HPV-16 AND -18 L1 VLP VACCINE

The present invention relates to virus-like particles (VLPs) from human papilloma virus (HPV) and to uses thereof in medicine, in particular to providing protection from infection and/or disease caused by heterologous HPV types.

Background of the Invention

Papillomaviruses are small DNA tumour viruses, which are highly species specific. So far, over 100 individual human papillomavirus (HPV) genotypes have been described. HPVs are generally specific either for the skin (e.g. HPV-1 and -2) or mucosal surfaces (e.g. HPV-6 and -11) and usually cause benign tumours (warts) that persist for several months or years. Such benign tumours may be distressing for the individuals concerned but tend not to be life threatening, with a few exceptions.

Some HPVs are also associated with cancers, known as oncogenic HPV types. The strongest positive association between an HPV and human cancer is that which exists between HPV-16 and HPV-18 and cervical carcinoma. Cervical cancer is the most common malignancy in developing countries, with about 500,000 new cases occurring in the world each year.

Other HPVs of particular interest with respect to cancer are types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68.

HPV virus like particles (VLPs) have been suggested as potential vaccines for treatment of HPV. Animal studies have shown that VLPs produce no cross protection against infection for other HPV types – see, for example Suzich, J. A., et al, Proc Natl Acad Sci, *92*: 11553-11557, 1995, and Breitburd, Seminars in Cancer Biology, vol 9, 1999, pp 431 – 445.

There is still a need for a vaccine that protects against multiple HPV types.

The present invention addresses this need.

Summary of the invention

In a first aspect the present invention relates to use of a mixture comprising HPV 16 and HPV 18 VLPs in the prevention of infection or disease caused by one or more of the group of oncogenic HPV types, excluding types 16 and 18.

The invention also relates to use of a mixture comprising HPV 16 and HPV 18 VLPs in the preparation of a medicament for the prevention of infection or disease caused by one or more of the group of oncogenic HPV types, excluding types 16 and 18.

In a second aspect the invention relates to use of a mixture comprising HPV 16 and HPV 18 VLPs in the prevention of infection or disease caused by one or more of the group of HPV types 31,33,35,39,45,51,52,56,58,59,66,68.

The invention also relates to use of a mixture of HPV 16 and HPV18 VLPs in the preparation of a medicament for the prevention of infection or disease caused by one or more of the group HPV types 31,33,35,39,45,51,52,56,58,59,66,68.

In a third aspect the present invention relates to use of an HPV 16 VLP in the prevention of infection or disease caused by one or more of the group of HPV types 31,33,35,39,45,51,52,56,58,59,66,68.

The invention also relates to use of an HPV 16 VLP in the preparation of a medicament for the prevention of infection or disease caused by one or more of the group of HPV types 31,33,35,39,45,51,52,56,58,59,66,68.

In a fourth aspect the present invention relates to use of an HPV 18 VLP in the prevention of infection or disease caused by one or more of the group of HPV types 31,33,35,39,45,51,52,56,58,59,66,68.

The invention also relates to use of an HPV 18 VLP in the preparation of a medicament for the prevention of infection or disease caused by one or more of the group HPV types 31,33,35,39,45,51,52,56,58,59,66,68.

More particularly, the invention pertains to a method of inducing an immune response against one or more HPV types selected from the group consisting of all oncogenic cancer types excluding types 16 and 18, the method comprising administering to a subject a composition comprising an HPV16 VLP. Preferred is a method wherein the HPV types are selected from the group consisting of types 18 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68. Also preferred is a method of inducing an immune response against one or more of the HPV types selected from the group consisting of HPV31, HPV33, HPV35, HPV52 and HPV58. Most preferred is a method of inducing an immune response against one or more of the HPV types selected from the group consisting of HPV31, HPV35 and HPV58. In another embodiment, the composition useful in the method further comprises an HPV18 VLP and/or an adjuvant. In yet another embodiment, the immune response which is induced results in the prevention of infection by the listed HPV types.

In a further embodiment, the invention pertains to a method of inducing an immune response against one or more HPV types selected from the group consisting of all oncogenic cancer types excluding types 16 and 18, the method comprising administering to a subject a composition comprising an HPV18 VLP. Preferred is a method of inducing an immune response against one or more of the HPV types selected from the group consisting of 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68, the method comprising administering to a subject a composition comprising an HPV18 VLP. Preferred is a method of inducing an immune response against one or more of the HPV types selected from the group consisting HPV45 and HPV58. In another embodiment, the composition useful in the method further comprises an HPV16 VLP and/or an adjuvant. In yet another embodiment, the immune response which is induced results in the prevention of infection by the listed HPV types.

Preferred is a method as described above, wherein an immune response is induced against any 1,2,3,4,5,6,7,8,9,10,11 or 12 HPV types of the specific groups identified, (as appropriate for the size of preferred group).

Detailed Description

In the present invention we have surprisingly found that use of HPV 16 and HPV 18 VLPs provides cross protection against infection by one or more of the group of oncogenic HPV types (excluding types 16 and 18), oncogenic types being those types capable of causing cancer. The group of oncogenic types comprises or consists of types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82, 26, 53, and 66.
In particular use of HPV 16 and HPV 18 VLPs provides cross protection against infection and /or disease caused by the group of HPV types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68, hereinafter called the group of 'high risk cancer types'. Protection against the whole group of high risk cancer types, and against certain specific types and combinations of types has been identified. The effect is in addition to the 'homologous' protective effect seen against HPV 16 and HPV 18 by use of HPV 16 and HPV 18 VLPs respectively. As such, HPV 16 VLPs and HPV 18 VLPs can be used both in a vaccine against HPV 16, HPV 18 and against other HPV types.

Cross protection is herein taken to mean that incidence of infection for a group of oncogenic HPV types (infection being incident or persistent infection) and/or oncogenic disease caused by HPV infection is lower in a group of individuals vaccinated with HPV 16 and/or 18 VLPs, preferably types 16 and 18, than in a non vaccinated group. Complete cross protection against a type, or group of types, is not required in the present invention – indeed, any level of cross protection provides a benefit. Preferably the level of cross protection observed is such that the vaccinated group has 5% less infection and/or disease than a comparable non vaccinated group, more preferably up to 10%, up to 15%, up to 20%, up to 25%, up to 30%, up to 35%, up to 40%, up to 45%, up to 50%, up to 55%, up to 60%, up to 65% up to 70%, up to 80%, up to 90% or even up to 100% less infection and/or disease.

Cross protection may be assessed by detecting the presence of nucleic acid specific for various oncogenic types in the vaccinees and control group. Detection may be carried out, for example, using techniques as described in WO03014402, and references therein, particularly for non-specific amplification of HPV DNA and subsequent detection of DNA types using a LiPA system as described in WO 99/14377, and in Kleter et al, [Journal of Clinical Microbiology (1999), 37 (8): 2508-2517], the whole contents of which are herein incorporated by reference. Any suitable method can, however, be used for the detection of HPV DNA in a sample, such as type specific PCR using primers specific for each HPV type of interest. Suitable primers are known to the skilled person, or can be easily constructed given that the sequences of the oncogenic HPV types are known.

Suitably cross protection is observed in the female population, preferably women who are seronegative for HPV infection, or seronegative for HPV 16 and 18, preferably adolescent women pre-sexual activity.

Cross protection (as assessed by protection seen in a vaccinated group vs a control group) is preferably seen against any one oncogenic type other than 16 or 18, for example any one of the group of high risk cancer types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 or 68 or, collectively, groups of high risk cancer types such as any 2,3,4,5,6,7,8, 9,10 or 11 types, or indeed all of the high risk cancer types. All possible combinations of 2,3,4,5,6,7,8,9,10 and 11 of the high risk cancer types are specifically contemplated, and as such there are various different 'groups' of VLP types individualised herein, for which the level of cross protection can be analysed in comparison to a placebo group. Use of HPV 16 and/or HPV 18 VLPS to provide prevention of HPV infection by any such group of HPV types is preferred.

Suitably the present invention provides cross- protection against infection and/or disease by the following preferred groups of HPV types:

A the group comprising one or more of HPV types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66,68.

B the group of statement A comprising type 31 and one or more of types 33, 35, 39, 45, 51, 52, 56, 58, 59, 66,68

C the group of statement A or B comprising type 33 and one or more of types 35, 39, 45, 51, 52, 56, 58, 59, 66,68

D the group of statement A - C comprising type 35 and one or more of types 39, 45, 51, 52, 56, 58, 59, 66,68

E the group of statement A-D comprising type 39 and one or more of types 45, 51, 52, 56, 58, 59, 66,68

F the group of statement A- E comprising type 45 and one or more of types 51, 52, 56, 58, 59, 66,68

G the group of statement A-F comprising type 51 and one or more of types 52, 56, 58, 59, 66,68

H the group of statement A-G comprising type 52 and one or more of types 56, 58, 59, 66,68

I the group of statement A-H comprising type 56 and one or more of types 58, 59, 66,68

J the group of statement A-I comprising type 58 and one or more of types 59, 66,68

K the group of statement A-J comprising type 59 and one or more of types 66, 68

L the group of statement A-K comprising type 66 and one or more of types 68

In particular we have determined that, across a population, a placebo group was 38% more likely to be infected with at least one of the group of high risk cancer types in comparison to those women vaccinated with a vaccine comprising HPV 16 and 18 VLPs. Thus the invention relates particularly to use of a composition comprising HPV 16 and 18 VLPs in prevention of infection in a population by one or more of the high risk cancer types. Preferably the composition is up to or at least 20% more effective than a placebo in prevention of infection in a population by the group consisting of high risk cancer types, preferably up to or at least 25 % more effective, most preferably up to or at least 30% more effective, suitably up to or at least 34% more effective in prevention of infection, most preferably up to or at least 38 % more effective in prevention of infection.

Preferably the present invention relates to a composition comprising HPV 16 and HPV 18 VLPs in the preparation of a medicament for the prevention of infection by one or more of HPV 31, 33, 35, 52 and 58.

We have determined that, across a population, a placebo group was 43% more likely to be infected with at least one of the group HPV types 31, 33, 35, 52 and 58 in comparison to those women vaccinated with HPV 16 and HPV 18 VLPs. Thus the invention relates particularly to use of a composition comprising HPV 16 and HPV 18 VLPs in prevention of infection in a population by one or more of HPV 31, 33, 35, 52 and 58. Preferably the combination is up to or at least 10% more effective than a placebo in prevention of infection in a population by the group consisting of HPV 31, 33 35, 52 or 58, more preferably up to or at least 15 % effective, more preferably up to or at least 20% effective, 25% effective, 30% effective, 37% effective or suitably up to or at least 43% effective in prevention of infection.

Preferably the present invention relates to a composition comprising HPV 16 and HPV 18 VLPs in the preparation of a medicament for the prevention of infection by one or more of HPV 31, 35 and 58.

We have determined that, across a population, a placebo group was 58% more likely to be infected with at least one of the group of HPV types 31, 35 and 58 in comparison to women vaccinated with HPV 16 and HPV 18 VLPs. Thus the invention relates particularly to use of a composition comprising HPV 16 and HPV 18 VLPs in prevention of infection within a population by one or more of HPV 31, 35 and 58. Preferably the composition is up to or at least 15% more effective than a placebo in prevention of infection in a population by the group consisting of HPV 31, 35 or 58, preferably up to or at least 20 % effective, preferably up to 25% effective, up to 30%, up to 35%, up to 40%, up to 45%, up to 49%, up to 55% or even more effective, suitably up to or at least 58% effective in prevention of infection.

Preferably the present invention relates to a composition comprising HPV 16 and HPV 18 VLPs in the preparation of a medicament for the prevention of infection by one or more of HPV 45 and 59.

In particular we have determined that, across a population, a placebo group was 33% more likely to be infected with at least one (or more) of the group of HPV types 45 and 59 in comparison to those women vaccinated with a vaccine comprising HPV 16 and 18 VLPs. Thus the invention relates particularly to use of a composition comprising HPV 16 and 18 VLPs in prevention of infection in a population by one or more of HPV 45 and HPV 59. Preferably the composition is up to or at least 20% more effective than a placebo in prevention of infection in a population by the group consisting of HPV 45 and HPV 59, preferably up to or at least 25 % more effective, more preferably up to 30% more effective, more preferably up to or at least 33% more effective in prevention of infection.

Preferably the present invention relates to a method of prevention of HPV infection within a population, wherein vaccination with HPV 16 VLPs, HPV 18 VLPs or a mixture thereof provides cross protection against infection by the following groups of HPV types: all oncogenic HPV types (excluding types 16 and 18), any of the groups identified in paragraphs A- L above, the group of high risk cancer types, the group of HPV 31, 35, 58; the group of HPV 31, 33, 35, 52, 58; the group of HPV 45 and 59,

the protection achieved against all members of the group collectively being greater than that which is seen with a placebo.

Preferably the composition comprising HPV 16 and HPV 18 VLPs provides cross-protection against at least one of HPV 35 and HPV 52 infection.

Preferably the composition of HPV 16 and HPV 18 VLPs also provides protection against infection by HPV 16 and/or HPV 18, preferably both HPV 16 and HPV 18.

The invention also relates to the use of an HPV16 VLP in the prevention of infection by one or more of the group of high risk cancer types, preferably the group consisting of HPV 31, 33, 35, 52, 58, or the group consisting of 31, 35, 58.

The invention also relates to use of an HPV 16 VLP in the preparation of a medicament for the prevention of infection by one or more of the group of high risk cancer types, preferably the group consisting of HPV 31, 33, 35, 52, 58, or the group consisting of HPV types 31, 35 and 58.

The invention also relates to the use of HPV18 VLPs in the prevention of infection by one or more of the group of high risk cancer types, preferably the group consisting of HPV 45 and 59.

The HPV 16 VLP of the invention may be used in the absence of any other HPV VLP type, or may be used in combination with another HPV VLP type. Preferably the HPV16 VLP is used in combination with an HPV 18 VLP.

The HPV 18 VLP of the invention may be used in the absence of any other HPV VLP type, or may be used in combination with another HPV VLP type. Preferably the HPV18 VLP is used in combination with an HPV 16 VLP.

Preferably the HPV 16 VLPs, HPV 18 VLPs and combinations thereof are used to provide protection against individual oncogenic types, preferably the high risk cancer types, specifically HPV types 31,33,35,39,45,51,52,56,58,59,66,68.

In addition to the cross protection effect, preferably the immunogenic composition of the invention, most preferably an HPV 16 and HPV 18 VLP combination, is used in the active immunization of adults and adolescent females from the age of 10 years onwards to prevent one or more of: HPV-16 and HPV-18 infection, persistent HPV-16 and HPV-18 infection and HPV-16 and HPV-18 associated cervical neoplasia.

Preferably the use of the immunogenic composition of the invention is used to prevent cervical neoplasia associated with infection by other (non HPV 16, 18) oncogenic types.

HPV VLPs and methods for the production of VLPs are well known in the art. VLPs typically are constructed from the L1 and optionally L2 structural proteins of the virus, see for example WO9420137 and WO9405792. Any suitable HPV VLP may be used in the present invention which provides cross protection, such as an L1 or L1 + L2 VLP.

Preferably the VLP is an L1 only VLP.

The VLP may comprise full length L1 protein.

Preferably the L1 protein used to form the VLP is a truncated L1 protein. Preferably the truncation removes a nuclear localisation signal. Preferably the truncation is a C terminal truncation. Preferably the C terminal truncation removes less than 50 amino acids, more preferably less than 40 amino acids. Where the VLP is an HPV 16 VLP then preferably the C terminal truncation removes 34 amino acids from HPV 16 L1. Where the VLP is an HPV 18 VLP then preferably the C terminal truncation removes 35 amino acids from HPV 18 L1.

Truncated L1 proteins are suitably functional L1 protein derivatives. Functional L1 protein derivatives are capable of raising an immune response (if necessary, when suitably adjuvanted), said immune response being capable of recognising a VLP consisting of the full length L1 protein and/or the HPV type from which the L1 protein was derived.

VLPs of the invention may also comprise other types of functional protein derivatives, including mutants of the full length or truncated HPV L1 proteins such as deletion, substitution, or insertion mutants. Suitable derivatives also include codon optimised sequences. The L1 protein or derivative may also be a fusion protein, such as the fusion of the L1 protein with L2 or an early protein. The L1 protein or functional protein derivative is able to form a VLP, and VLP formation can be assessed by standard techniques such as, for example, electron microscopy and dynamic laser light scattering.

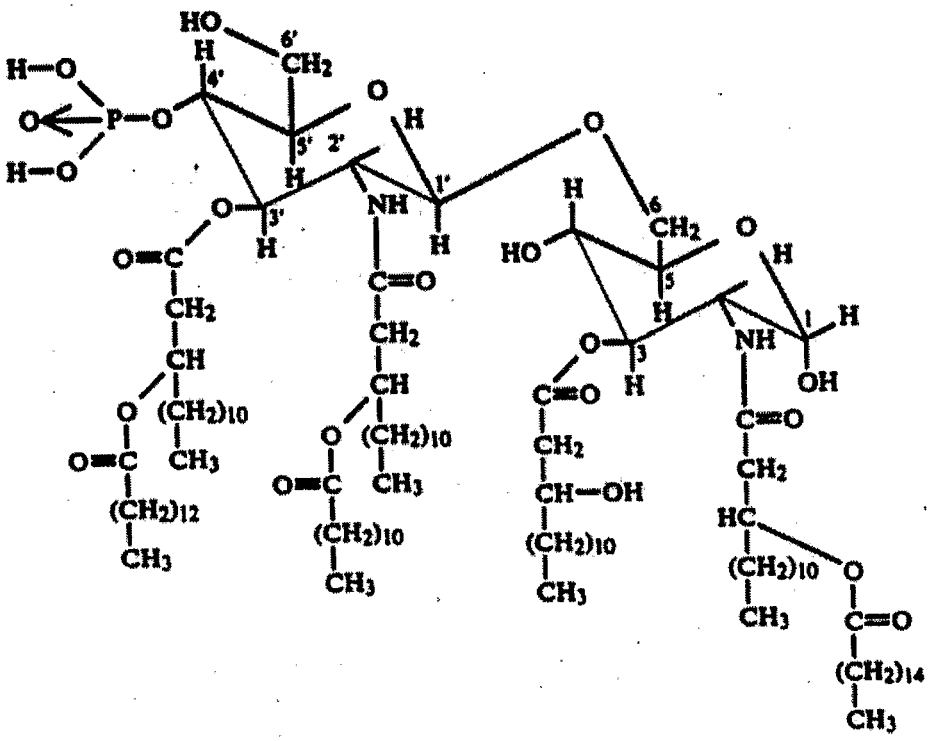
VLPs may be made in any suitable cell substrate such as yeast cells or insect cells e.g. baculovirus cells, and techniques for preparation of VLPS are well known in the art, such as WO9913056 and US6245568, and references therein, the entire contents of which are hereby incorporated by reference.

VLPS are preferably made by disassembly and reassembly techniques, which can provide for more stable and/or homogeneous papillomavirus VLPs. For example, McCarthy et al, 1998 "Quantitative Disassembly and Reassembly of Human Papillomavirus Type 11 Viruslike Particles in Vitro" J. Virology 72(1):33-41, describes the disassembly and reassembly of recombinant L1 HPV 11 VLPs purified from insect cells in order to obtain a homogeneous preparation of VLP's. WO9913056 and US6245568 also describe disassembly/reassembly processes for making HPV VLPs.

Preferably the HPV VLPS of the invention are made as described WO9913056 or US6245568

The VLPs of the invention may be combined with an adjuvant. The vaccines of the invention may comprise a suitable adjuvant or imunostimulant such as, but not limited to, detoxified lipid A from any source and non-toxic derivatives of lipid A, saponins and other reagents capable of stimulating a TH1 type response.

It has long been known that enterobacterial lipopolysaccharide (LPS) is a potent stimulator of the immune system, although its use in adjuvants has been curtailed by its toxic effects. A non-toxic derivative of LPS, monophosphoryl lipid A (MPL), produced by removal of the core carbohydrate group and the phosphate from the reducing-end glucosamine, has been described by Ribi et al (1986, Immunology and Immunopharmacology of bacterial endotoxins, Plenum Publ. Corp., NY, p407-419) and has the following structure:



A further detoxified version of MPL results from the removal of the acyl chain from the 3-position of the disaccharide backbone, and is called 3-O-Deacylated monophosphoryl lipid A (3D-MPL). It can be purified and prepared by the methods taught in GB 2122204B, which reference also discloses the preparation of diphosphoryl lipid A, and 3-O-deacylated variants thereof.

A preferred form of 3D-MPL is in the form of an emulsion having a small particle size less than 0.2μm in diameter, and its method of manufacture is disclosed in WO 94/21292. Aqueous formulations comprising monophosphoryl lipid A and a surfactant have been described in WO9843670A2.

The bacterial lipopolysaccharide derived adjuvants to be formulated in the compositions of the present invention may be purified and processed from bacterial sources, or alternatively they may be synthetic. For example, purified monophosphoryl lipid A is described in Ribi et al 1986 (supra), and 3-O-Deacylated monophosphoryl or diphosphoryl lipid A derived from *Salmonella sp.* is described in GB 2220211 and US 4912094. Other purified and synthetic lipopolysaccharides have been described (Hilgers *et al.*, 1986, *Int.Arch.Allergy.Immunol.*, 79(4):392-6; Hilgers *et al.*, 1987, Immunology, 60(1):141-6; and EP 0 549 074 B1). A particularly preferred bacterial lipopolysaccharide adjuvant is 3D-MPL.

Accordingly, the LPS derivatives that may be used in the present invention are those immunostimulants that are similar in structure to that of LPS or MPL or 3D-MPL. In another aspect of the present invention the LPS derivatives may be an acylated monosaccharide, which is a sub-portion to the above structure of MPL.

Saponins are taught in: Lacaille-Dubois, M and Wagner H. (1996. A review of the biological and pharmacological activities of saponins. Phytomedicine vol 2 pp 363-386). Saponins are steroid or triterpene glycosides widely distributed in the plant and marine animal kingdoms. Saponins are noted for forming colloidal solutions in water which foam on shaking, and for precipitating cholesterol. When saponins are near cell membranes they create pore-like structures in the membrane which cause the membrane to burst. Haemolysis of erythrocytes is an example of this phenomenon, which is a property of certain, but not all, saponins.

Saponins are known as adjuvants in vaccines for systemic administration. The adjuvant and haemolytic activity of individual saponins has been extensively studied in the art (Lacaille-Dubois and Wagner, *supra*). For example, Quil A (derived from

the bark of the South American tree Quillaja Saponaria Molina), and fractions thereof, are described in US 5,057,540 and "Saponins as vaccine adjuvants", Kensil, C. R., *Crit Rev Ther Drug Carrier Syst*, 1996, 12 (1-2):1-55; and EP 0 362 279 B1. Particulate structures, termed Immune Stimulating Complexes (ISCOMS), comprising fractions of Quil A are haemolytic and have been used in the manufacture of vaccines (Morein, B., EP 0 109 942 B1; WO 96/11711; WO 96/33739). The haemolytic saponins QS21 and QS17 (HPLC purified fractions of Quil A) have been described as potent systemic adjuvants, and the method of their production is disclosed in US Patent No.5,057,540 and EP 0 362 279 B1. Other saponins which have been used in systemic vaccination studies include those derived from other plant species such as Gypsophila and Saponaria (Bomford *et al.*, Vaccine, 10(9):572-577, 1992).

An enhanced system involves the combination of a non-toxic lipid A derivative and a saponin derivative particularly the combination of QS21 and 3D-MPL as disclosed in WO 94/00153, or a less reactogenic composition where the QS21 is quenched with cholesterol as disclosed in WO 96/33739.

A particularly potent adjuvant formulation involving QS21 and 3D-MPL in an oil in water emulsion is described in WO 95/17210 and is a preferred formulation.

Accordingly in one embodiment of the present invention there is provided a vaccine adjuvanted with detoxified lipid A or a non-toxic derivative of lipid A, more preferably adjuvanted with a monophosphoryl lipid A or derivative thereof.

Preferably the vaccine additionally comprises a saponin, more preferably QS21.

Preferably the formulation additionally comprises an oil in water emulsion. The present invention also provides a method for producing a vaccine formulation comprising mixing an L2 peptide of the present invention together with a pharmaceutically acceptable excipient, such as 3D-MPL.

Additional components that are preferably present in an adjuvanted vaccine formulation according to the invention include non-ionic detergents such as the octoxynols and polyoxyethylene esters as described herein, particularly t-octylphenoxy polyethoxyethanol (Triton X-100) and polyoxyethylene sorbitan monooleate (Tween 80); and bile salts or cholic acid derivatives as described herein, in particular sodium deoxycholate or taurodeoxycholate. Thus, a particularly preferred formulation comprises 3D-MPL, Triton X-100, Tween 80 and sodium deoxycholate, which may be combined with an L2 antigen preparation to provide a suitable vaccine.

In one preferred embodiment of the present invention, the vaccine comprises a vesicular adjuvant formulation comprising cholesterol, a saponin and an LPS derivative. In this regard the preferred adjuvant formulation comprises a unilamellar vesicle comprising cholesterol, having a lipid bilayer preferably comprising dioleoyl phosphatidyl choline, wherein the saponin and the LPS derivative are associated with, or embedded within, the lipid bilayer. More preferably, these adjuvant formulations comprise QS21 as the saponin, and 3D-MPL as the LPS derivative, wherein the ratio of QS21:cholesterol is from 1:1 to 1:100 weight/weight, and most preferably 1:5 weight/weight. Such adjuvant formulations are described in EP 0 822 831 B, the disclosure of which is incorporated herein by reference.

Suitably the vaccines of the invention are used in combination with aluminium, and are suitably adsorbed or partially adsorbed onto aluminium adjuvants. Suitably the adjuvant is an aluminium salt, preferably in combination with 3D MPL, such as aluminium phosphate and 3D MPL. Aluminium hydroxide, optionally in combination with 3D MPL is also preferred.

Most preferred in the present invention is the combination of VLPs with an aluminium salt or with an aluminium salt + 3D MPL. Aluminium hydroxide is preferred as the aluminium salt.

The vaccine may also comprise aluminium or an aluminium compound as a stabiliser.

The vaccines of the invention may be provided by any of a variety of routes such as oral, topical, subcutaneous, mucosal (typically intravaginal), intraveneous, intramuscular, intranasal, sublingual, intradermal and via suppository.

Optionally the vaccine may also be formulated or co-administered with other HPV antigens such as early antigens or non-HPV antigens. Suitably these non-HPV antigens can provide protection against other diseases, most preferably sexually transmitted diseases such as herpes simplex virus, EBV, chlamydia and HIV. We particularly prefer that the vaccine comprises gD or a truncate thereof from HSV. In this way the vaccine provides protection against both HPV and HSV.

The dosage of the vaccine components will vary with the condition, sex, age and weight of the individual, the administration route and HPV of the vaccine. The quantity may also be varied with the number of VLP types. Suitably the delivery is of an amount of vaccine suitable to generate an immunologically protective response. Suitably each vaccine dose comprises 1-100 μg of each VLP, preferably 5-80μg, more preferably 5- 30 μg each VLP, most preferably 5-20 μg of each VLP with 5μg, 6μg, 10μg, 15 μg or 20μg especially preferred.

For all vaccines of the invention, it is preferred that the vaccine is used for the vaccination of adolescent girls aged 10-15, preferably 10-13 years. The vaccine may also be administered to women following an abnormal pap smear or after surgery following removal of a lesion caused by HPV, or who are seronegative and DNA negative for HPV cancer types.

Preferably the vaccine is delivered in a 2 or 3 dose regime, for example in a 0, 1 month regime or 0,1 and 6 month regime respectively. Suitably the vaccination regime incorporates a booster injection after 5 to10 years, preferably 10 years.

Preferably the vaccine is a liquid vaccine formulation, although the vaccine may be lyophilised and reconstituted prior to administration.

Preferably the immunogen used in the present invention (HPV 16 and or HPV 18 VLPs) is used as a vaccine and may be formulated into a vaccine using appropriate excipients. Thus the invention particularly relates to use of an HPV 16 or HPV 18 vaccine, such as an LI only vaccine, in the prevention of HPV infection.

The present invention is hereby illustrated by reference to the following non-limiting example.

Example 1

Healthy women between the ages of 15 and 25 years were immunised with a mixture of HPV 16 and HPV 18 L1 VLPs. The women at enrolment were: 1) seronegative for HPV-16 and HPV-18; 2) negative for high risk HPV infection of the cervix (detected by HPV PCR); 3) had 6 or fewer lifetime sexual partners and 4) had normal PAP smears.

The mixture comprised, per 0.5 ml dose, 20 μg of HPV-16 L1 VLP, 20 μg of HPV-18 L1 VLP and was adjuvanted with 500 μg of aluminum hydroxide and 50 μg of 3D MPL. The placebo group was injected with 500 μg of aluminum hydroxide alone.

The vaccine efficacy (V.E.) against high risk cancer HPV types was assessed, wherein the V.E. is the % improvement in protection against infection by the vaccine compared to a placebo group.

Cross protection was assessed by detecting the presence of nucleic acid specific for various oncogenic types in the vaccinees and control group. Detection was carried out using techniques as described in WO03014402, and references therein, particularly for non-specific amplification of HPV DNA and subsequent detection of DNA types using a LiPA system as described in WO 99/14377, and in Kleter et al, [Journal of Clinical Microbiology (1999), 37 (8): 2508-2517], the whole contents of which are herein incorporated by reference.

Any suitable method can, however, be used for the detection of HPV DNA in a sample, such as type specific PCR using primers specific for each HPV type of interest. Suitable primers are known to the skilled person, or can be easily constructed given that the sequences of the oncogenic HPV types are known.

Vaccine efficacy was assessed against infections for all of the 12 high risk cancer types, HPV-16 phylogenetic-related types (the groups of; 31, 35, and 58; 31, 33, 35, 52 and 58) and HPV-18 phylogenetic related types (45 and 59).

An initial analysis was carried out on an "ITT" (Intention To Treat cohort, representing all individuals who received at least one dose of vaccine). This data is shown in Table 1.

The results presented in Tables 2 and 3 relate to the "ATP" (According To Protocol) group for those patients who complied with all the criteria of the trial. Table 2 is a midpoint analysis with data taken from all patients at the timepoint at which at least 50% of the cohort were 18 months after their first vaccination. Table 3 gives the final results, all data being from subjects at 18 months post first vaccination (month 0). In the ATP group all patients received 3 doses of vaccine at 0, 1 and 6 months and were seronegative at 6 months.

As demonstrated by the data presented in table 1, immunization with a mixture of HPV16 and HPV18 VLPs provided apparent cross-protection against other HPV types. At this point the sample sizes are too small to provide for a rigorous statistical analysis, however the data demonstrate a positive trend and suggest that immunization with HPV16 and HPV18 VLPs will be efficacious against infection with other HPV types.

This was confirmed as the study progressed.

Table 2 demonstrates that HPV 16 and HPV 18 provide statistically significant cross protection against the group of high risk cancer types 31,33,35,39,45,51,52,56,58,59, 66 and 68.

Table 3 demonstrates that, except for the HPV-18 related types (which show a very strong trend), there is statistically significant cross-protection against the groups of: HPV 31, 35, 58; HPV 31, 33, 35, 52, 58; and the 12 high risk (non HPV-16/18) types evaluated.

Table 1

HPV types analysed	Number of women infected (vaccine group)	% women infected (vaccine group) = A	Number of women infected (placebo group)	% women infected (placebo group) = B	% vaccine efficacy 1 – (A/B) x 100, adjusted for relative size of vaccine and placebo group	95% confidence limits -lower limit	95% confidence limits -upper limit	P
HPV 31,35, 58	5	1.1	11	2.4	55.1	-29.1	84.4	0.127
HPV 31, 33, 35, 52, 58	17	3.8	24	5.4	30.3	-29.7	62.6	0.252
HPV 45, 59	3	0.7	6	1.3	50.6	-97.7	87.6	0.309
HPV31,33,35, 39,45,51,52,56 ,58,59,66,68.	27	6.3	40	9.4	34.6	-6.5	59.9	0.086

Samples were taken at 9, 12, 15 and 18 months from patients and tested for HPV infection by the types specified above.

Table 2 - vaccine efficacy after three doses in preventing incident heterologous infections.

Table 2: Vaccine efficacy against infection with HPV-16 phylogenetically related types, HPV-18 phylogenetically related types, HPV-16 and/or HPV-18 phylogenetically related types and all high-risk types exclusive of HPV-16 and HPV-18 – ATP cohort (month 6-18)

Infection Type	Attack rate						Vaccine efficacy		
	Vaccine			Placebo					
	N	n	AR	N	n	AR	%	95% CI	p-value
HPV-16 related	433	12	2.8	438	24	5.5	49.4	0.2 74.4	0.060
HPV-16 related*	423	29	6.9	423	46	10.9	37.0	1.6 59.6	0.052
HPV-18 related	442	9	2.0	449	16	3.6	42.9	-27.9 74.5	0.223
HPV-16/18 related	433	21	4.9	438	41	9.4	48.2	13.8 68.9	0.012
HPV-16/18 related*	423	34	8.0	423	56	13.2	39.3	9.0 59.5	0.019
High-risk**	385	53	13.8	386	88	22.8	39.6	17.7 55.7	0.001

N = number of subjects in specific cohort

n = number of subjects with incident HPV infection

AR = Attack rate = n / N

95% CI = 95% confidence interval

lower limit = 1- exp (log (arv / arp) + 1.96 * sqrt (1/nv – 1/Nv + 1/np – 1/Np))

upper limit = 1- exp (log (arv / arp) – 1.96 * sqrt (1/nv – 1/Nv + 1/np – 1/Np))

when number of cases in vaccine = 0 :

lower limit* = 1- exp (log (arv* / arp*) + 1.96 * sqrt (1/(nv+0.5) – 1/(Nv+0.5) + 1/(np+0.5) – 1/(Np+0.5)))

upper limit* = 1- exp (log (arv* / arp*) – 1.96 * sqrt (1/(nv+0.5) – 1/(Nv+0.5) + 1/(np+0.5) – 1/(Np+0.5)))

with: arv = attack rate in vaccine recipients
arp = attack rate in placebo recipients
nv = number of cases in vaccine recipients
Nv = number of cases and non-cases in vaccine recipients
np = number of cases in placebo recipients
Np = number of cases and non-cases in placebo recipients

HPV-16 related: HPV-16 phylogenetically related types 35, 31, 58 without considering other HPV types
HPV-16 related*: HPV-16 phylogenetically related types 35, 31, 58, 33, 52 without considering other HPV types
HPV-18 related: HPV-18 phylogenetically related types 45, 59 without considering other HPV types
HPV-16 and/or HPV-18 related: HPV-16 and/or HPV-18 phylogenetically related types 35, 31, 58, 45, 59 without considering other HPV types
HPV-16 and/or HPV-18 related*: HPV-16 and/or HPV-18 phylogenetically related types 35, 31, 58, 33, 52, 45, 59 without considering other HPV types
** = High-risk types exclusive of HPV-16 and HPV-18

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Table 3

HPV types analysed	Total number of number of subjects with information available per group	Number of women infected (vaccine group)	% women infected (vaccine group) = A	Number of women infected (placebo group)	% women infected (placebo group) = B	% vaccine efficacy 1 – (A/B) x 100, adjusted for relative size of vaccine and placebo group	95% confidence limits -lower limit	95% confidence limits -upper limit	P
HPV 31,35, 58	412	11	2.7	26	6.3	57.9	15.9	78.9	0.012
HPV 31, 33, 35, 52, 58	403	28	6.9	48	12.2	43.0	11.0	63.5	0.015
HPV 45, 59	421	10	2.4	15	3.6	33.5	-46.3	69.8	0.319
HPV31,33,35, 39,45,51,52,56 ,58,59,66,68.	368	58	15.8	90	25.3	37.7	16.2	53.6	0.002

Samples were taken at 18 months from patients and tested for HPV infection by the types specified above.

Claims

1 Use of a composition comprising HPV 16 and HPV 18 VLPs in the preparation of a medicament for the prevention of infection and/or disease caused by one or more of the group of oncogenic HPV types, the group excluding types HPV 16 and HPV 18.

2 Use of a composition according to claim 1 for the prevention of infection and/or disease by one or more of the group of HPV types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66,68.

3 Use according to claim 2 for prevention of infection and/or disease by one or more of the group of HPV 31, HPV 33, HPV 35, HPV 52 and HPV 58.

4 Use according to claim 3 for prevention of infection and/or disease by one or more of the group of HPV 31, HPV 35, and HPV 58.

5 Use according to any preceding claim wherein the extent of prevention is determined by comparing a vaccinated population with a non-vaccinated population (placebo group) in respect of all members of the group of HPV types.

6 Use according to any preceding claim wherein the prevention afforded is at least 15% better than a placebo.

7 Use according to any preceding claim wherein the HPV VLPs are combined with an adjuvant.

8 Use according to claim 7 wherein the adjuvant is a combination of an aluminium salt and 3D-MPL.

9 Use according to claim 8 wherein the adjuvant is a combination of an aluminium hydroxide and 3D MPL.

10 Use according to any preceding claim wherein the VLP comprises an L1 protein or immunogenic fragment thereof but no L2 protein.

11 A method of inducing an immune response against one or more of the group of oncogenic HPV types, the group excluding types HPV 16 and HPV 18, the method comprising administering to a subject a composition comprising an HPV16 VLP and an HPV 18 VLP.

12 A method for preventing infection and/or disease caused by one or more of the group of oncogenic HPV types, the group excluding types HPV 16 and HPV 18, the method comprising administering to a subject a composition comprising an HPV16 VLP and an HPV 18 VLP.

13 The method of Claim 11 or 12 wherein the HPV types are selected from the group consisting of types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68.

14 The method of any of claims 11 – 13 wherein the HPV types are selected from the group consisting of HPV types HPV31, HPV33, HPV35, HPV52 and HPV58.

15 The method of any of claims 11- 14 wherein the HPV types are selected from the group consisting of HPV31, HPV35 and HPV58.

16 The method of any of claims 11-15 wherein the composition further comprises an adjuvant.

17 The method of Claim 16 wherein the adjuvant comprises an aluminum salt and 3D MPL.

18 The method of Claim 17 wherein the aluminum salt is aluminum hydroxide.

19 A method for preventing infection of a population of subjects by one or more HPV types selected from the group consisting of oncogenic HPV types, excluding types 16 and 18, the method comprising administering to a subject a composition comprising an HPV16 VLP

and an HPV 18 VLP, wherein administration of the composition prevents infection and or disease in up to or at least 15% more of the population than administration of a placebo.

20 Use of a mixture of HPV 16 and HPV18 VLPs in the preparation of a medicament for the prevention of infection and/or disease in a group of humans by the group of oncogenic HPV types excluding types 16 and 18, wherein the level of infection and/or disease seen in the group is significantly lower than that which is seen with a placebo.

21 A vaccine composition comprising an HPV16 VLP and an HPV 18 VLP which is cross protective against infection and/or disease caused by oncogenic HPV types other than type 16 and 18.