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Castillo Grau & Asociados
ABOGADOS

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Folios: 2

Señor Jefe

DIVISION DE NUEVAS CREACIONES

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

Santa Fe de Bogotá, D.C.

Re.: Expediente No. 000-66.605

Solicitud Patente

SMITHKLINE BEECHAM BIOLOGICALS s.a.

Debidamente traducida, adjunto acompaño la copia oficial de la solicitud de patente básica Inglesa No. 9921146.8 la cual le solicito agregar a este expediente para los efectos legales respectivos.

De Usted atentamente,

GERMAN CASTILLO GRAU

T.P.#2.978 del C.S.J.

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INVESTOR IN PEOPLE

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Dated 22nd August 2000

-7 SEP 1999 75 73

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08SEP99 E475125-1 D02029
P01/7700 0.00 - 9921146.8

Your Reference: KLP/RH/ B45198

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Form 1/77 **Patents Act 1977**

❶ Title of invention

1 Please give the title of the invention: Novel Composition

❷ Applicant's details

☐ **First or only applicant**

2a If you are applying as a corporate body please give:

Corporate Name: SmithKline Beecham Biologicals s.a.

Country (*and State of incorporation, if appropriate*): Belgium

2b If you are applying as an individual or one of a partnership please give in full:

Surname

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2c **In all cases,** please given the following details:

Address: rue de l'Institut 89, B-1330 Rixensart
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postcode (*if applicable*) 5781117ac1

Country: Belgium

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3a Have you appointed an agent to deal with your application?

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U

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Agent's name: Kathryn L Privett
Agent's address: SmithKline Beecham
Corporate Intellectual Property
New Horizons Court
Great West Road
Brentford, Middlesex

Postcode: TW8 9EP
Agent's ADP number: 7135830001

3b: If you have appointed an agent, all correspondence concerning your application will be sent to the agent's United Kingdom address.

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76 74

❹ Reference number

4. Agent's or applicant's reference number *(if applicable)* KLP/RH B45198

❺ Claiming an earlier application date

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7. Are you (the applicant or applicants) the sole inventor or the joint inventors?
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Yes ☐ No ☒ ➲ *A Statement of Inventorship on Patents form 7/77 will need to be filed (see Rule 15).*

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Please supply duplicates of claim(s), abstract, description and drawings).

8a Please fill in the number of sheets for each of the following types of document contained in this application

Continuation sheets for this Patents Form 1/77	–
Claim(s) 3	Description 16
Abstract	Drawing(s)

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Novel Composition

This invention relates to novel vaccine formulations, methods for preparing them and their use in therapy. In particular the present invention relates to combination vaccines for administration to adolescents.

Papillomaviruses are small DNA tumour viruses, which are highly species specific. So far, over 70 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. 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. It is now technically feasible to actively combat primary HPV-16 infections, and even established HPV-16-containing cancers, using vaccines. For a review on the prospects for prophylactic and therapeutic vaccination against HPV-16 see Cason J., Clin. Immunother. 1994; 1(4) 293-306 and Hagenesee M.E., Infections in Medicine 1997 14(7) 555-556,559-564.

Other HPVs of particular interest are serotypes 31,33 and 45.

Today, the different types of HPVs have been isolated and characterised with the help of cloning systems in bacteria and more recently by PCR amplification. The molecular organisation of the HPV genomes has been defined on a comparative basis with that of the well-characterised bovine papillomavirus type 1 (BPV1).

Although minor variations do occur, all HPVs genomes described have at least seven early genes, E1 to E7 and two late genes L1 and L2. In addition, an upstream regulatory region harbors the regulatory sequences which appear to control most transcriptional events of the HPV genome.

E1 and E2 genes are involved in viral replication and transcriptional control, respectively and tend to be disrupted by viral integration. E6 and E7, and recent evidence implicate also E5 are involved in viral transformation.

In the HPVs involved in cervical carcinoma such as HPV 16 and 18, the oncogenic process starts after integration of viral DNA. The integration results in the inactivation of genes coding for the capsid proteins L1 and L2 and in installing continuous over expression of the two early proteins E6 and E7 that will lead to gradual loss of the normal cellular differentiation and the development of the carcinoma.

Carcinoma of the cervix is common in women and develops through a pre-cancerous intermediate stage to the invasive carcinoma which frequently leads to death. The intermediate stages of the disease is known as cervical intraepithelial neoplasia and is graded I to III in terms of increasing severity.

Clinically, HPV infection of the female anogenital tract manifests as cervical flat condylomas, the hallmark of which is the koilocytosis affecting predominantly the superficial and intermediate cells of the cervical squamous epithelium.

Koilocytes which are the consequence of a cytopathic effect of the virus, appear as multinucleated cells with a perinuclear clear halo. The epithelium is thickened with abnormal keratinisation responsible for the warty appearance of the lesion.

Such flat condylomas when positive for the HPV 16 or 18 serotypes, are high-risk factors for the evolution toward cervical intraepithelial neoplasia (CIN) and

carcinoma in situ (CIS) which are themselves regarded as precursor lesions of invasive cervix carcinoma.

WO 96/19496 discloses variants of human papilloma virus E6 and E7 proteins, particularly fusion proteins of E6/E7 with a deletion in both the E6 and E7 proteins. These deletion fusion proteins are said to be immunogenic.

HPV L1 based vaccines are disclosed in WO94/00152, WO94/20137, WO93/02184 and WO94/05792. Such a vaccine can comprise the L1 antigen as a monomer, a capsomer or a virus like particle. Such particles may additionally comprise L2 proteins. L2 based vaccines are described for example in WO93/00436. Other HPV vaccines are based on the Early proteins, such as E7 or fusion proteins such as L2-E7.

HSV-2 is the primary etiological agent of herpes genitalis. HSV-2 and HSV-1 (the causative agent of herpes labialis) are characterised by their ability to induce both acute diseases and to establish a latent infection, primarily in neuronal ganglia cells.

Genital herpes is estimated to occur in about 5 million people in the U.S.A. alone with 500,000 clinical cases recorded every year (primary and recurrent infection). Primary infection typically occurs after puberty and is characterised by the localised appearance of painful skin lesions, which persist for a period of between 2 to 3 weeks. Within the following six months after primary infection 50% of patients will experience a recurrence of the disease. About 25% of patients may experience between 10-15 recurrent episodes of the disease each year. In immunocompromised patients the incidence of high frequency recurrence is statistically higher than in the normal patient population.

Both HSV-1 and HSV-2 virus have a number of glycoprotein components located on the surface of the virus. These are known as gB, gC, gD and gE etc.

There is a need for effective combination vaccines to prevent diseases to which adolescents are particularly prone.

The present invention provides a vaccine composition comprising:

(a) a herpes simplex virus (HSV) antigen; and

(b) a human papillomavirus (HPV) antigen

in combination with an adjuvant which is a preferential stimulator of TH1 cell response.

The vaccine composition of the invention is of great benefit for administration to adolescents who may be particularly at risk of HSV, and/or HPV infection.

Optionally the vaccine composition of the invention additionally comprises one or more of a number of other antigens as described below.

It has been found that the vaccine compositions according to the invention surprisingly show no interference, that is to say that the immune response to each antigen in the composition of the invention is essentially the same as that which is obtained by each antigen given individually in conjunction with an adjuvant which is a preferential stimulator of TH1 cell response.

Vaccines for the prophylaxis of hepatitis B infections, comprising one or more hepatitis B antigens, are well known. For example the vaccine Engerix-B (Trade Mark) from SmithKline Beecham Biologicals is used to prevent Hepatitis B. This vaccine comprises hepatitis B surface antigen (specifically the 226 amino acid S-antigen described in Harford et. al. in Postgraduate Medical Journal, 1987, 63 (Suppl. 2), p65-70) and is formulated using aluminium hydroxide as adjuvant.

The vaccine Havrix (Trade Mark), also from SmithKline Beecham Biologicals is an example of a vaccine that can be used to prevent hepatitis A infections. It is formulated with aluminium hydroxide as adjuvant. This vaccine comprises an

attenuated strain of the HM-175 Hepatitis A virus inactivated with formol (formaldehyde); see Andre et. al. (Prog. med. Virol., vol. 37, p1-24).

As used herein, the term hepatitis A viral (HAV) antigen is used to refer to either a protein derived from hepatitis A virus or an attenuated strain of HAV, optionally inactivated, e.g. with formaldehyde. If the HAV antigen is a protein derived from hepatitis A virus it may optionally be a recombinant protein.

The vaccine Twinrix (Trade Mark) is a combination of a recombinant hepatitis B anitgen with the aforementioned inactivated attenuated hepatitis A virus. The vaccine may be used to protect against hepatitis A and hepatitis B simultaneously.

European patent 0 339 667 (Chemo Sero) describes the general concept of combining a hepatitis A antigen and a hepatitis B antigen to make a combination vaccine. In that specification it is stated that the adjuvant which is used is not critical: it must only be capable of enhancing the immune activity to a desired extent and not cause any side-effects. It is stated that aluminium gel may be used, in particular aluminium hydroxide gel and aluminium phosphate gel.

The vaccine composition according to the invention may comprises, in addition the HPV and HSV antigens, an HAV antigen or a HBV antigen or more preferably a combination of both an HAV and an HBV antigen

Such a vaccine is of great benefit for administration to adolescents who may be particularly at risk of HSV, and/or HPV infection, and/or HAV infection, and/or HBV infection.

An immune response may be broadly divided into two extreme catagories, being a humoral or cell mediated immune response (traditionally characterised by antibody and cellular effector mechanisms of protection respectively). These categories of response have been termed TH1-type responses (cell-mediated response), and TH2-type immune responses (humoral response).

Extreme TH1-type immune responses may be characterised by the generation of antigen specific, haplotype restricted cytotoxic T lymphocytes, and natural killer cell responses. In mice TH1-type responses are often characterised by the generation of antibodies of the IgG2a subtype, whilst in the human these correspond to IgG1 type antibodies. TH2-type immune responses are characterised by the generation of a broad range of immunoglobulin isotypes including in mice IgG1, IgA, and IgM.

It can be considered that the driving force behind the development of these two types of immune responses are cytokines. High levels of TH1-type cytokines tend to favour the induction of cell mediated immune responses to the given antigen, whilst high levels of TH2-type cytokines tend to favour the induction of humoral immune responses to the antigen.

The distinction of TH1 and TH2-type immune responses is not absolute. In reality an individual will support an immune response which is described as being predominantly TH1 or predominantly TH2. However, it is often convenient to consider the families of cytokines in terms of that described in murine CD4 +ve T cell clones by Mosmann and Coffman (*Mosmann, T.R. and Coffman, R.L. (1989) TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annual Review of Immunology, 7, p145-173*). Traditionally, TH1-type responses are associated with the production of the INF-γ and IL-2 cytokines by T-lymphocytes. Other cytokines often directly associated with the induction of TH1-type immune responses are not produced by T-cells, such as IL-12. In contrast, TH2- type responses are associated with the secretion of IL-4, IL-5, IL-6, IL-10 and tumour necrosis factor-β(TNF-β).

It is known that certain vaccine adjuvants are particularly suited to the stimulation of either TH1 or TH2 - type cytokine responses. Traditionally the best indicators of the TH1:TH2 balance of the immune response after a vaccination or infection includes direct measurement of the production of TH1 or TH2 cytokines by T lymphocytes

in vitro after restimulation with antigen, and/or (at least in mice) the measurement of the IgG1:IgG2a ratio of antigen specific antibody responses.

Thus, a TH1-type adjuvant is one which stimulates isolated T-cell populations to produce high levels of TH1-type cytokines when re-stimulated with antigen *in vitro*, and induces antigen specific immunoglobulin responses associated with TH1-type isotype.

Adjuvants which are capable of preferential stimulation of the TH1 cell response are described in International Patent Application No. WO 94/00153 and WO 95/17209.

3 De-O-acylated monophosphoryl lipid A (3D-MPL) is one such adjuvant. This is known from GB 2220211 (Ribi). Chemically it is a mixture of 3 De-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains and is manufactured by Ribi Immunochem, Montana. A preferred form of 3 De-O-acylated monophosphoryl lipid A is disclosed in European Patent 0 689 454 B1 (SmithKline Beecham Biologicals SA).

Preferably, the particles of 3D-MPL are small enough to be sterile filtered through a 0.22micron membrane (as described in European Patent number 0 689 454). 3D-MPL will be present in the range of 10μg - 100μg preferably 25-50μg per dose wherein the antigen will typically be present in a range 2-50μg per dose.

Another preferred adjuvant comprises QS21, an Hplc purified non-toxic fraction derived from the bark of Quillaja Saponaria Molina. Optionally this may be admixed with 3 De-O-acylated monophosphoryl lipid A (3D-MPL), optionally together with an carrier.

The method of production of QS21 is disclosed in US patent No. 5,057,540.

Non-reactogenic adjuvant formulations containing QS21 have been described previously (WO 96/33739). Such formulations comprising QS21 and cholesterol

have been shown to be successful TH1 stimulating adjuvants when formulated together with an antigen. Thus vaccine compositions which form part of the present invention may include a combination of QS21 and cholesterol.

Further adjuvants which are preferential stimulators of TH1 cell response include immunomodulatory oligonucleotides, for example unmethylated CpG sequences as disclosed in WO 96/02555.

Combinations of different TH1 stimulating adjuvants, such as those mentioned hereinabove, are also contemplated as providing an adjuvant which is a preferential stimulator of TH1 cell response. For example, QS21 can be formulated together with 3D-MPL. The ratio of QS21 : 3D-MPL will typically be in the order of 1 : 10 to 10 : 1; preferably 1:5 to 5 : 1 and often substantially 1 : 1. The preferred range for optimal synergy is 2.5 : 1 to 1 : 1 3D-MPL: QS21.

Preferably a carrier is also present in the vaccine composition according to the invention. The carrier may be an oil in water emulsion, or an aluminium salt, such as aluminium phosphate or aluminium hydroxide.

A preferred oil-in-water emulsion comprises a metabolisible oil, such as squalene, alpha tocopherol and Tween 80. Additionally the oil in water emulsion may contain span 85 and/or lecithin and/or tricaprylin.

In a particularly preferred aspect the antigens in the vaccine composition according to the invention are combined with 3D-MPL and alum.

Typically for human administration QS21 and 3D-MPL will be present in a vaccine in the range of 1μg - 200μg, such as 10-100μg, preferably 10μg - 50μg per dose. Typically the oil in water will comprise from 2 to 10% squalene, from 2 to 10% alpha tocopherol and from 0.3 to 3% tween 80. Preferably the ratio of squalene: alpha tocopherol is equal to or less than 1 as this provides a more stable emulsion. Span 85 may also be present at a level of 1%. In some cases it may be

advantageous that the vaccines of the present invention will further contain a stabiliser.

Non-toxic oil in water emulsions preferably contain a non-toxic oil, e.g. squalane or squalene, an emulsifier, e.g. Tween 80, in an aqueous carrier. The aqueous carrier may be, for example, phosphate buffered saline.

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

The HPV antigen in the composition of the invention is preferably derived from HPV 16 and/or 18, or from HPV 6 and/or 11, or HPV 31, 33 or 45.

In one preferred embodiment the HPV antigen in the vaccine composition according to the invention comprises the major capsid protein L1 of HPV and optionally the L2 protein, particularly from HPV 16 and/or HPV 18. In this embodiment, the preferred form of the L1 protein is a truncated L1 protein. Preferably the L1, optionally in a L1-L2 fusion, is in the form of a virus-like particle (VLP). The L1 protein may be fused to another HPV protein, in particular E7 to form an L1-E7 fusion. Chimeric VLPs comprising L1-E or L1-L2-E are particularly preferred.

In another preferred embodiment, the HPV antigen in the composition of the invention is derived from an E6 or E7 protein, in particular E6 or E7 linked to an immunological fusion partner having T cell epitopes.

In a preferred form of this embodiment of the invention, the immunological fusion partner is derived from protein D of *Heamophilus influenza* B. Preferably the protein D derivative comprises approximately the first 1/3 of the protein, in particular approximately the first N-terminal 100-110 amino acids.

Preferred fusion proteins in this embodiment of the invention comprise Protein D - E6 from HPV 16, Protein D - E7 from HPV 16 Protein D - E7 from HPV 18 and

Protein D - E6 from HPV 18. The protein D part preferably comprises the first 1/3 of protein D.

In still another embodiment of the invention, the HPV antigen is in the form of an L2-E7 fusion, particularly from HPV 6 and/or HPV 11.

The proteins of the present invention preferably are expressed in E. coli. In a preferred embodiment the proteins are expressed with a Histidine tail comprising between 5 to 9 and preferably six Histidine residues. These are advantageous in aiding purification. The description of the manufacture of such proteins is fully described in co-pending UK patent application number GB 9717953.5.

The HSV antigen in the composition of the invention is preferably derived from HSV-2, typically glycoprotein D. Glycoprotein D is located on the viral membrane, and is also found in the cytoplasm of infected cells (Eisenberg R.J. et al; J of Virol 1980, 35, 428-435). It comprises 393 amino acids including a signal peptide and has a molecular weight of approximately 60 kD. Of all the HSV envelope glycoproteins this is probably the best characterised (Cohen et al; J. of Virology, 60, 157-166). In vivo it is known to play a central role in viral attachment to cell membranes. Moreover, glycoprotein D has been shown to be able to elicit neutralising antibodies in vivo (Eing et al J. Med. Virology 127: 59-65). However, latent HSV-2 virus can still be reactivated and induce recurrence of the disease despite the presence of high neutralising antibodies titre in the patients sera.

In a preferred embodiment of the invention the HSV antigen is a truncated HSV-2 glycoprotein D of 308 amino acids which comprises amino acids 1 through 306 naturally occurring glycoprotein with the addition Asparagine and Glutamine at the C terminal end of the truncated protein devoid of its membrane anchor region. This form of the protein includes the signal peptide which is cleaved to yield a mature 283 amino acid protein. The production of such a protein in Chinese Hamster ovary cells has been described in Genentech's European patent EP-B-139 417.

The recombinant mature HSV-2 glycoprotein D truncate is preferably used in the vaccine formulations of the present invention and is designated rgD2t.

A combination of this HSV-2 antigen in combination with the adjuvant 3D-MPL has been described in WO 92/16231

When a hepatitis B viral (HBV) antigen is included in the composition of the invention this is typically hepatitis B surface antigen.

The preparation of Hepatitis B surface antigen (HBsAg) is well documented. See for example, Harford et.al. in Develop. Biol. Standard 54, page 125 (1983), Gregg et.al. in Biotechnology, 5, page 479 (1987), EP-A- 0 226 846, EP-A-0 299 108 and references therein.

As used herein the expression 'Hepatitis B surface antigen', abbreviated herein to 'HBsAg' or 'HBS' includes any HBsAg antigen or fragment thereof displaying the antigenicity of HBV surface antigen. It will be understood that in addition to the 226 amino acid sequence of the HBsAg S antigen (see Tiollais et. al. Nature, 317, 489 (1985) and references therein) HBsAg as herein described may, if desired, contain all or part of a pre-S sequence as described in the above references and in EP-A- 0 278 940. HBsAg as herein described can also refer to variants, for example the 'escape mutant' described in WO 91/14703. In a further aspect the HBsAg may comprise a protein described as L* in European Patent Application Number 0 414 374, that is to say a protein, the amino acid sequence of which consists of parts of the amino acid sequence of the hepatitis B virus large (L) protein (ad or ay subtype), characterised in that the amino acid sequence of the protein consists of either:

(a) residues 12 - 52, followed by residues 133 - 145, followed by residues 175 - 400 of the said L protein; or

(b) residue 12, followed by residues 14 - 52, followed by residues 133 - 145, followed by residues 175 - 400 of the said L protein.

HBsAg may also refer to polypeptides described in EP 0 198 474 or EP 0 304 578.

Normally the HBsAg will be in particle form. It may comprise S protein alone or may be as composite particles, for example (L*,S) wherein L* is as defined above and S denotes the S-protein of hepatitis B surface antigen.

The HBsAg may be adsorbed on aluminium phosphate as described in WO93/24148.

Preferably an hepatitis B (HBV) antigen used in the formulation of the invention is HBsAg S-antigen as used in the commercial product Engerix-B (Trade Mark; SmithKline Beecham Biologicals).

A vaccine comprising hepatitis B surface antigen in conjunction with 3D-MPL was described in European Patent Application 0 633 784.

Examples of antigens from additional pathogens which may be included in the compositions according to the invention are now described.

Epstein Barr Virus (EBV), a member of the herpesvirus group, causes infectious mononucleosis as a primary disease in humans. Predominantly it affects children or young adults. More than 90% of the average adult population is infected by EBV that persists for lifetime in peripheral B-lymphocytes. The virus is lifelong produced in the parotid gland and spread primarily by exchange of saliva from individuals who shed the virus. Children infected with EBV are largely asymptomatic or have very mild symptoms, while adolescents and adults who become infected develop typical infectious mononucleosis, characterised by fever, pharyngitis, and adenopathy. People who have been infected maintain anti-EBV antibodies for the remainder of their lives, and are thus immune to further infection.

In addition to its infectious qualities, EBV has been shown to transform lymphocytes into rapidly dividing cells and has therefore been implicated in several different

lymphomas, including African Burkitt's lymphoma (BL). EBV may also be involved in causing nasopharyngeal carcinoma (NPC). Worldwide it is estimated that 80,000 cases of nasopharyngeal carcinoma occur and it is more prevalent in ethnic Chinese populations. Infectious mononucleosis is a consequence of primary infection by EBV. It is not a life-threatening disease if additional risk factors are absent.

Four proteins of the EBV viral envelope constituting the so-called membrane antigen complex have been described. They are usually referred to as gp 220/350 or gp 250/350 or simply as gp 250 or 350 (see EP-A-151079). There is convincing evidence that gp 350 and gp 250 induce the production of neutralising antibodies and that antibodies against gp 350 and gp 250 have neutralising capacity. These proteins are thus candidates for a possible EBV vaccine. For further information about the application of gp 250/350 for prophylaxis and treatment of EBV-related diseases see EP 0 173 254.

The major EBV surface glycoprotein gp350/220 infects human target cells through interaction with the cellular membrane protein, CD21. Gp350/220 is the primary target for EBV-neutralising antibodies in humans and some forms of gp350/220 have been shown to protect against EBV-related disease. Preferably a vaccine composition according to the invention comprises gp 350 of EBV although other protective antigens may be used.

In a preferred aspect the vaccine composition of the invention additionally comprises a Varicella Zoster viral antigen (VZV antigen). Suitable antigens of VZV for inclusion in the vaccine formulation include gpI-V described by Longnecker et al., Proc Natl Acad Sci USA 84, 4303-4307 (1987).

In a preferred embodiment gpI (see Ellis et al., US patent 4,769,239) is used. See also European Patent No. 0 405 867 B1.

In another preferred aspect the vaccine composition of the invention additionally comprises a human cytomegalovirus (HCMV) antigen. HCMV is a human DNA virus belonging to the family of herpes viruses. HCMV is endemic in most parts of the world. Among two populations, HCMV is responsible for serious medical conditions. HCMV is a major cause of congenital defects in new borns. The second population at risk are immunocompromised patients such as those suffering from HIV infection and those patients undergoing transplantations. The clinical disease causes a variety of symptoms including fever, hepatitis, pneumonitis and infectious mononucleosis. A preferred antigen for use in a vaccine against HCMV is gB685** as described in WO 95/31555. Immunogens for use in HCMV vaccines are also provided by pp65, an HCMV Matrix Protein as described in WO 94/00150 (City of Hope).

In one preferred aspect the vaccine composition of the invention additionally comprises both a VZV and an HCMV antigen, in particular those antigens described above.

In another preferred aspect the vaccine composition of the invention additionally comprises a *Toxoplasma gondii* antigen. *Toxoplasma gondii* is an obligate intracellular protozoan parasite responsible for toxoplasmosis in warm-blooded animals, including man. Although it is generally clinically asymptomatic in healthy individuals, toxoplasmosis may cause severe complications in pregnant women and immunocompromised patients. A preferred antigen for use in a vaccine against *Toxoplasma gondii* is SAG1 (also known as P30) as described in WO96/02654 or Tg34 as described in WO92/11366.

In one preferred aspect the vaccine composition of the invention additionally comprises either a VZV antigen or an HCMV antigen combined with a *Toxoplasma gondii* antigen, in particular those antigens described above.

In a preferred aspect the vaccine composition of the invention is a multivalent vaccine, for example a tetra- or pentavalent vaccine.

The formulations of the present invention are very effective in inducing protective immunity, even with very low doses of antigen (e.g. as low as 5μg rgD2t).

They provide excellent protection against primary infection and stimulate, advantageously both specific humoral (neutralising antibodies) and also effector cell mediated (DTH) immune responses.

The present invention in a further aspect provides a vaccine formulation as herein described for use in medical therapy, particularly for use in the treatment or prophylaxis of human papillomavirus infections and herpes simplex virus infections.

The vaccine of the present invention will contain an immunoprotective quantity of the antigens and may be prepared by conventional techniques.

Vaccine preparation is generally described in Pharmaceutical Biotechnology, Vol.61 Vaccine Design - the subunit and adjuvant approach, edited by Powell and Newman, Plenum Press, 1995. New Trends and Developments in Vaccines, edited by Voller et al., University Park Press, Baltimore, Maryland, U.S.A. 1978. Encapsulation within liposomes is described, for example, by Fullerton, U.S. Patent 4,235,877. Conjugation of proteins to macromolecules is disclosed, for example, by Likhite, U.S. Patent 4,372,945 and by Armor et al., U.S. Patent 4,474,757.

The amount of protein in each vaccine dose is selected as an amount which induces an immunoprotective response without significant, adverse side effects in typical vaccinees. Such amount will vary depending upon which specific immunogen is employed. Generally, it is expected that each dose will comprise 1-1000μg of protein, preferably 2-100μg, most preferably 4-40μg. An optimal amount for a particular vaccine can be ascertained by standard studies involving observation of antibody titres and other responses in subjects. Following an initial vaccination, subjects may receive a boost in about 4 weeks.

In addition to vaccination of persons susceptible to HPV or HSV infections, the pharmaceutical compositions of the present invention may be used to treat, immunotherapeutically, patients suffering from the said viral infections.

In a further aspect of the present invention there is provided a method of manufacture as herein described, wherein the method comprises mixing a human papilloma virus antigen and a herpes simplex virus antigen with a TH-1 inducing adjuvant, for example 3D-MPL and, preferably, a carrier, for example alum.

If desired, other antigens may be added, in any convenient order, to provide multivalent vaccine compositions as described herein.

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CLAIMS

1. A vaccine composition comprising:

(a) a herpes simplex virus (HSV) antigen; and

(b) a human papillomavirus (HPV) antigen

in conjunction with an adjuvant which is a preferential stimulator of TH1 cell response.

2. A vaccine composition according to claim 1 which additionally comprises a carrier.

3. A vaccine composition according to claim 1 or claim 2 in which the preferential stimulator of TH1-cell response is selected from the group of adjuvants comprising: 3D-MPL, 3D-MPL wherein the size of the particles of 3D-MPL is preferably about or less than 100nm, QS21, a mixture of QS21 and cholesterol, and a CpG oligonucleotide.

4. A vaccine composition according to claim 3 in which the preferential stimulator of TH1-cell response is 3D-MPL.

5.. A vaccine compostion according to any one of claims 1 to 4 in which the HSV antigen is HSV-2 gD or a truncate thereof.

6. A vaccine composition according to any one of claims 1 to 5 which comprises at least one HPV antigen selected from the group consisting of L1, L2, E6 and E7, optionally in the form of a fusion protein and/or a truncate.

7. A vaccine composition according to any one of claims 1 to 6 in which an EBV antigen is additionally present.

8. A vaccine composition as defined in claim 7 in which the EBV antigen is gp 350.

9. A vaccine composition according to any one of claims 1 to 6 in which a hepatitis A antigen is additionally present.

10. A vaccine composition according to claim 9 in which the HAV antigen is derived from the HM-175 strain.

11. A vaccine composition according to any one of claims 1 to 6 in which a hepatitis B virus (HBV) antigen is additionally present.

12. A vaccine composition according to claim 11 in which the HBV antigen is hepatitis B surface antigen.

13. A vaccine composition according to any one of claims 1 to 12 in which the carrier is selected from the group comprising aluminium hydroxide, aluminium phosphate and tocopherol and an oil in water emulsion.

14. A vaccine composition according to any one of claims 1 to 13 which additionally comprises a VZV antigen.

15. A vaccine composition according to claim 14 in which the VZV antigen is gpI.

16. A vaccine composition according to any one of claims 1 to 15 which additionally comprises a HCMV antigen.

17. A vaccine composition according to claim 16 in which the HCMV antigen is gB685** or pp65.

18. A vaccine composition according to any one of claims 1 to 17 which additionally comprises a *Toxoplasma gondii* antigen.

19. A vaccine composition according to claim 18 in which the *Toxoplasma gondii* antigen is SAG1 or TG34.

20. A vaccine composition according to any one of claims 1 to 4 comprising HSV-2 gDt antigen and an HPV antigen chosen from the group consisting of L1, L2, E6, E7, protein D-E6, protein D-E7 or L2-E7 of HPV and optionally in addition one or more of HBsAg S antigen; EBVgp 350; VZVgpI; HAV HM-175 inactivated strain; gB685** or pp65 of HCMV and SAG1 or TG34 antigens of *Toxoplasma gondii*.

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OFICINA DE PATENTES

Referencia No. KLP/RH/B45198 No. 9921146.8 18 de noviembre de 1999

OFICINA DE PATENTES: Solicitud de adjudicación de una Patente.

Forma 1/77 Ley de Patentes de 1977

1. TITULO DEL INVENTO: "COMPOSICIÓN NOVEDOSA"

2. INFORMACIÓN DEL SOLICITANTE:

2a. Nombre: SmithKline Beecham Biologicals S.A.

Pais: BELGICA

2b (N/D)

2c Dirección: Rue de l'Institut 89, B-1330 Rixensart

Código Postal:

PAIS. BELGICA

ADP No. 5781117001

3. Ha nombrado Ud. a alguien que le represente en su solicitud? SI

Nombre del Agente: KATHRYN L PRIVETT
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No. ADP 7735830001

4. Número de Referencia: KLP/RH/B45198

5. Solicita Ud. que esta solicitud sea tratada como si hubiera sido radicada en la fecha de presentación de una solicitud anterior: NO

6. No aplica

7. Es usted el único inventor o inventor conjunto? NO

8. Cuántas hojas describen esta solicitud? 16 - Reivindicaciones: - 3

Resumen: - - Dibujos - -

9. Solicitud: Solicitamos la adjudicación de una patente teniendo en cuenta esta solicitud.

(FIRMA) KATHRYN L. PRIVETT Fecha: 07/09/99

Favor devolver la forma diligenciada y sus anexos y duplicados, junto con su valor a:

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87 89

LEGALIZACIONES

OFICINA DE PATENTES

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El suscrito, un funcionario debidamente autorizado conforme a los Artículos 74(1) y (4) de la Ley de Desreglamentación y Contratación de 1994 para firmar y expedir certificados en nombre del Contralor General, certifico que la copia adjunta es una copia correcta de los documentos archivados originalmente con respecto a la solicitud de patente que allí se identifica.

De acuerdo con las Reglamentaciones de Patentes de 1982 (Re-registro de Compañías), si una compañía nombrada en este certificado y en los documentos adjuntos se ha vuelto a registrar según la Ley de Compañías de 1980 con el mismo nombre con el que fue registrada inmediatamente antes del re-registro, salvo la sustitución o inclusión como la última parte del nombre de las palabras "sociedad anónima" o su equivalente en galés, las referencias al nombre de la compañía y sus documentos adjuntos serán tratadas como referencias al nombre con el cual fue re-registrada.

De acuerdo con las reglas, las palabras "sociedad anónima" (o en inglés *public limited company)* pueden ser reemplazadas por las letras p.l.c, plc. P.L.C ó PLC.

El re-registro bajo la Ley de Compañías no constituye una nueva entidad legal sino simplemente somete a la compañía a ciertas normas de ley adicionales.

(FIRMA) R. MAHONEY FECHA: 22 de agosto del 2000

(SELLO DE LACRE)

COMPOSICIÓN NOVEDOSA

REIVINDICACIONES:

1. Una composición de vacuna, que comprende:

a) un antígeno del virus herps simplex (HSV); y

b) un antígeno de papillomavirus humano

junto con un adyuvante que es un estimulador preferencial de la respuesta de la célula TH1.

2. Una composición de vacuna según la reivindicación 1 que adicionalmente comprende un vehículo.

3. Una composición de vacuna según la reivindicación 1 ó 2, en la que el estimulador preferencial de la respuesta de la célula TH1 es seleccionada del grupo de adyuvantes que comprenden; 3D-MPL, 3D-MPL, en donde el tamaño de las partículas de 3D-MPL es preferiblemente aproximadamente o menos de 100 nm, QS21, una mezcla de QS21 y colesterol, y un oligonucleótido CpG.

4. Una composición de vacuna según la reivindicación 3, en la que el estimulador preferencial de la respuesta de la célula TH1 es 3D-MPL.

5. Una composición de vacuna según cualquiera de las reivindicaciones 1 a 4, en la que el antígeno HSV es HSV-2 gD o un truncado de lo mismo.

6. Una composición de vacuna según cualquiera de las reivindicaciones 1 a 5, que comprende al menos un antígeno HPV seleccionado del grupo compuesto por L1, L2, E6 y E7, opcionalmente en la forma de una proteína de fusión y/o un truncado.

7. Una composición de vacuna según cualquiera de las reivindicaciones 1 a 6, en donde un antígeno EBV está adicionalmente presente.

8. Una composición de vacuna según se define en la reivindicación 7, en la que el antígeno es gp 350.

9. Una composición de vacuna según cualquiera de las reivindicaciones 1 a 6, en la que un antígeno de hepatitis A está adicionalmente presente.

10. Una composición de vacuna según la reivindicación 9, en la que el antígeno HAV se deriva de la cepa HM-175.

11. Una composición de vacuna según cualquiera de las reivindicaciones 1 a 6, en la que un antígeno del virus de hepatitis B (HBV) está adicionalmente presente.

12. Una composición de vacuna según la reivindicación 11, en la que el antígeno HBV es un antígeno de superficie de hepatitis B.

13. Una composición de vacuna según cualquiera de las reivindicaciones 1 a 12, en la que el vehículo es seleccionado del grupo compuesto por hidróxido de aluminio, fosfato de aluminio y tocoferol en una emulsión aceite en agua.

14. Una composición de vacuna según cualquiera de las reivindicaciones 1 a 13 que adicionalmente comprende un antígeno VZV.

15. Una composición de vacuna según la reivindicación 14 en la que el antígeno VZV es gpI.

16. Una composición de vacuna según cualquiera de las reivindicaciones 1 a 15 que adicionalmente comprende un antígeno HCMV.

17. Una composición de vacuna según la reivindicación 16, en la que el antígeno HCMV es gB685* o pp65.

18. Una composición de vacuna según cualquiera de las reivindicaciones 1 a 17 que adicionalmente comprende un antígeno de *Toxoplasma gondii*

19. Una composición de vacuna según la reivindicación 18, en la que el antígeno de *Toxoplasma gondii* es SAG1 o TG34.

20. Una composición de vacuna según cualquiera de las reivindicaciones 1 a 4, que comprende un antígeno HSV-2 gDt y un antígeno HPV escogido del grupo compuesto por L1, L2, E6, E7, proteína D-E6, proteína D-E7 o L2-E7 de HPV y opcionalmente en adición uno o más del antígeno HbsAg; EBV gp 350; VZV gpI; cepa inactivada de HAV HM-175; gB685** o pp65 de HCMV y antígenos SAG1 o TG34 de *Toxoplasma gondii.*