide or a DNA sequence coding for the polypeptide, substantially as described above. The vaccine may be for prophylactic or therapeutic treatment of HPV infection, in particular HPV 6, 16 and 18.

Preferably, the vaccine will be capable of inducing an immunogenic response to HPV and to the introduced peptide in a suitable host.

The vaccine may further include a pharmaceutical excipient and/or adjuvant.

DESCRIPTION OF THE DRAWINGS

Figure 1 shows a diagrammatic representation of the genomic organisation of HPV 16;

Figure 2 shows the monomer structure of HPV 16 L1;

Figure 3 shows the nucleotide sequence of the native sequence of the HPV 16 L1 gene into which the L2 epitope was inserted to produce the chimaeric constructs of Figures 5 to 14 (SEQ I.D. NO: 2);

Figure 4 shows the amino acid sequence of Figure 3 (SEQ I.D. NO: 3);

Figure 5 shows the nucleotide sequence of chimaeric construct A (SEQ I.D. NO: 4);

Figure 6 shows the amino acid sequence of chimaeric construct A (SEQ I.D. NO: 5);

Figure 7 shows the nucleotide sequence of chimaeric construct C (SEQ I.D. NO: 6);

Figure 8 shows the amino acid sequence of chimaeric construct C (SEQ I.D. NO: 7);

Figure 9 shows the nucleotide sequence of chimaeric construct E (SEQ I.D. NO: 8);

Figure 10 shows the amino acid sequence of chimaeric construct E (SEQ I.D. NO: 9);

Figure 11 shows the nucleotide sequence of chimaeric construct F (SEQ I.D. NO: 10);

Figure 12 shows the amino acid sequence of chimaeric construct F (SEQ I.D. NO: 11);

Figure 13 shows the nucleotide sequence of chimaeric construct H (SEQ I.D. NO: 12);

Figure 14 shows the amino acid sequence of chimaeric construct H (SEQ I.D. NO: 13);

Figure 15 shows a diagrammatic representation of the chimaeric construct A;

Figure 16 shows a diagrammatic representation of the chimaeric construct C;

Figure 17 shows a diagrammatic representation of the chimaeric construct E;

Figure 18 shows a diagrammatic representation of the chimaeric construct F;

Figure 19 shows a diagrammatic representation of the chimaeric construct H;

Figure 20 shows data obtained from end point titrations of mice inoculated with chimaeric VLPs obtained from construct A;

Figure 21 shows data obtained from end point titrations of mice inoculated with chimaeric VLPs obtained from construct C;

Figure 22 shows data obtained from end point titrations of mice inoculated with chimaeric VLPs obtained from construct E;

Figure 23 shows data obtained from end point titrations of mice inoculated with chimaeric VLPs obtained from construct F;

Figure 24 shows data obtained from end point titrations of mice inoculated with chimaeric VLPs obtained from construct H;

Figure 25 shows data obtained from end point titrations of mice inoculated with VLPs obtained from a non-chimaeric HPV 16 L1 (SA-opt);

Figure 26 shows the response of mice to boosters following initial immunisation;

Figure 27 shows the nucleotide sequence of the L2 peptide which was inserted into the L1 sequence (SEQ I.D. NO: 14); and

Figure 28 shows the amino acid sequence of the L2 peptide which was inserted into the L1 sequence (SEQ I.D. NO: 1).

DETAILED DESCRIPTION OF AN EMBODIMENT OF THE INVENTION

Design of chimaeric constructs

Primers encoding the peptide:

LVEETSFIDAGAP (SEQ I.D. NO: 1)

which is a common neutralising epitope for HPV 6 and 16 in the region (aa) 108-120 of the HPV minor capsid protein, L2, were synthesised.

According to the HPV 16 L1 monomer structure published by Chen et al. (2000) (Figure 2), various surface loops and regions are exposed when pentamers and high order structures are formed.

Based on the epitope mapping of the V5 antibody (neutralising antibody raised to L1), various regions/loops were selected for the insertion of the L2 peptide so as to maintain the V5 antibody binding region of the L1 VLPs.

The major antigenic region (V5 binding region) of the L1 molecule has been mapped with amino acid residues A266, (with F50 being located beneath the surface residue V271) and S282 (Roden *et al.*, 1996, White *et al.*, 1999). Based on these residues, the inventors decided not to alter the B loop of the molecule, as this would alter the antigenic region and possibly destroy vital L1 epitopes.

The following L1 regions were therefore selected for insertion of the L2 peptide:

- A: E-F loop (SEQ I.D. NO: 4 and 5)
- C: D-E loop (SEQ I.D. NO: 6 and 7)
- E: region between the h4 helix and J region of L1 (SEQ I.D. NO: 8 and 9)
- F: h4 helix (SEQ I.D. NO: 10 and 11)
- H: internal C-D loop (SEQ I.D. NO: 12 and 13)

Synthesis of chimaeric constructs

Chimaeric constructs were prepared by PCR with the primers being designed with the 3' end coding for the L2 peptide. The HPV 16 SA-opt L1 gene (Figures 3 and 4) cloned in the pSK plasmid vector was used as a template. The following DNA sequence was used to encode the L2 peptide:
5'-TTAGTGGAAGAAACTAGTTTTATTGATGCTGGTGCACCA-3' (SEQ I.D. NO: 14).

The L2 peptide was inserted into the gene by replacing the regions shown in Table 1. This method of replacing the existing nucleotides, rather than merely inserting the L2 nucleotides into the L1 sequence without any replacement, is advantageous in that disturbances of the tertiary structure are kept to a minimum, and the possibility of steric effects or interference with antibody binding to nearby sequences due to extra peptide "loops" is minimised.

The position of the inserted L2 epitope is depicted for the chimaeric constructs in figures 15 to 19 respectively.

The chimaeric genes from the sequenced pSK constructs were cloned into a pFastbac1 vector (Sal I / Xba I site). The DNA from the pFastbac1 clones was used to transfect DH10bac cells to prepare bacmid clones.

The chimaeric constructs were expressed in insect cells using the Bac-to-Bac® baculovirus expression system (Life Technologies).

Table 1: Insertion of L2 peptide

Chimaeric construct	Nucleotide position of L2 peptide	Shown in Figure No. (SEQ I.D. NO)	Amino acid position of L2 peptide	Shown in Figure No. (SEQ I.D. NO)
A	520 - 558	5 (4)	174-186	6 (5)
C	391 -429	7 (6)	131-143	8 (7)
E	1291 -1329	9 (8)	431-443	10 (9)
F	1240 - 1278	11 (10)	414-426	12 (11)
H	241 -279	13 (12)	81-93	14 (13)

The bacmid DNA was transferred into *sf21 (Spodoptera frugiperda)* insect cells using cellfectin. The basic Bac-to-Bac® protocol was followed to amplify the recombinant virus and infect the *sf21* insect cells for expression of the chimaeric VLPs.

A basic HPV 16 L1 VLP purification protocol was followed. The infected insect cells were spun down at 3 000 rpm and resuspended in phosphate buffered saline (PBS) with 0.5M NaCl. The suspension was sonicated 4 times at 5 second intervals. This was then overlayed

onto a 40% sucrose cushion and pelleted at 100 000 x g for 3 hrs. The pellet was resuspended in CsCl buffer (PBS with 0.4g/ml CsCl) and resuspended by drawing up through 18 and 26 gauge needles to reduce the viscosity before sonication (4 times at 5 second intervals). The suspension was centrifuged at 100 000 x g at 10°C for 24 hrs.

No distinct bands were observed in CsCl gradients. 500 µl fractions were therefore collected and analysed by ELISA using conformation specific V5 and D9 (linear epitope) monoclonal antibodies. Fractions that were found to react to the V5 and/or D9 antibodies were pooled and dialysed against PBS at 4°C overnight.

Results

Western blots showed that a polyclonal L2 antibody raised to the peptide in rabbits (obtained from Dr. Neil Christensen of The Jake Gittlen Cancer Research Institute, The Milton S. Hershey Medical Centre, Hersey Pennsylvania, USA) bound to the chimaeric L1 particles (55 kD), showing that the L2 epitope is expressed by the chimaeric constructs.

Antibody characterisation of the purified VLPs was carried out by ELISA using a panel of antibodies provided by Dr. Neil Christensen (Chistensen *et al.*, 1996, 2001). Table 2 summarises the data.

Table 2

	V5	E70	U4	9A	D9	I23	L2
A	+	-	+	+	+	+	+
C	-	-	+	+	+	+	+
E	+	+	+	+	+	+	+
F	+	+	+	+	+	+	+
H	-	-	-	-	+	+	+
SA-opt L1	+	+	+	+	-	+	-

A brief description of the antibodies and their binding regions is given in table 3 below.

Table 3

V5	Monoclonal, conformation specific antibody; aa 266 and 282 being critical
E70	Monoclonal, conformation specific antibody; aa 50, 266 and 282 being critical
U4	Monoclonal, conformation specific antibody

9A	Monoclonal, binds a linear region in the are aa 1-171
D9	Monoclonal, binds denatured L1 protein
I23	Monoclonal, binds in the region aa 111-130
L2	Polyclonal antibody that binding the L2 epitope (aa108-120)

Electron microscopy of the chimaeric particles

EM results showed that the particles formed from the chimaeric constructs are not identical to those produced by the wild type HPV L1 gene. The particles formed are mainly in a partially broken down or partially disassembled state and are generally seen to clump together

Animal experimentation with chimaeric antigen

Six sets of 5 Balb/c mice were used for the animal experimentation to determine if inoculation with the chimaeric VLPs elicited an immune response. Chimaeric VLPs produced from the 5 chimaeric constructs and VLPs produced from a non-chimaeric HPV 16 L1 (SA-opt) were injected at a concentration of 100 µg at two sub-cutaneous sites. The animals were inoculated at weeks 0, 2 and 4. Blood samples and vaginal washes were taken at various time periods.

The mouse sera from each group was pooled and analysed by ELISA using VLPs produced in insect cells by recombinant baculoviruses. End point titrations for each group of mice were carried out to determine the extent of the response and give a better reflection of the response (Figures 20 to 26).

The results indicate that a high immune response was achieved.

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2003232951 12 Jun 2009

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method for producing a chimaeric human papillomavirus (HPV) L1 polypeptide containing a HPV L2 peptide, the method comprising the steps of:

introducing a DNA sequence coding for the L2 peptide into a DNA sequence coding for the L1 polypeptide;

introducing the DNA sequence including the sequences for the L1 polypeptide and L2 peptide into a host cell in which the DNA sequence can be expressed;

causing expression of the DNA sequence; and

recovering the resulting chimaeric L1 polypeptide which includes the L2 peptide.

2. A method according to claim 1, wherein the HPV L2 peptide has the following amino acid sequence: LVEETSFIDAGAP (SEQ I.D. NO: 1), or a sequence which is a modification or derivative thereof, with the proviso that the modified or derived sequence has at least 80% homology to the sequence of SEQ I.D. NO: 1 and codes for a peptide capable of eliciting an immunogenic response against HPV.

3. A method according to claims 1 or 2, wherein nucleotides of the L1 DNA sequence are replaced by the nucleotides of the L2 DNA sequence.

4. A method according to any one of claims 1 to 3, wherein the chimaeric polypeptide has an amino acid sequence set out in any one of Figures 6, 8, 10, 12 and 14 (SEQ I.D. NOS: 5, 7, 9, 11 and 13), or a sequence which is at least 80% homologous to any one of the sequences set out in SEQ I.D. NOS: 5, 7, 9, 11 and 13 and which is capable of eliciting an immunogenic response against HPV.

5. A method according to any one of claims 1 to 4, wherein the DNA sequence coding for the chimaeric polypeptide has a DNA sequence set out in any one of Figures 5, 7, 9, 11 and 13 (SEQ I.D. NOS: 4, 6, 8, 10 and 12), or a sequence having at least 80% homology to any one of these sequences and which codes for a polypeptide capable of eliciting an immunogenic response against HPV.

6. A method according to any one of claims 1 to 5, wherein the chimaeric L1 polypeptide is capable of assembling into virus-like particles and/or capsomers.

7. A method according to claim 6, wherein the virus-like particles or capsomers are immunogenic.

8. A chimaeric human papilloma virus (HPV) L1 DNA sequence into which a DNA sequence coding for a HPV L2 peptide (SEQ I.D. NO: 1) has been inserted, which is capable of expressing the HPV L2 peptide.

9. A HPV L1 DNA sequence according to claim 8, wherein nucleotides of the L1 DNA sequence are replaced by the nucleotides of the L2 DNA sequence.

10. A human papillomavirus (HPV) DNA sequence having a DNA sequence set out in any one of SEQ I.D. NOS: 4, 6, 8, 10 and 12, or a DNA sequence which is a modification or derivative thereof, with the proviso that the modified or derived DNA sequence has at least 80% homology any one of SEQ I.D. NOS: 4, 6, 8, 10 and 12 and codes for a chimeric L1 peptide presenting an HPV L2 peptide, the chimeric L1 peptide being capable of eliciting an immunogenic response against HPV.

11. A vector including a DNA sequence as described in any one of claims 8 to 10.

12. A host cell including a vector as claimed in claim 11.

13. A chimaeric human papilloma virus (HPV) L1 polypeptide including a peptide having the amino acid sequence:

LVEETSFIDAGAP (SEQ I.D. NO: 1).

14. A polypeptide according to claim 13, which is a chimaeric HPV L1 virus-like particle or capsomer.

15. A human papilloma virus (HPV) polypeptide having an amino acid sequence set out in any one of SEQ I.D. NOS: 5, 7, 9, 11 and 13, or a sequence which is a modification or derivative thereof, with the proviso that the modified or derived amino acid sequence has at least 80% homology to any one of the sequences set out in SEQ I.D. NOS: 5, 7, 9, 11 and 13 and is a chimeric L1 peptide presenting an HPV L2 peptide, the chimeric L1 peptide being capable of eliciting an immunogenic response against HPV.

16. A method for producing a chimaeric human papillomavirus (HPV) L1 polypeptide containing a heterologous peptide, the method comprising the steps of:

introducing a DNA sequence coding for the heterologous peptide into a DNA sequence coding for the L1 polypeptide at any one of nucleotide positions 241-279, 391-429, 520-558, 1240-1278 or 1291-1329;

introducing the DNA sequence including the sequences for the L1 polypeptide and heterologous peptide into a host cell in which the DNA sequence can be expressed;

causing expression of the DNA sequence; and

recovering the resulting chimaeric L1 polypeptide which includes the heterologous peptide.

17. A method according to claim 16, wherein the heterologous peptide sequence is any other HPV sequence or is derived from any antigenic epitope, B-cell or T-cell specific.

18. A method according to either one of claims 16 or 17, wherein nucleotides of the L1 DNA sequence are replaced by the nucleotides of the heterologous DNA sequence.

19. A vaccine including a chimaeric human papilloma virus (HPV) L1 polypeptide as described in any one of claims 13 to 15 or a DNA sequence as described in any one of claims 8 to 10.

20. A vaccine according to claim 19, which is for use in the prophylactic or therapeutic treatment of HPV infection.

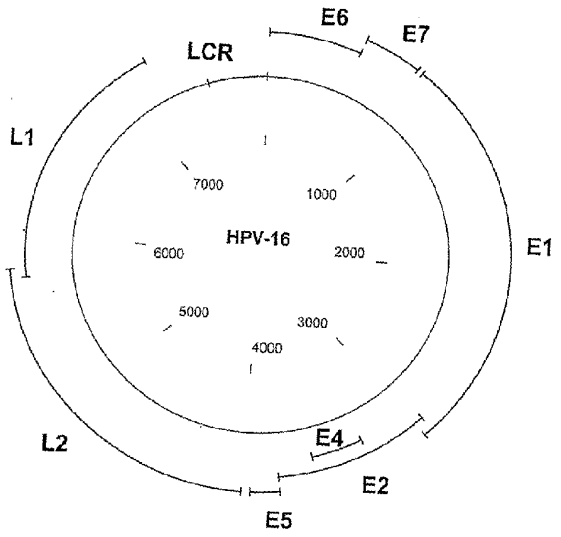
E6
E7
LCR
L1
1
7000
1000
HPV-16
6000
2000
E1
5000
3000
4000
E4
L2
E2
E5

Figure 1

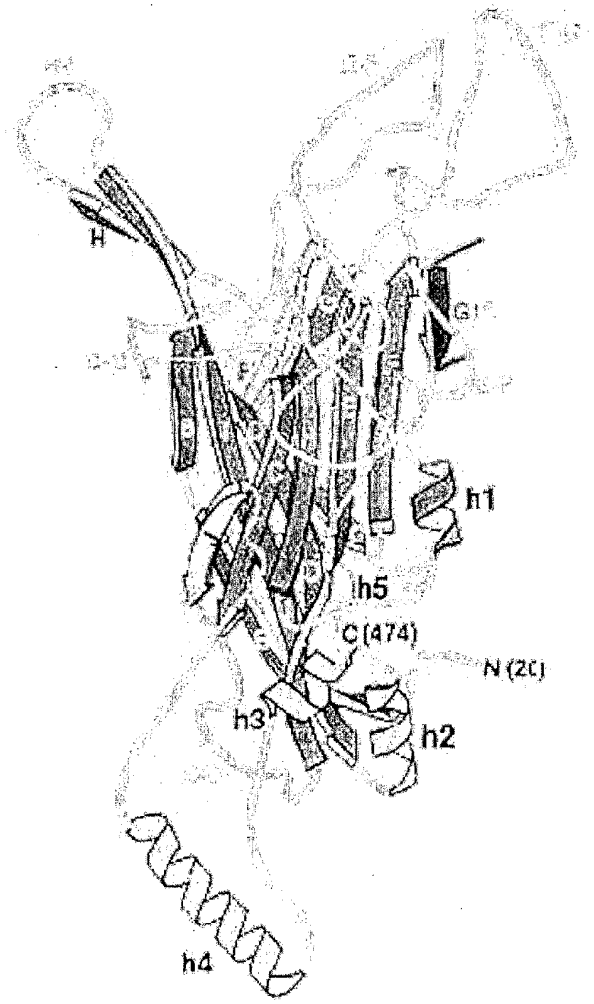

Figure 2 (Chen et al., 2000 and 2001)

```
SEQ  Sa-11: 1518 bp;
Composition  486  A; 293  C; 287  G; 452  T; 0 OTHER
Percentage:  32%  A; 19%  C; 19%  G; 30%  T; 0%OTHER

Molecular Weight (kDa): ssDNA: 468.63    dsDNA: 935.7
ORIGIN
1       ATGTCTCTTT GGCTGCCTAG TGAGGCCACT GTCTACTTGC CTCCTGTCCC AGTATCTAAG
61      GTTGTAAGCA CGGATGAATA TGTTGCACGC ACAAACATAT ATTATCATGC AGGGACATCC
121     AGACTACTTG CAGTTGGACA TCCCTATTTT CCTATTAAAA AACCTAACAA TAACAAAATA
181     TTAGTTCCTA AAGTATCAGG ATTACAATAC AGGGTATTTA GAATACATTT ACCTGACCCC
241     AATAAGTTTG GTTTTCCTGA CACCTCATTT TATAATCCAG ATACACAGCG GCTGGTTTGG
301     GCCTGTGTAG GTGTTGAGGT AGGCCGTGGT CAGCCATTAG GTGTGGGCAT TAGTGGCCAT
361     CCTTTATTAA ATAAATTGGA TGACACAGAA AATGCTAGTG CTTATGCAGC AAATGCAGGT
421     GTGGATAATA GAGAATGTAT ATCTATGGAT TACAAACAAA CACAATTGTG TTTAATTGGT
481     TGCAAACCAC CTATAGGGGA ACACTGGGGC AAAGGATCCC CATGTACCAA TGTTGCAGTA
541     AATCCAGGTG ATTGTCCACC ATTAGAGTTA ATAAACACAG TTATTCAGGA TGGTGATATG
601     GTTGATACTG GCTTTGGTGC TATGGACTTT ACTACATTAC AGGCTAACAA AAGTGAAGTT
661     CCACTGGATA TTTGTACATC TATTTGCAAA TATCCAGATT ATATTAAAAT GGTGTCAGAA
721     CCATATGGCG ACAGCTTATT TTTTTATTTA CGGAGGGAAC AAATGTTTGT TAGACATTTA
781     TTTAATAGGG CTGGTACTGT TGGTGAAAAT GTACCAGACG ATTTATACAT TAAAGGCTCT
841     GGGTCTACTG CAAATTTAGC CAGTTCAAAT TATTTTCCTA CACCTAGTGG TTCTATGGTT
901     ACCTCTGATG CCCAAAIATT CAATAAACCT TATTGGTTAC AACGAGCACA GGGCCACAAT
961     AATGGCATTT GTTGGGGTAA CCAACTATTT GTTACTGTTG TTGATACTAC ACGCAGTACA
1021    AATATGTCAT TATGTGCTGC CATATCTACT TCAGAAACTA CATATAAAAA TACTAACTTT
1081    AAGGAGTACC TACGACATGG GGAGGAATAT GATTTACAGT TTATTTTTCA ACTGTGCAAA
1141    ATAACCTTAA CTGCAGACGT TATGACATAC ATACATTCTA TGAATTCCAC TATTTTGGAG
1201    GACTGGAATT TTGGTCTACA ACCTCCCCCA GGAGGCACAC TAGAAGATAC TTATAGGTTT
1261    GTAACATCCC AGGCAATTGC TTGTCAAAAA CATACACCTC CAGCACCTAA AGAAGATCCC
1321    CTTAAAAAAT ACACTTTTTG GGAAGTAAAT TTAAAGGAAA AGTTTTCTGC AGACCTAGAT
1381    CAGTTTCCTT TAGGACGCAA ATTTTTACTA CAAGCAGGAT TGAAGGCCAA ACCAAAATTT
1441    ACATTAGGAA AACGAAAAGC TACACCCACC ACCTCATCTA CCTCTACAAC TGCTAAACGC
1501    AAAAAACGTA AGCTGTAA
```

Figure 3 (SEQ I.D. NO: 2)

```
Translation of Sa-11(1-1518)
Universal code
Total amino acid number: 505, MW=56196
Max ORF: 1-1515, 505 AA, MW=56196

ORIGIN
1         MSLWLPSEAT VYLPPVPVSK VVSTDEYVAR TNIYYHAGTS RLLAVGHPYF PIKKPNNNKI
61        LVPKVSGLQY RVFRIHLPDP NKFGFPDTSF YNPDTQRLVW ACVGVEVGRG QPLGVGISGH
121       PLLNKLDDTE NASAYAANAG VDNRECISMD YKQTQLCLIG CKPPIGEHWG KGSPCTNVAV
181       NPGDCPPLEL INTVIQDGDM VDTGFGAMDF TTLQANKSEV PLDICTSICK YPDYIKMVSE
241       PYGDSLFFYL RREQMFVRHL FNRAGAVGEN VPDDLYIKGS GSTANLASSN YFPTPSGSMV
301       TSDAQIFNKP YWLQRAQGHN NGICWGNQLF VTVVDTTRST NMSLCAAIST SETTYKNTNF
361       KEYLRHGEEY DLQFIFQLCK ITLTADVMTY IHSMNSTILE DWNFGLQPPP GGTLEDTYRF
421       VTSQAIACQK HTPPAPKEDP LKKYTFWEVN LKEKFSADLD QFPLGRKFLL QAGLKAKPKF
481       TLGKRKATPT TSSTSTTAKR KKRKL*
```

Figure 4 (SEQ I.D. NO: 3)

```
SEQ  Sa-Aopt: 1518 bp;
Composition  486  A; 289  C; 289  G; 454  T; 0 OTHER
Percentage:  32%  A; 19%  C; 19%  G; 30%  T; 0%OTHER

Molecular Weight (kDa): ssDNA: 468.74    dsDNA: 935.7
ORIGIN
1     ATGTCTCTTT GGCTGCCTAG TGAGGCCACT GTCTACTTGC CTCCTGTCCC AGTATCTAAG
61    GTTGTAAGCA CGGATGAATA TGTTGCACGC ACAAACATAT ATTATCATGC AGGGACATCC
121   AGACTACTTG CAGTTGGACA TCCCTATTTT CCTATTAAAA AACCTAACAA TAACAAAATA
181   TTAGTTCCTA AAGTATCAGG ATTACAATAC AGGGTATTTA GAATACATTT ACCTGACCCC
241   AATAAGTTTG GTTTTCCTGA CACCTCATTT TATAATCCAG ATACACAGCG GCTGGTTTGG
301   GCCTGTGTAG GTGTTGAGGT AGGCCGTGGT CAGCCATTAG GTGTGGGCAT TAGTGGCCAT
361   CCTTTATTAA ATAAATTGGA TGACACAGAA AATGCTAGTG CTTATGCAGC AAATGCAGGT
421   GTGGATAATA GAGAATGTAT ATCTATGGAT TACAAACAAA CACAATTGTG TTTAATTGGT
481   TGCAAACCAC CTATAGGGGA ACACTGGGGC AAAGGATCCT TAGTGGAAGA AACTAGTTTT
541   ATTGATGCTG GTGCACCACC ATTAGAGTTA ATAAACACAG TTATTCAGGA TGGTGATATG
601   GTTGATACTG GCTTTGGTGC TATGGACTTT ACTACATTAC AGGCTAACAA AAGTGAAGTT
661   CCACTGGATA TTTGTACATC TATTTGCAAA TATCCAGATT ATATTAAAAT GGTGTCAGAA
721   CCATATGGCG ACAGCTTATT TTTTTATTTA CGGAGGGAAC AAATGTTTGT TAGACATTTA
781   TTTAATAGGG CTGGTACTGT TGGTGAAAAT GTACCAGACG ATTTATACAT TAAAGGCTCT
841   GGGTCTACTG CAAATTTAGC CAGTTCAAAT TATTTTCCTA CACCTAGTGG TTCTATGGTT
901   ACCTCTGATG CCCAAATATT CAATAAACCT TATTGGTTAC AACGAGCACA GGGCCACAAT
961   AATGGCATTT GTTGGGGTAA CCAACTATTT GTTACTGTTG TTGATACTAC ACGCAGTACA
1021  AATATGTCAT TATGTGCTGC CATATCTACT TCAGAAACTA CATATAAAAA TACTAACTTT
1081  AAGGAGTACC TACGACATGG GGAGGAATAT GATTTACAGT TTATTTTTCA ACTGTGCAAA
1141  ATAACCTTAA CTGCAGACGT TATGACATAC ATACATTCTA TGAATTCCAC TATTTTGGAG
1201  GACTGGAATT TTGGTCTACA ACCTCCCCCA GGAGGCACAC TAGAAGATAC TTATAGGTTT
1261  GTAACATCCC AGGCAATTGC TTGTCAAAAA CATACACCTC CAGCACCTAA AGAAGATCCC
1321  CTTAAAAAAT ACACTTTTTG GGAAGTAAAT TTAAAGGAAA AGTTTTCTGC AGACCTAGAT
1381  CAGTTTCCTT TAGGACGCAA ATTTTTACTA CAAGCAGGAT TGAAGGCCAA ACCAAAATTT
1441  ACATTAGGAA AACGAAAAGC TACACCCACC ACCTCATCTA CCTCTACAAC TGCTAAACGC
1501  AAAAAACGTA AGCTGTAA
```

Figure 5 (SEQ I.D. NO: 4)

```
Translation of ChiA(1-1518)
Universal code
Total amino acid number: 505, MW=56288
Max ORF: 1-1515, 505 AA, MW=56288

ORIGIN
1         MSLWLPSEAT VYLPPVPVSK VVSTDEYVAR TNIYYHAGTS RLLAVGHPYF PIKKPNNNKI
61        LVPKVSGLQY RVFRIHLPDP NKFGFPDTSF YNPDTQRLVW ACVGVEVGRG QPLGVGISGH
121       PLLNKLDDTE NASAYAANAG VDNRECISMD YKQTQLCLIG CKPPIGEHWG KGSLVEETSF
181       IDAGAPPLEL INTVIQDGDM VDTGFGAMDF TTLQANKSEV PLDICTSICK YPDYIKMVSE
241       PYGDSLFFYL RREQMFVRHL FNRAGTVGEN VPDDLYIKGS GSTANLASSN YFPTPSGSMV
301       TSDAQIFNKP YWLQRAQGHN NGICWGNQLF VTVVDTTRST NMSLCAAIST SETTYKNTNF
361       KEYLRHGEEY DLQFIFQLCK ITLTADVMTY IHSMNSTILE DWNFGLQPPP GGTLEDTYRF
421       VTSQAIACQK HTPPAPKEDP LKKYTFWEVN LKEKFSADLD QFPLGRKFLL QAGLKAKPKF
481       TLGKRKATPT TSSTSTTAKR KKRKL*
```

Figure 6 (SEQ I.D. NO: 5)

```
SEQ  Sa-Copt: 1518 bp;
Composition  485  A; 293  C; 286  G; 454  T; 0 OTHER.
Percentage:  32% A; 19%  C; 19%  G; 30%  T; 0%OTHER

Molecular Weight (kDa): ssDNA: 468.59    dsDNA: 935.7
ORIGIN
1     ATGTCTCTTT GGCTGCCTAG TGAGGCCACT GTCTACTTGC CTCCTGTCCC AGTATCTAAG
61    GTTGTAAGCA CGGATGAATA TGTTGCACGC ACAAACATAT ATTATCATGC AGGGACATCC
121   AGACTACTTG CAGTTGGACA TCCCTATTTT CCTATTAAAA AACCTAACAA TAACAAAATA
181   TTAGTTCCTA AAGTATCAGG ATTACAATAC AGGGTATTTA GAATACATTT ACCTGACCCC
241   AATAAGTTTG GTTTTCCTGA CACCTCATTT TATAATCCAG ATACACAGCG GCTGGTTTGG
301   GCCTGTGTAG GTGTTGAGGT AGGCCGTGGT CAGCCATTAG GTGTGGGCAT TAGTGGCCAT
361   CCTTTATTAA ATAAATTGGA TGACACAGAA TTAGTGGAAG AAACTAGTTT TATTGATGCT
421   GGTGCACCAA GAGAATGTAT ATCTATGGAT TACAAACAAA CACAATTGTG TTTAATTGGT
481   TGCAAACCAC CTATAGGGGA ACACTGGGGC AAAGGATCCC CATGTACCAA TGTTGCAGTA
541   AATCCAGGTG ATTGTCCACC ATTAGAGTTA ATAAACACAG TTATTCAGGA TGGTGATATG
601   GTTGATACTG GCTTTGGTGC TATGGACTTT ACTACATTAC AGGCTAACAA AAGTGAAGTT
661   CCACTGGATA TTTGTACATC TATTTGCAAA TATCCAGATT ATATTAAAAT GGTGTCAGAA
721   CCATATGGCG ACAGCTTATT TTTTTATTTA CGGAGGGAAC AAATGTTTGT TAGACATTTA
781   TTTAATAGGG CTGGTACTGT TGGTGAAAAT GTACCAGACG ATTTATACAT TAAAGGCTCT
841   GGGTCTACTG CAAATTTAGC CAGTTCAAAT TATTTTCCTA CACCTAGTGG TTCTATGGTT
901   ACCTCTGATG CCCAAATATT CAATAAACCT TATTGGTTAC AACGAGCACA GGGCCACAAT
961   AATGGCATTT GTTGGGGTAA CCAACTATTT GTTACTGTTG TTGATACTAC ACGCAGTACA
1021  AATATGTCAT TATGTGCTGC CATATCTACT TCAGAAACTA CATATAAAAA TACTAACTTT
1081  AAGGAGTACC TACGACATGG GGAGGAATAT GATTTACAGT TTATTTTTCA ACTGTGCAAA
1141  ATAACCTTAA CTGCAGACGT TATGACATAC ATACATTCTA TGAATTCCAC TATTTTGGAG
1201  GACTGGAATT TTGGTCTACA ACCTCCCCCA GGAGGCACAC TAGAAGATAC TTATAGGTTT
1261  GTAACATCCC AGGCAATTGC TTGTCAAAAA CATACACCTC CAGCACCTAA AGAAGATCCC
1321  CTTAAAAAAT ACACTTTTTG GGAAGTAAAT TTAAAGGAAA AGTTTTCTGC AGACCTAGAT
1381  CAGTTTCCTT TAGGACGCAA ATTTTTACTA CAAGCAGGAT TGAAGGCCAA ACCAAAATTT
1441  ACATTAGGAA AACGAAAAGC TACACCCACC ACCTCATCTA CCTCTACAAC TGCTAAACGC
1501  AAAAAACGTA AGCTGTAA
```

Figure 7 (SEQ I.D. NO: 6)

```
Translation of ChiC(1-1518)
Universal code
Total amino acid number: 505, MW=56337
Max ORF: 1-1515, 505 AA, Mw=56337

ORIGIN
1        MSLWLPSEAT VYLPPVPVSK VVSTDEYVAR TNIYYHAGTS RLLAVGHPYF PIKKPNNNKI
61       LVPKVSGLQY RVFRIHLPDP NKFGFPDTSF YNPDTQRLVW ACVGVEVGRG QPLGVGISGH
121      PLLNKLDDTE LVEETSFIDA GAPRECISMD YKQTQLCLIG CKPPIGEHWG KGSPCTNVAV
181      NPGDCPPLEL INTVIQDGDM VDTGFGAMDF TTLQANKSEV PLDICTSICK YPDYIKMVSE
241      PYGDSLFFYL RREQMFVRHL FNRAGTVGEN VPDDLYIKGS GSTANLASSN YFPTPSGSMV
301      TSDAQIFNKP YWLQRAQGHN NGICWGNQLF VTVVDTTRST NMSLCAAIST SETTYKNTNF
361      KEYLRHGEEY DLQFIFQLCK ITLTADVMTY IHSMNSTILE DWNFGLQPPP GGTLEDTYRF
421      VTSQAIACQK HTPPAPKEDP LKKYTFWEVN LKEKFSADLD QFPLGRKFLL QAGLKAKPKF
481      TLGKRKATPT TSSTSTTAKR KKRKL*
```

Figure 8 (SEQ I.D. NO: 7)

```
SEQ  Sa-eopt: 1518 bp;
Composition  480  A; 285  C; 294  G; 459  T; 0 OTHER
Percentage:  32%  A; 19%  C; 19%  G; 30%  T; 0%OTHER

Molecular Weight (kDa): ssDNA: 468.87    dsDNA: 935.7
ORIGIN
1     ATGTCTCTTT GGCTGCCTAG TGAGGCCACT GTCTACTTGC CTCCTGTCCC AGTATCTAAG
61    GTTGTAAGCA CGGATGAATA TGTTGCACGC ACAAACATAT ATTATCATGC AGGGACATCC
121   AGACTACTTG CAGTTGGACA TCCCTATTTT CCTATTAAAA AACCTAACAA TAACAAAATA
181   TTAGTTCCTA AAGTATCAGG ATTACAATAC AGGGTATTTA GAATACATTT ACCTGACCCC
241   AATAAGTTTG GTTTTCCTGA CACCTCATTT TATAATCCAG ATACACAGCG GCTGGTTTGG
301   GCCTGTGTAG GTGTTGAGGT AGGCCGTGGT CAGCCATTAG GTGTGGGCAT TAGTGGCCAT
361   CCTTTATTAA ATAAATTGGA TGACACAGAA AATGCTAGTG CTTATGCAGC AAATGCAGGT
421   GTGGATAATA GAGAATGTAT ATCTATGGAT TACAAACAAA CACAATTGTG TTTAATTGGT
481   TGCAAACCAC CTATAGGGGA ACACTGGGGC AAAGGATCCC CATGTACCAA TGTTGCAGTA
541   AATCCAGGTG ATTGTCCACC ATTAGAGTTA ATAAACACAG TTATTCAGGA TGGTGATATG
601   GTTGATACTG GCTTTGGTGC TATGGACTTT ACTACATTAC AGGCTAACAA AAGTGAAGTT
661   CCACTGGATA TTTGTACATC TATTTGCAAA TATCCAGATT ATATTAAAAT GGTGTCAGAA
721   CCATATGGCG ACAGCTTATT TTTTTATTTA CGGAGGGAAC AAATGTTTGT TAGACATTTA
781   TTTAATAGGG CTGGTACTGT TGGTGAAAAT GTACCAGACG ATTTATACAT TAAAGGCTCT
841   GGGTCTACTG CAAATTTAGC CAGTTCAAAT TATTTTCCTA CACCTAGTGG TTCTATGGTT
901   ACCTCTGATG CCCAAATATT CAATAAACCT TATTGGTTAC AACGAGCACA GGGCCACAAT
961   AATGGCATTT GTTGGGGTAA CCAACTATTT GTTACTGTTG TTGATACTAC ACGCAGTACA
1021  AATATGTCAT TATGTGCTGC CATATCTACT TCAGAAACTA CATATAAAAA TACTAACTTT
1081  AAGGAGTACC TACGACATGG GGAGGAATAT GATTTACAGT TTATTTTTCA ACTGTGCAAA
1141  ATAACCTTAA CTGCAGACGT TATGACATAC ATACATTCTA TGAATTCCAC TATTTTGGAG
1201  GACTGGAATT TTGGTCTACA ACCTCCCCCA GGAGGCACAC TAGAAGATAC TTATAGGTTT
1261  GTAACATCCC AGGCAATTGC TTGTCAAAAA TTAGTGGAAG AAACTAGTTT TATTGATGCT
1321  GGTGCACCAT ACACTTTTTG GGAAGTAAAT TTAAAGGAAA AGTTTTCTGC AGACCTAGAT
1381  CAGTTTCCTT TAGGACGCAA ATTTTTACTA CAAGCAGGAT TGAAGGCCAA ACCAAAATTT
1441  ACATTAGGAA AACGAAAAGC TACACCCACC ACCTCATCTA CCTCTACAAC TGCTAAACGC
1501  AAAAAACGTA AGCTGTAA
```

Figure 9 (SEQ I.D. NO: 8)

```
Translation of ChiE(1-1518)
Universal code
Total amino acid number: 505, Mw=56117
Max ORF: 1-1515, 505 AA, MW=56117

ORIGIN
1       MSLWLPSEAT VYLPPVPVSK VVSTDEYVAR TNIYYHAGTS RLLAVGHPYF PIKKPNNNKI
61      LVPKVSGLQY RVFRIHLPDP NKFGFPDTSF YNPDTQRLVW ACVGVEVGRG QPLGVGISGH
121     PLLNKLDDTE NASAYAANAG VDNRECISMD YKQTQLCLIG CKPPIGEHWG KGSPCTNVAV
181     NPGDCPPLEL INTVIQDGDM VDTGFGAMDF TTLQANKSEV PLDICTSICK YPDYIKMVSE
241     PYGDSLFFYL RREQMFVRHL FNRAGTVGEN VPDDLYIKGS GSTANLASSN YFPTPSGSMV
301     TSDAQIFNKP YWLQRAQGHN NGICWGNQLF VTYVDTTRST NMSLCAAIST SETTYKNTNF
361     KEYLRHGEEY DLQFIFQLCK ITLTADVMTY IHSMNSTILE DWNFGLQPPP GGTLEDTYRF
421     VTSQALACQK LVEETSFIDA GAPYTFWEVN LKEKFSADLD QFPLGRKFLL QAGLKAKPKF
481     TLGKRKATPT TSSTSTTAKR KKRKL*
```

Figure 10 (SEQ I.D. NO: 9)

```
SEQ  Sa-Fopt: 1518 bp;
Composition  484  A; 291  C; 290  G; 453  T; 0 OTHER
Percentage:  32%  A; 19%  C; 19%  G; 30%  T; 0%OTHER

Molecular Weight (kDa): ssDNA: 468.71    dsDNA: 935.7
ORIGIN
1     ATGTCTCTTT GGCTGCCTAG TGAGGCCACT GTCTACTTGC CTCCTGTCCC AGTATCTAAG
61    GTTGTAAGCA CGGATGAATA TGTTGCACGC ACAAACATAT ATTATCATGC AGGGACATCC
121   AGACTACTTG CAGTTGGACA TCCCTATTTT CCTATTAAAA AACCTAACAA TAACAAAATA
181   TTAGTTCCTA AAGTATCAGG ATTACAATAC AGGGTATTTA GAATACATTT ACCTGACCCC
241   AATAAGTTTG GTTTTCCTGA CACCTCATTT TATAATCCAG ATACACAGCG GCTGGTTTGG
301   GCCTGTGTAG GTGTTGAGGT AGGCCGTGGT CAGCCATTAG GTGTGGGCAT TAGTGGCCAT
361   CCTTTATTAA ATAAATTGGA TGACACAGAA AATGCTAGTG CTTATGCAGC AAATGCAGGT
421   GTGGATAATA GAGAATGTAT ATCTATGGAT TACAAACAAA CACAATTGTG TTTAATTGGT
481   TGCAAACCAC CTATAGGGGA ACACTGGGGC AAAGGATCCC CATGTACCAA TGTTGCAGTA
541   AATCCAGGTG ATTGTCCACC ATTAGAGTTA ATAAACACAG TTATTCAGGA TGGTGATATG
601   GTTGATACTG GCTTTGGTGC TATGGACTTT ACTACATTAC AGGCTAACAA AAGTGAAGTT
661   CCACTGGATA TTTGTACATC TATTTGCAAA TATCCAGATT ATATTAAAAT GGTGTCAGAA
721   CCATATGGCG ACAGCTTATT TTTTTATTTA CGGAGGGAAC AAATGTTTGT TAGACATTTA
781   TTTAATAGGG CTGGTACTGT TGGTGAAAAT GTACCAGACG ATTTATACAT TAAAGGCTCT
841   GGGTCTACTG CAAATTTAGC CAGTTCAAAT TATTTTCCTA CACCTAGTGG TTCTATGGTT
901   ACCTCTGATG CCCAAATATT CAATAAACCT TATTGGTTAC AACGAGCACA GGGCCACAAT
961   AATGGCATTT GTTGGGGTAA CCAACTATTT GTTACTGTTG TTGATACTAC ACGCAGTACA
1021  AATATGTCAT TATGTGCTGC CATATCTACT TCAGAAACTA CATATAAAAA TACTAACTTT
1081  AAGGAGTACC TACGACATGG GGAGGAATAT GATTTACAGT TTATTTTTCA ACTGTGCAAA
1141  ATAACCTTAA CTGCAGACGT TATGACATAC ATACATTCTA TGAATTCCAC TATTTTGGAG
1201  GACTGGAATT TTGGTCTACA ACCTCCCCCA GGAGGCACAT TAGTGGAAGA AACTAGTTTT
1261  ATTGATGCTG GTGCACCAGC TTGTCAAAAA CATACACCTC CAGCACCTAA AGAAGATCCC
1321  CTTAAAAAAT ACACTTTTTG GGAAGTAAAT TTAAAGGAAA AGTTTTCTGC AGACCTAGAT
1381  CAGTTTCCTT TAGGACGCAA ATTTTTACTA CAAGCAGGAT TGAAGGCCAA ACCAAAATTT
1441  ACATTAGGAA AACGAAAAGC TACACCCACC ACCTCATCTA CCTCTACAAC TGCTAAACGC
1501  AAAAAACGTA AGCTGTAA
```

Figure 11 (SEQ I.D. NO: 10)

```
Translation of ChiF(1-1518)
Universal code
Total amino acid number: 505, MW=56032
Max ORF: 1-1515, 505 AA, MW=56032

ORIGIN
1         MSLWLPSEAT VYLPPVPVSK VVSTDEYVAR TNIYYHAGTS RLLAVGHPYF PIKKPNNNKI
61        LVPKVSGLQY RVFRIHLPDP NKFGFPDTSF YNPDTQRLVW ACVGVEVGRG QPLGVGISGH
121       PLLNKLDDTE NASAYAANAG VDNRECISMD YKQTQLCLIG CKPPIGEHWG KGSPCTNVAV
181       NPGDCPPLEL INTVIQDGDM VDTGFGAMDF TTLQANKSEV PLDICTSICK YPDYIKMVSE
241       PYGDSLFFYL RREQMFVRHL FNRAGTVGEN VPDDLYIKGS GSTANLASSN YFPTPSGSMV
301       TSDAQIFNKP YWLQRAQGHN NGICWGNQLF VTVVDTTRST NMSLCAAIST SETTYKNTNF
361       KEYLRHGEEY DLQFIFQLCK ITLTADVMTY IHSMNSTILE DWNFGLQPPP GGTLVEETSF
421       IDAGAPACQK HTPPAPKEDP LKKYTFWEVN LKEKFSADLD QFPLGRKFLL QAGLKAKPKF
481       TLGKRKATPT TSSTSTTAKR KKRKL*
```

Figure 12 (SEQ I.D. NO: 11)

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SEQ  Sa-hopt: 1518 bp;
Composition  486  A; 290  C; 293  G; 449  T; 0 OTHER
Percentage:  32%  A; 19%  C; 19%  G; 30%  T; 0%OTHER

Molecular Weight (kDa): ssDNA: 468.82    dsDNA: 935.7
ORIGIN
1       ATGTCTCTTT GGCTGCCTAG TGAGGCCACT GTCTACTTGC CTCCTGTCCC AGTATCTAAG
61      GTTGTAAGCA CGGATGAATA TGTTGCACGC ACAAACATAT ATTATCATGC AGGGACATCC
121     AGACTACTTG CAGTTGGACA TCCCTATTTT CCTATTAAAA AACCTAACAA TAACAAAATA
181     TTAGTTCCTA AAGTATCAGG ATTACAATAC AGGGTATTTA GAATACATTT ACCTGACCCC
241     TTAGTGGAAG AAACTAGTTT TATTGATGCT GGTGCACCAG ATACACAGCG GCTGGTTTGG
301     GCCTGTGTAG GTGTTGAGGT AGGCCGTGGT CAGCCATTAG GTGTGGGCAT TAGTGGCCAT
361     CCTTTATTAA ATAAATTGGA TGACACAGAA AATGCTAGTG CTTATGCAGC AAATGCAGGT
421     GTGGATAATA GAGAATGTAT ATCTATGGAT TACAAACAAA CACAATTGTG TTTAATTGGT
481     TGCAAACCAC CTATAGGGGA ACACTGGGGC AAAGGATCCC CATGTACCAA TGTTGCAGTA
541     AATCCAGGTG ATTGTCCACC ATTAGAGTTA ATAAACACAG TTATTCAGGA TGGTGATATG
601     GTTGATACTG GCTTTGGTGC TATGGACTTT ACTACATTAC AGGCTAACAA AAGTGAAGTT
661     CCACTGGATA TTTGTACATC TATTTGCAAA TATCCAGATT ATATTAAAAT GGTGTCAGAA
721     CCATATGGCG ACAGCTTATT TTTTTATTTA CGGAGGGAAC AAATGTTTGT TAGACATTTA
781     TTTAATAGGG CTGGTACTGT TGGTGAAAAT GTACCAGACG ATTTATACAT TAAAGGCTCT
841     GGGTCTACTG CAAATTTAGC CAGTTCAAAT TATTTTCCTA CACCTAGTGG TTCTATGGTT
901     ACCTCTGATG CCCAAATATT CAATAAACCT TATTGGTTAC AACGAGCACA GGGCCACAAT
961     AATGGCATTT GTTGGGGTAA CCAACTATTT GTTACTGTTG TTGATACTAC ACGCAGTACA
1021    AATATGTCAT TATGTGCTGC CATATCTACT TCAGAAACTA CATATAAAAA TACTAACTTT
1081    AAGGAGTACC TACGACATGG GGAGGAATAT GATTTACAGT TTATTTTTCA ACTGTGCAAA
1141    ATAACCTTAA CTGCAGACGT TATGACATAC ATACATTCTA TGAATTCCAC TATTTTGGAG
1201    GACTGGAATT TTGGTCTACA ACCTCCCCCA GGAGGCACAC TAGAAGATAC TTATAGGTTT
1261    GTAACATCCC AGGCAATTGC TTGTCAAAAA CATACACCTC CAGCACCTAA AGAAGATCCC
1321    CTTAAAAAAT ACACTTTTTG GGAAGTAAAT TTAAAGGAAA AGTTTTCTGC AGACCTAGAT
1381    CAGTTTCCTT TAGGACGCAA ATTTTTACTA CAAGCAGGAT TGAAGGCCAA ACCAAAATTT
1441    ACATTAGGAA AACGAAAAGC TACACCCACC ACCTCATCTA CCTCTACAAC TGCTAAACGC
1501    AAAAAACGTA AGCTGTAA
```

Figure 13 (SEQ I.D. NO: 12)

```
Translation of ChiH(1-1518)
Universal code
Total amino acid number: 505, MW=56041
Max ORF: 1-1515, 505 AA, MW=56041

ORIGIN
1         MSLWLPSEAT VYLPPVPVSK VVSTDEYVAR TNIYYHAGTS RLLAVGHPYF PIKKPNNNKI
61        LVPKVSGLQY RVFRTHLPDP LVEETSFIDA GAPDTQRLVW ACVGVEVGRG QPLGVGISGH
121       PLLNKLDDTE NASAYAANAG VDNRECISMD YKQTQLCLIG CKPPIGEHWG KGSPCTNVAV
181       NPGDCPPLEL INTVIQDGDM VDTGFGAMDF TTLQANKSEV PLDICTSICK YPDYIKMVSE
241       PYGDSLFFYL RREQMFVRHL FNRAGTVGEN VPDDLYIKGS GSTANLASSN YFPTPSGSMV
301       TSDAQIFNKP YWLQRAQGHN NGICWGNQLF VTVVDTTRST NMSLCAAIST SETTYKNTNF
361       KEYLRHGEEY DLQFIFQLCK ITLTADVMTY IHSMNSTILE DWNFGLQPPP GGTLEDTYRF
421       VTSQAIACQK HTPPAPKEDP LKKYTFWEVN LKEKFSADLD QFPLGRKFLL QAGLKAKPKF
481       TLGKRKATPT TSSTSTTAKR KKRKL*
```

Figure 14 (SEQ I.D. NO: 13)

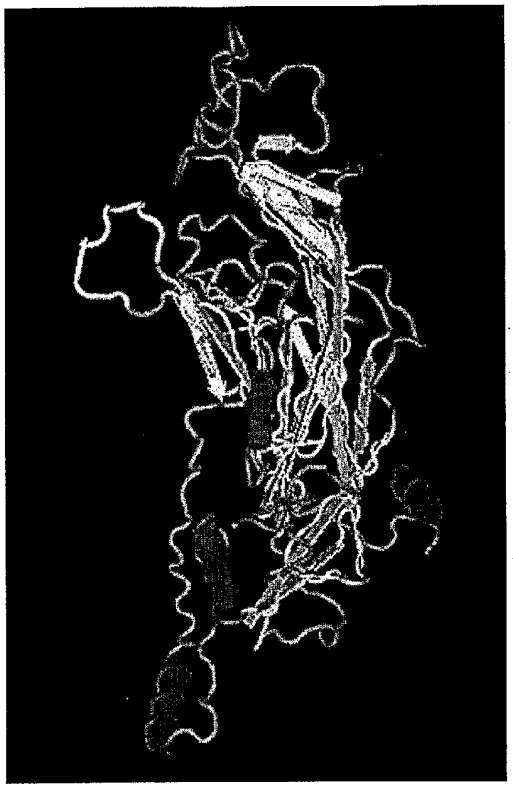

Figure 15

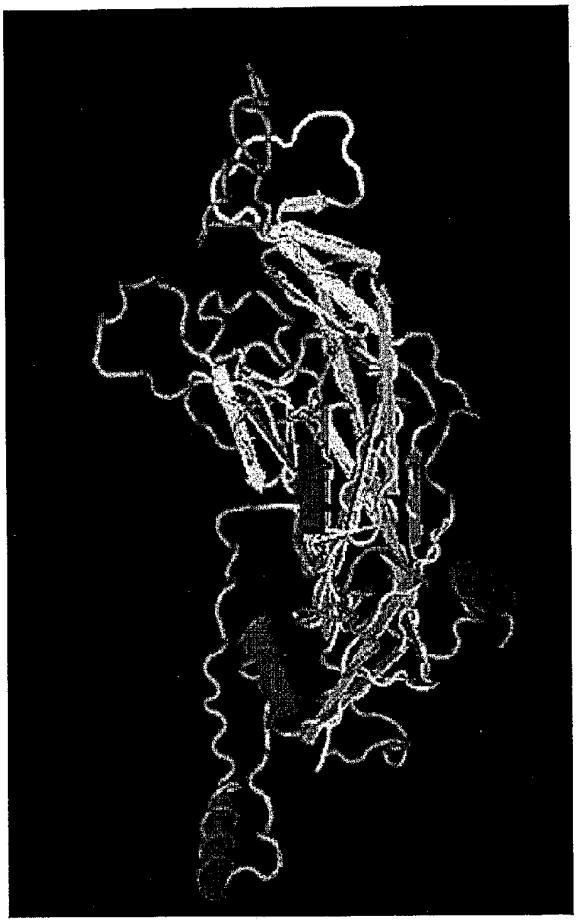

Figure 16

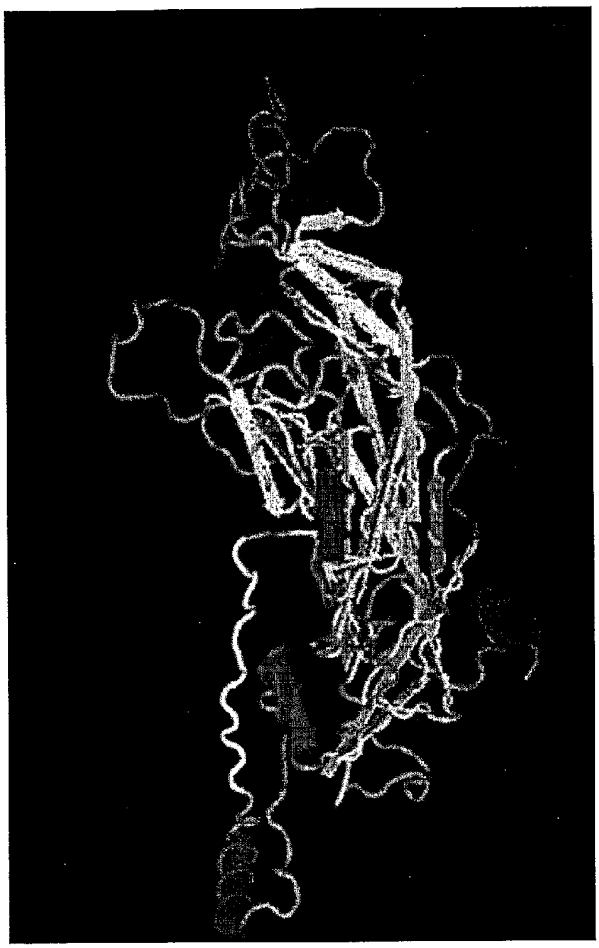

Figure 17

Figure 18

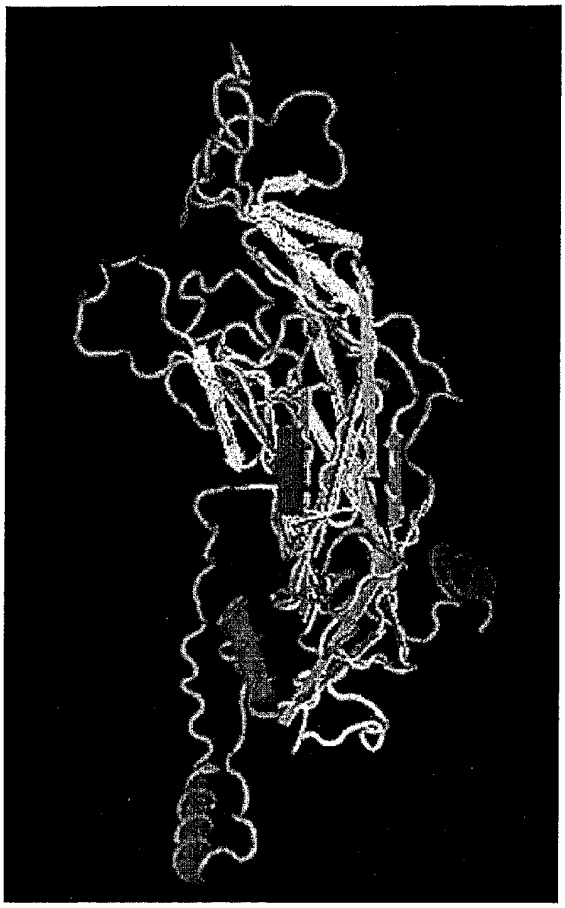

Figure 19

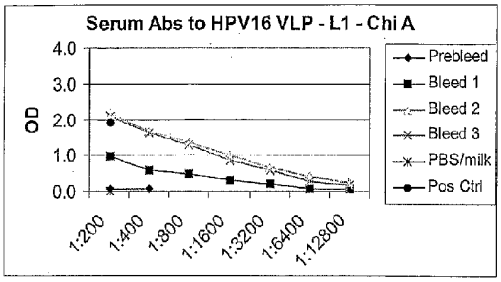

Figure 20

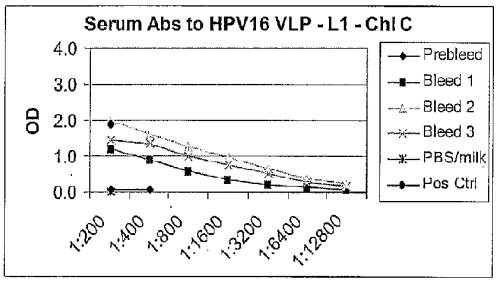

Figure 21

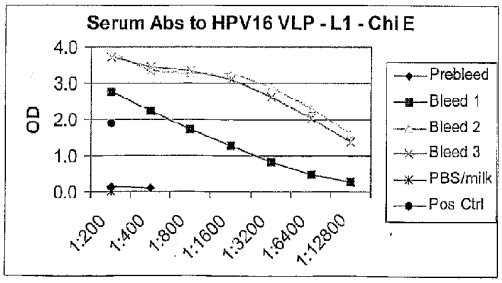

Figure 22

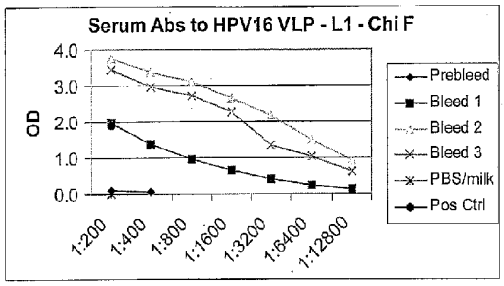

Figure 23

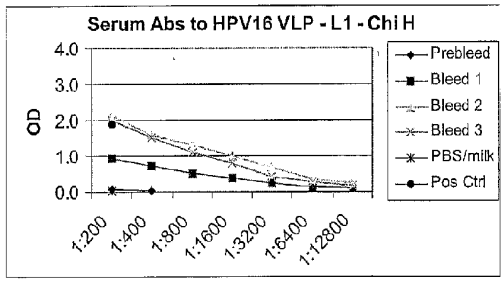

Figure 24

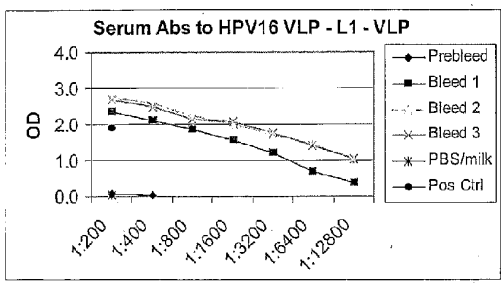

Figure 25

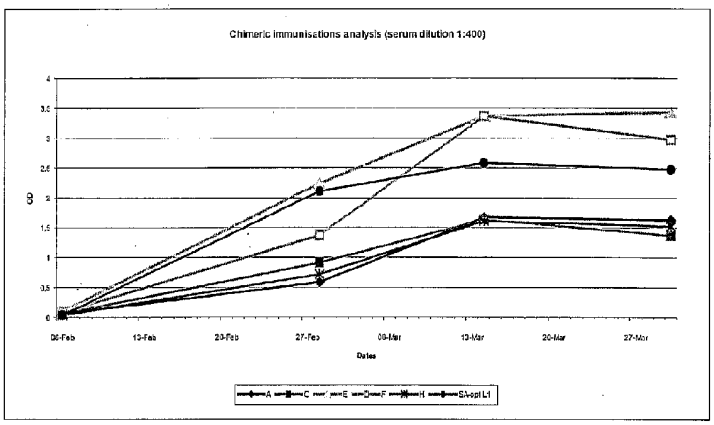

Figure 26

TTAGTGGAAG AAACTAGTTT TATTGATGCT GGTCCACCA

Figure 27 (SEQ I.D. NO: 14)

LVEETSFIDA GAP

Figure 28 (SEQ I.D. NO: 1)