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Castillo Grau & Asociados
ABOGADOS

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Señora Jefe
DIVISION DE NUEVAS CREACIONI
SUPERINTENDENCIA DE INDUSTI
Bogotá, D.C.

SUPERINTENDENCIA Y COMERCIO
Radicacion : 00066605 00000003 Folios: 2
Fecha (AMD): 2003-07-02 10:40:26
Tramite : 002 PATENTES D 1 REGISTRO/ 538 PETICION
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

Re: Expediente No. 00 66.605
Solicitud de Patente a nombre de
SmithKline Beecham Biologicals s.a.
Código 02 – 01 - 538

En relación con la solicitud contenida en el Expediente de la referencia y, en particular, con la publicación del extracto de la misma en la Gaceta de la Propiedad Industrial No. 528 de 30 de Mayo de 2003, por medio del presente escrito muy respetuosamente me permito solicitarle se sirva proceder a efectuar el **EXAMEN DE PATENTABILIDAD** de la solicitud aquí contenida.

En consecuencia, acompaño el comprobante de pago de la tasa correspondiente, Recibo No. 03 – 45540

De Usted atentamente,

GERMAN CASTILLO GRAU
T.P. No. 2978 del C.S.J.

SUPERINDUSTRIA Y COMERCIO
Radicacion : 00066605 00000003 Folios: 2
Fecha (AMD): 2003-07-02 10:40:36
Tramite : 002 PATENTES D 1 REGISTRO/ 536 PETICION
NIT : 800176089 Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

RECIBO OFICIAL DE CAJA : 03 - 45,540
FECHA : JULIO 2 DE 2003

C O N S I G N A C I O N

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
CASTILLO GRAU Y ASOCIADOS	CONSIGNACION	BANCO POPULAR	050-00110-6	8771294	02/07/2003	1,074,000.00

C O N C E P T O

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-01 SOLICITUDES	85 EXAMEN DE PATENTABILIDAD DE INVEN	335,000.00
		TOTAL : 0	335,000.00

SON: TRESCIENTOS TREINTA Y CINCO MIL PESOS

RESPONSABLE : _____

RECIBO DE CAJA APLICADO AL EXPEDIENTE No._____

DIVISION DE NUEVAS CREACIONES

RADICACIÓN EXPEDIENTE No. **00-66605**

EL EXTRACTO DE LA PRESENTE SOLICITUD DE **PATENTE DE INVENCION** FUE PUBLICADO EN LA GACETA DE PROPIEDAD INDUSTRIAL No **528**, DE FECHA **30** DE **MAYO** DE **2003**, EL TERMINO HABIL SEÑALADO POR LA LEY EXPIRA EL **29** DE **AGOSTO** DE **2003**.

NOTA: Dentro del plazo de los seis (6) meses siguientes a partir de la fecha de publicación en la gaceta de Propiedad Industrial, deberá solicitar y cancelar el examen de patentabilidad so pena de que la solicitud caiga en abandono, de acuerdo con lo establecido en el Artículo 44 de la Decisión 486/2000.

SE PRESENTARON OPOSICIONES POR PARTE DE TERCEROS.

SI ___ _____ NO ___ **X** _____

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Industria y Comercio
SUPERINTENDENCIA

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Doctor
GERMAN CASTILLO GRAU
Smithkline Beecham Biologicals
(Apoderado)

ASUNTO

Radicación 00-66605
Trámite 002
Evento 001
Actuación 430
Oficio 7737
Folios

Como resultado del examen de fondo, realizado con fundamento en el artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica:

Documentos estudiados:

Los textos estudiados fueron la descripción (Folios: 4 a 51) y las reivindicaciones 1 a 20(folios: 52 a 55).

La presente solicitud reivindica prioridad GB Nº 9921146,8. de 7 de septiembre de 1999 y cumple con lo establecido en los artículos 9, 10 y 11 de la Decisión 486 de la Comisión de la Comunidad Andina.

Objeto de invención:

El objeto de la presente solicitud se refiere a una composición de vacuna que comprende un antígeno del virus del herpes simple (HSV), antígeno de papiloma humano (HPV) más un coadyuvante estimulador Th1 y un vehículo. En donde el antígeno HSV es gDt de HSV-2, el antígeno HPV puede ser L1, L2, E6, E7, Proteína D-E6 o L2-E7, el adyuvante estimulador Th1 es 3DMPL y el vehículo se selecciona entre $Al(OH)_3$, $AlPO_4$, tocoferol o emulsión O/W Las composiciones pueden contener además 1 o más antígenos seleccionados entre HBsAg, gp350 de EBV, gpI de VZV, cepa inactivada HM175 de HAV, gB685** o pp65 de HCMV y SAG1 o TG34 de Toxoplasma gondii.

El objeto de la solicitud definido anteriormente se clasificó en la IPC A61K 39/00, 39/245, 31/295.

Determinación del estado de la técnica:

El artículo 16 de la Decisión 486 de la Comisión de la Comunidad Andina establece que "El estado de la técnica comprenderá todo lo que haya sido accesible al público por una descripción escrita u oral, utilización, comercialización o cualquier otro medio antes de la fecha de presentación de la solicitud de Patente o, en su caso, de la prioridad reconocida.

Sólo para el efecto de la determinación de la novedad, también se considerará dentro del estado de la técnica, el contenido de una solicitud de patente en trámite ante la oficina nacional competente, cuya fecha de presentación o de prioridad fuese anterior a la fecha de presentación o de prioridad de la solicitud de patente que se estuviese examinando, siempre que dicho contenido esté incluido en la solicitud de fecha anterior cuando ella se publique o hubiese transcurrido el plazo previsto en el artículo 40".

Considerando que la fecha de presentación de la primera solicitud de patente de la cual se reivindica prioridad es: 7 de septiembre de 1999, se buscaron documentos del estado de la técnica relacionados, con fecha de publicación anterior a esta.

Se hizo la búsqueda de documentos relevantes en el estado de la técnica, entendiendo como estado de la técnica la definición provista en el artículo 16 de la Decisión 486 de la Comisión de la Comunidad Andina y las excepciones fijadas en el artículo 17 de la misma.

Se encontró como anterioridades (documentos cuya fecha de publicación es anterior a la fecha de presentación de la primera solicitud de patente sobre la cual se reivindica prioridad o de la fecha de presentación de esta solicitud) que pueden afectar la patentabilidad del objeto de la presente solicitud:

Nº	Documento	Título	Fecha de Publicación
1	US 5855891	ICHIMERIC PAPILLOMAVIRUS-LIKE PARTICLES	9 de enero de 1997
2	WO95/17209	VACCINES	29 de junio de 1995

Evaluación de los requisitos de Patentabilidad:

El Artículo 14 de la Decisión 486 de la Comisión de la Comunidad Andina el cual establece: "Los Países Miembros otorgarán Patentes para las invenciones, sean de producto o de procedimiento en todos los campos de la tecnología siempre que sean nuevas, tengan nivel inventivo y sean susceptibles de aplicación industrial".

Se procedió a evaluar los requisitos de novedad, nivel inventivo y aplicación industrial con el fin de establecer la patentabilidad de la invención reivindicada en la solicitud en estudio.

Evaluación de Nivel Inventivo:

El artículo 18 de la Decisión 486 de la Comisión de la Comunidad Andina establece que "Se considerará que una invención tiene nivel inventivo, si para una persona del oficio normalmente versada en la materia técnica correspondiente, esa invención no hubiese resultado obvia ni se hubiese derivado de manera evidente del estado de la técnica".

El Nivel Inventivo se evaluó por comparación entre lo solicitado y encontrado en el estado de la técnica y que se relaciona con el objeto reivindicado y de acuerdo con la definición de nivel inventivo del Artículo 18 de la Decisión 486 de la Comisión de la Comunidad Andina.

Las reivindicaciones 1 a 6 de la solicitud en estudio reclaman composiciones de vacunas que comprenden un antígeno de Herpes simplex (HSV), un antígeno de papiloma humano (HPV) más un coadyuvante estimulador Th1 y un vehículo. En donde el antígeno HSV es gDt de HSV-2, el antígeno HPV puede ser L1, L2, E6, E7,

proteína D-E6 o L2-E7, y el adyuvante estimulador Th1 se selecciona del grupo 3DMPL <100nm, QS21, QS21+colesterol+CpG.

El documento US 5,855,891 de ahora en adelante D1, divulga partículas quiméricas de virus en las que se emplean epítopes conformacionales L1 y L2 de papiloma humano específicamente HPV6, fusionados con otras proteínas para obtener un mayor espectro de acción. También se menciona el empleo de epítopes E6 y E7 de HPV en la generación de las partículas virales. Adicionalmente uno de los socios de fusión sugeridos en D1 es Herpes simplex (HSV).

De acuerdo con lo anterior las vacunas de las reivindicaciones 1 a 6 se diferencian de las divulgadas en D1, en el adyuvante estimulador de la respuesta Th1.

Sin embargo en la práctica es común el empleo de adyuvantes estimuladores de la respuesta Th1, tales como 3DMPL, saponinas, QS21 para potenciar la respuesta inmune a un antígeno dado. Esto debido a que la respuesta Th1 a través de interferón alfa esta asociada con respuestas protectivas frente a patógenos intracelulares. De hecho, el Documento WO95/17209 divulga el empleo de 3DMPL y QS21 en vacunas que contienen antígenos de HSV (gD) en forma de emulsión O/W con el objeto de obtener respuestas inmunológicas mejoradas.

Siendo que en la anterioridad D2 ya había sido divulgado el efecto de los adyuvantes 3DMPL y QS21 en vacunas conteniendo el antígeno gD de HSV, resulta obvio para una persona medianamente versada en el desarrollo de vacunas, adicionar estos mismos adyvantes a composiciones que contienen el antígeno gD de HSV + el epítope L1 o L2 de HPV.

Por otra parte, el documento D1 revela que el producto de fusión no está limitado a un único socio de fusión por molécula y que se pueden incluir socios adicionales. De esta forma un técnico en la materia podría de manera evidente crear proteínas fusionadas a partir de sus epítopes de interés y de antígenos de patógenos ampliamente conocidos en el estado del arte. De esta forma la materia contenida en las reivindicaciones 7 a12 y 14 a 20 carecería de nivel inventivo.

Por último los vehículos de hidróxido y fosfato de aluminio son ampliamente empleados en vacunas y en formulaciones de emulsiones tipo O/W y por ende la reivindicación 13 no sería considerada como inventiva.

La respuesta al problema técnico que se pretendía resolver, que era proveer vacunas combinadas para la prevención frente a infecciones por virus de papiloma humano (HPV) resulta derivado de la combinación de las anterioridades D1 y D2.

Como se observa, el objeto de esta solicitud podría resultar obvio para una persona del oficio normalmente versada en la materia y derivarse de manera evidente del estado de la técnica.

En conclusión los requisitos de patentabilidad de la presente solicitud se encuentran afectados así:

Nº	Documento	Reivindicaciones afectadas	Requisito que afecta
1	US 5855891	1-12, 14-20	Nivel Inventivo
2	WO95/17209	3-4	Nivel Inventivo

101

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta días contados a partir de la fecha de la notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única N° 10 de 2001.

ALIX CARMENZA CÉSPEDES DE VERGEL
Jefe de la División de Nuevas Creaciones

El anterior requerimiento fue notificado por fijación en lista, de fecha 01 SET. 2005

CONCEPTO TÉCNICO N°: 1274	**EXAMINADOR TÉCNICO Código N°: 202063**	**EXAMINADOR JURÍDICO Código N°: N.A**

US005855891A

United States Patent [19]

Lowy et al.

[11] Patent Number: 5,855,891

[45] Date of Patent: Jan. 5, 1999

[54] **ICHIMERIC PAPILLOMAVIRUS-LIKE PARTICLES**

[75] Inventors: **Douglas R. Lowy**, Bethesda; **John T. Schiller**; **Heather Greenstone**, both of Silver Spring, all of Md.

[73] Assignee: **The United States of America as represented by the Department of Health and Human Services**, Washington, D.C.

[21] Appl. No.: **781,084**

[22] Filed: **Jan. 9, 1997**

Related U.S. Application Data

[60] Division of Ser. No. 319,467, Oct. 6, 1994, Pat. No. 5,618,536, which is a continuation-in-part of Ser. No. 32,869, Mar. 16, 1993, Pat. No. 5,437,951, which is a continuation-in-part of Ser. No. 941,371, Apr. 3, 1992.

[51] **Int. Cl.**[6] **A61K 39/00**; A61K 39/12; C12P 21/06; C12N 7/00

[52] **U.S. Cl.** **424/192.1**; 424/204.1; 435/69.1; 435/235.1; 435/236

[58] **Field of Search** 435/235.1, 240.2, 435/69.1, 254.2, 320.1, 236, 317.1; 530/412, 413; 424/192.1, 204.1

[56] **References Cited**

FOREIGN PATENT DOCUMENTS

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WO9302184	2/1993	WIPO .
WO9405792	3/1994	WIPO .

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Kirnbauer et al. (1992) Papillomavirus L1 major capsid protein self–assembles into virus–like particles that are highly immunogenic. Proc. Natl. Acad. Sci. 89:12180–12184.

Laga et al. (1992) Genital papillomavirus infection and cervical dysplasia–opportunistic complications of HIV infection. Int. J. Cancer 50:45–48.

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(List continued on next page.)

Primary Examiner—Ponnathapura Achutamurthy
Assistant Examiner—Hankyel T. Park
Attorney, Agent, or Firm—Knobbe, Martens, Olson & Bear, LLP

[57] **ABSTRACT**

The present invention provides a papillomavirus-like particle having conformational epitopes comprising a papillomavirus L1 fusion product and, optionally, a papillomavirus L2 product; and related DNA molecules, host cells, and methods.

10 Claims, No Drawings

-continued

(v i) ORIGINAL SOURCE:

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GCTCCGCGGG GTACCTGCAG GATCAGCC 28

What is claimed is:

1. A papillomavirus-like particle having conformational epitopes comprising a papillomavirus L1 fusion product.

2. The papillomavirus-like particle of claim 1 wherein said papillomavirus L1 fusion product is a human papillomavirus L1 fusion product or a bovine papillomavirus L1 fusion product.

3. The papillomavirus-like particle of claim 2 wherein said human papillomavirus L1 fusion product is a HPV16 L1 fusion product and said bovine papillomavirus L1 fusion product is a BPV1 L1 fusion product.

4. The papillomavirus-like particle of claim 1 wherein said papillomavirus L1 fusion product comprises a fusion partner that is a peptide or a full-length protein, or comprises fusion partners that include peptides or full-length proteins or combinations thereof linked in tandem.

5. The papillomavirus-like particle of claim 4 wherein said fusion partner is a papillomavirus E6 or E7 product.

6. The papillomavirus-like particle of claim 1 wherein said papillomavirus L1 fusion product is fused at its N-terminus or C-terminus to a fusion partner.

7. The papillomavirus-like particle of claim 1 wherein said papillomavirus L1 fusion product has a fusion partner inserted between L1 amino acids.

8. The papillomavirus-like particle of claim 1 further comprising a papillomavirus L2 product.

9. The papillomavirus-like particle of claim 8 wherein said papillomavirus L2 product is a HPV16 L2 product or a BPV1 L2 product.

10. A composition comprising the papillomavirus-like particle of claim 1.

* * * * *

ICHIMERIC PAPILLOMAVIRUS-LIKE PARTICLES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a division of U.S. patent application Ser. No. 08/319,467, filed Oct. 6, 1994, U.S. Pat. No. 5,618,536, which was a continuation-in-part of U.S. patent application Ser. No. 08/032,869, filed Mar. 16, 1993, now U.S. Pat. No. 5,437,951, which was a continuation-in-part of U.S. patent application Ser. No. 07/941,371, filed Sep. 3, 1992. These applications and all patent applications, patents and publications cited hereunder are incorporated herein by reference.

FIELD OF THE INVENTION

The present invention relates to chimeric papillomavirus-like particles and related synthetic DNA molecules, host cells, methods and vaccines.

BACKGROUND OF THE INVENTION

Papillomaviruses infect the epithelia of humans and a wide variety of animals, where they generally induce benign proliferation at the site of infection. However, in some cases the lesions induced by certain papillomaviruses undergo malignant progression. There is a strong association between malignant progression of human genital lesions and certain human papillomavirus (HPV) types, such as HPV16. Infection by one of these types is considered the most significant risk factor in the development of cervical cancer, one of the most common cancers of women worldwide (zur Hausen, H., Science 254:1167 (1991); Schiffman, M. H., J. Natl. Cancer Inst. 84:394 (1992)). The majority of cervical carcinomas contain and express HPV early genes, such as E6 and E7, and these genes have been shown to have potent transforming and immortalizing activity in cultured cells (Werness, B. A., Munger, K. & Howley, P. M. (1991) Advances in Oncology, eds. DeVita V. T., Hellman, S. & Rosenberg, S. A. (Lipponcott, Philadelphia) pp. 3–18).

Papillomaviruses are non-enveloped double-stranded DNA viruses about 55 nm in diameter with an approximately 8 kb genome in the nucleohistone core (Baker, et al., Biophys J 60:1445 (1991)). The capsids are composed of two virally-encoded proteins, L1 and L2, that migrate on SDS-PAGE gels at approximately 55 kDa and 75 kDa, respectively (Mose Larson et al., J. Virol. 61:3596 (1987)). L1, which is the major capsid protein, is arranged in 72 pentameters which associate with T=7 icosahedral symmetry. The function and position within the virion of L2 are unclear (Baker, et al., Biophys J 60:1445 (1991)).

The L1 protein has the capacity to self-assemble so that large amounts of virus-like particles (VLPs) may be generated by expression of the L1 protein from a number of species of papillomavirus in a variety of recombinant expression systems (Hagensee et al., J Virol 67:315 (1993); Kirnbauer et al., Proc Natl Acad Sci USA 89:12180 (1992); Kirnbauer et al., J Virol 67:6929 (1993); Rose et al., J Virol 67:1936 (1993)). Although not required for assembly, L2 is incorporated into VLPs when co-expressed with L1 (L1/L2 VLPs) in cells.

Immunization of rabbits with native virions or L1 VLPs, but not with non-assembled L1 expressed in *E. coli*, induces high titers of neutralizing serum antibodies (Christensen, N. D. and Kreider, J. W., J Virol 64:3151 (1990); Kirnbauer et al., Proc Natl Acad Sci USA 89:12180 (1992); Pilacinski et al., Bio/Technology 2:356 (1984); Segre et al., Am. J. Vet. Res. 16:517 (1955)). The polyclonal and monoclonal antibodies generated against native particles recognize conformationally dependent epitopes (Christensen, N. D. and Kreider, J. W., Virus Res 28:195 (1993); Christensen et al., J Virol 64:5678 (1990); Christensen et al., Virology 181:572 (1991)).

Neutralizing antibodies generated against VLPs also recognize conformationally dependent epitopes. Using infectious BPV1, which can be readily obtained from bovine lesions, and a quantitative in vitro BPV1 infectivity assay (Dvoretzky et al., Virology 103:369 (1980)), workers showed VLPs from bovine papillomavirus induced high levels of neutralizing antibodies (Kirnbauer et al., Proc Natl Acad Sci USA 89:12180 (1992)). The neutralizing antibodies were directed against conformationally dependent epitopes, in that denaturation of the particles prior to immunization abolished the ability of the preparation to induce neutralizing activity (id.).

When the L1 gene of a HPV16 isolate derived from a nonprogressed lesion was used to express the L1 major capsid protein in insect cells via recombinant baculoviruses, L1 self-assembled into VLPs at a yield 3 orders of magnitude higher than what had been obtained using L1 derived from the prototype HPV16 (originally isolated from a cancerous lesion), and formed VLPs that were morphologically similar to native virions (Kirnbauer et al., J Virol 67:6929 (1993)). DNA sequence comparison identified a single non-conserved amino acid change to be responsible for the inefficient self-assembly of the prototype L1 (id.). The L1 of the assembly-competent clone is thus considered to be the wild-type gene, and the prototype L1 of the assembly-defective clone a mutant.

Using HPV16 VLPs of the wild-type L1 protein as antigens, an ELISA was developed that detected serum antibodies in patients infected with HPV16 (Kirnbauer et al., J. Natl. Cancer Inst. 86:494 (1994)). In contrast, neither denatured HPV16 particles nor preparations of the prototype L1 protein could detect these antibodies (id.). These results demonstrate that the prototype L1 protein does not present conformational epitopes.

Rabbit serum raised against self-assembled wild-type HPV16 L1/L2 virus-like particles was discovered to prevent HPV16 VLP binding to cell surface molecules (Roden et al., J. Virol., in press, (November, 1994)). In contrast, serum raised against the prototype strain of HPV16 L1/L2 did not prevent such binding (id.). The data show that the prototype HPV16 strain lacks conformational epitopes.

Rabbits immunized with intact cottontail rabbit papillomavirus (CRPV) virus-like particles composed of L1 or L1/L2 were protected from subsequent experimental challenge by infectious CRPV (Breitburd et al., 12th International Papillomavirus Workshop in Amsterdam, in press, (October 1994)). In contrast, those immunized with denatured particles were not protected (id.). These findings are consistent with the conclusion that VLPs presenting conformational epitopes are able to induce protective immunity.

VLPs composed of capsid proteins are attractive candidates for prophylactic vaccines to prevent papillomavirus infection. However, it is unlikely that these VLP vaccines would have therapeutic effects against established papillomavirus infections. The capsid proteins, unlike E6 and E7, are not detectably expressed in progressed lesions or in infected basal epithelial cells, which are the presumed targets in immune regression of papillomas.

There is evidence from experimental models that immunity against papillomavirus proteins other than L1 and L2

might help control papillomavirus infection. Since E6 and E7 are selectively maintained during oncogenic progression, there is the possibility that peptides derived from these oncoproteins could serve as targets for cell-mediated immune responses to HPV-containing tumor cells. Studies in animal models suggest the E7 protein of HPV16 acts as a tumor rejection antigen (Chen et al., Proc. Natl. Acad. Sci. USA 88:110 (1991); Feltkemp et al., Eur. J. Immunol. 23:2242 (1993)). Moreover, the frequency of HPV infection, persistence of HPV infection, and risk of developing cervical cancer and other HPV-related cancers is increased in patients with depressed cellular immunity (Allout et al., Br. Med. J. 298:153 (1989); Laga et al., Int. J. Cancer 50:45 (1992)). These observations suggest cell-mediated immunity is important in the defense against HPV infection and its associated tumor development. The induction of such immunity might be therapeutic, as well as prophylactic.

It has been demonstrated that foreign peptides can be incorporated into viral capsid-like structures and these chimeric particles can be used to present foreign antigens to the immune system. Published examples include hepatitis B core antigen particle presentation of human rhinovirus type 2 epitopes (Francis et al., Proc. Natl. Acad. Sci. USA 87:2545 (1990)), gp41 of HIV (Borisova et al., FEBS Lett. 259:121 (1989)), and B19 parvovirus particle presentation of peptides from herpes simplex virus 1 and murine hepatitis virus (Brown et al., Virology 198:477 (1994)). The parvovirus chimeras protected mice from experimental challenge with the corresponding virus. In all of the above systems, foreign sequences have been inserted in proteins integral to the capsid structure and have been limited to less than 20 amino acids. However a recent study (Miyamura et al., Proc. Natl. Acad. Sci. USA 91:8507 (1994)) has demonstrated that the entire 147 aa hen egg white lysozyme protein can be incorporated into B19 parvovirus particles when fused to the parvovirus L1 minor capsid protein. The lysozyme remained biologically active and elicited an immune response when injected into rabbits. In perhaps less relevant studies, hepatitis B virus surface antigen particles (which are lipid membrane structures) containing 84 aa of HIV-1 envelope glycoprotein (Michel et al., J. Virol. 64:2452 (1990)) and yeast Ty virus-like particles containing a portion of HIV-1 V3 loop (Griffiths et al., J. Virol. 65:450 (1991)) have also been shown to produce an immune response to the inserted peptides when inoculated into animals. With respect to papillomaviruses, it was recently reported that hepatitis B core antigen particles containing HPV16 E7 peptides (all less than 20 aa) induced peptide specific antibodies and T-helper responses in mice (Tindle et al., Virology 200:547 (1994)).

Chimeric particles based on self-assembled papillomavirus L1 have not been reported, nor has the use of L2 as a viral fusion partner for purposes of generating chimeric VLPs been described. The chimeric particle studies cited above involve viruses that are unrelated to papillomaviruses and thus cannot predict the results of chimeric particle studies involving papillomaviruses. Indeed, in the papillomavirus study by Kimbauer et al., J Virol 67:6929 (1993), supra, it was demonstrated that a single nonconserved amino acid change in L1 is responsible for inefficient self-assembly of L1 into VLPs and the presentation of conformational epitopes, which seem to be required for induction and detection of clinically relevant immune reactivity. Thus, the studies using viruses unrelated to papillomavirus cannot predict whether a papillomavirus L2 containing a foreign peptide or protein can co-assemble with papillomavirus L1 into particles, given that a single amino acid substitution in L1 can abolish efficient self-assembly. Neither can these studies predict whether any resulting chimeric particles will retain the ability to induce or detect neutralizing antibodies or other immune related responses, given that a single amino acid substitution in L1 can bar the presentation of conformational epitopes.

It is an object of the present invention to provide chimeric papillomavirus-like particles. These chimeric particles may function as platforms for multivalent antigen presentation. Or they may serve for delivery into cells of proteins for processing into peptides and subsequent presentation of these peptides within the context of MHC molecules to elicit a cell-mediated immune response. The chimeric particles represent a cost effective way to generate an effective papillomavirus vaccine with a broad spectrum of utility. Alternatively, the chimeric papillomavirus-like particles may be applied to VLP and/or fusion partner purification. Or, the particles may operate to deliver into cells intact and active proteins, for example, enzymes, or toxins or drugs.

SUMMARY OF THE INVENTION

According to one aspect of the invention, there is provided a papillomavirus-like particle, characterized as having conformational epitopes, comprising a papillomavirus L1 product and a papillomavirus L2 fusion product.

The papillomavirus L2 fusion product may be characterized as being a human papillomavirus L2 fusion product or a bovine papillomavirus L2 fusion product.

The human papillomavirus L2 fusion product may be characterized as being a HPV16 L2 fusion product and the bovine papillomavirus L2 fusion product may be characterized as being a BPV1 L2 fusion product.

The papillomavirus L2 fusion product may comprise a fusion partner characterized as being a peptide or a full-length protein, or may comprise fusion partners that include peptides or full-length proteins or combinations thereof linked in tandem.

The fusion partner may be a papillomavirus E6 or E7 product.

The papillomavirus L2 fusion product may be characterized as being fused at its N-terminus or C-terminus to a fusion partner.

The papillomavirus L2 fusion product may be characterized as having a fusion partner inserted between L2 amino acids.

The papillomavirus L1 product may be characterized as being a human papillomavirus L1 product or a bovine papillomavirus L1 product.

The human papillomavirus L1 product may be characterized as being a HPV16 L1 product and the bovine papillomavirus L1 product may be characterized as being a BPV1 L1 product.

The papillomavirus-like particle may consist essentially of HPV16L1 and HPV16L2-HPV16E7 (full-length), where HPV16E7 is fused to the C-terminus of HPV16L2, BPVL1 and BPVL2-HPV16E7 (full-length), where HPV16E7 is fused to the C-terminus of BPVL2, or BPVL1 and BPVL2-HPV16E7 (amino acids 1–30), where HPV16E7 is fused to BPVL2 between L2 amino acids 274 and 275.

According to another aspect of the invention, there is provided one or more synthetic DNA molecule or molecules characterized as singly or doubly encoding a papillomavirus L1 product and a papillomavirus L2 fusion product where the molecule or molecules direct expression in a transformed host cell of a papillomavirus-like particle, characterized as

amino acid residues, advantageously at least about 10 amino acid residues, and preferably at least about 5 amino acid residues. Type, subgroup and strain variations of L2 (and L1), and human allelic and species variations of the fusion partner protein, are expressly contemplated as falling within the scope of the invention. The invention also includes conservative variants of the full-length L2 protein (and L1 protein) and the full-length fusion partner protein, and their peptide fragments, where conservative amino acids are substituted for amino acid residues of wild-type L2 (and L1) and the fusion partner protein. To account for degeneracy of the genetic code, the invention also includes DNA coding for the same amino acid residues as does the DNA of the L2 (and L1) and fusion partner gene.

The chimeric papillomavirus-like particle itself is envisioned as incorporating any L2 fusion product with any L1product from any papillomavirus, whether the genomes are closely related, or are distantly related, so long as incorporation into particles occurs. Thus, a L2 fusion product, for example, related to any of BPV-1, BPV-2, BPV-4, CRPV, DPV, EEPV, HPV-1, HPV-5, HPV-6, HPV-8, HPV-11, HPV-16, HPV-18, HPV-31 or HPV-33, can be incorporated into particles with any L1 product, for example, from any of the above virus, or any type, subgroup or strain variation of papillomavirus.

Because VLPs present conformationally dependent epitopes required for the induction of high titer neutralizing serum antibodies, VLP chimeras containing L2 fusion products can operate prophylactically as optimized subunit vaccines for the stimulation of humoral immunity to prevent papillomavirus infection and thereby preclude the development of papillomavirus associated cancers and other papillomavirus associated pathologies.

Because it is unlikely VLPs will prove effective as therapeutic vaccines to induce regression of existing papillomavirus proliferative lesions, as discussed above, chimeric VLPs can function to address this long-felt and heretofore unsatisfied need to develop a therapeutic vaccine.

Chimeric VLPs are expected to bind specific cell surface receptors, get internalized and be released into the cytoplasm, and thus be more likely to promote the presentation of peptides in conjunction with class I MHC molecules for display to cytotoxic T cells for the generation of cell-mediated immunity. This is in contrast to uncomplexed proteins that would not be expected to specifically enter cells, or to promote the presentation of peptides in the context of Class I MHC molecules to elicit a cytotoxic T cell response (being more likely, if at all, to promote the presentation of peptides to be linked to Class II MHC molecules and displayed to helper T cells).

Inclusion of one or more fusion partners as L2 fusion products would clearly be a cost effective way to generate an effective papillomavirus vaccine with a broad spectrum of utility, including therapy and improved prevention of clinical lesions.

The fusion partner may be selected from the list consisting of those fusion partners that would provide a method for expanding the potential targets of a VLP-based vaccine, for example, E6 or E7 peptide or full-length E6 or E7, other papillomavirus peptides or proteins, or peptides or proteins of other STD or infectious agents, e.g., Herpes simplex, HIV, *Chlamydia trachomatis, Neisseria gonorrhoeae*, and *Treponema pallidum*.

The L2 fusion product is not limited to a single fusion partner per L2 molecule, and may include additional fusion partners, for example, additional peptides or full-length proteins or combinations thereof derived from the same or different proteins linked in tandem. Also, more than one L2 fusion product may be co-assembled into a single VLP. For example, a L2 fusion product or chimeric VLP containing a viral target epitope may also be engineered to contain a binding domain of a co-stimulatory protein or an accessory receptor or ligand involved in immune reactivity, e.g., B7. (which interacts with CD28 on T cells), an intercellular adhesion molecule (ICAM), a lymphocyte functional antigen (LFA), a vascular cell adhesion molecule (VCAM-1), and a heat stable antigen (HSA). (To target the co-stimulators or ligands to the surface of VLPs, see Example 14.)

It will be appreciated that the actual preferred amounts of chimeric papillomavirus-like particles as vaccines in the prevention and/or treatment of disease will vary according to the specific compositions formulated, the mode of application, the particular situs and the organism being treated. Dosages for a given host can be determined using conventional considerations, e.g., by means of an appropriate, conventional vaccination protocol.

Alternatively, chimeric VLPs can used in a method of purification of VLPS. VLPs are useful per se as prophylactic vaccines and in immunodiagnostics (supra). Briefly, antibodies to the fusion partner are generated using standard immunological techniques, an affinity chromatography column is constructed using the antibodies, and the VLPs are subsequently purified in an affinity chromatography step.

Or, chimeric VLPs can be used in a method of purification of a fusion partner. For example, VLPs containing a L2-E7 fusion product may be useful as a means of obtaining correctly folded E7. It has been reported that a greater percentage of cervical cancer patients are seropositive for conformationally correct E7 than for denatured E7, as isolated from bacteria (Viscidi et al., Int. J. Cancer 55:780 (1993)). The in vitro transcription-translation system used to generate E7 in this report is laborious and expensive, and uses radio-labeled E7. It would be advantageous to purify chimeric E7 VLPs and use the material in an E7 ELISA. To avoid complications of seroreactivity with human L1 or L2, BPV-based particles could be used. Monitoring E7 seroreactivity, which correlates with cancer as opposed to premalignant disease, has been proposed to be useful to follow the course of disease in cervical cancer patients and to screen for reoccurrences (id.).

Optionally, chimeric VLPs can be used in a method of delivery of an intact and active protein into cells. This protein may be, for example, an enzyme, or a toxin or a drug. The chimeric VLPs are administered to the cells, either in vitro, in vivo, in situ, or ex vivo, and the protein is subsequently delivered into the cell where it functions for its intended purpose, for example, as an enzyme, or a toxin or a drug.

We have succeeded at generating chimeric papillomavirus-like particles incorporating E7 protein or peptide fused to L2.

A papillomavirus E7 gene when fused in frame to the 3' end of the L2 gene or within part of the L2 open reading frame expressed an L2-E7 fusion protein that, in the presence of L1 protein, was incorporated into virus-like particles. Efficiency and authenticity of incorporation of L2-E7 into VLPs was similar to that of the wild-type L2 protein. BPV L1/L2-E7 virus-like particles were found to induce neutralizing antibodies as effectively as BPV L1/L2 particles. Chimeric particles were observed to elicit humoral immunity specific for fusion partner epitopes in that rabbits

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Title: VACCINES

Abstract

The present invention provides vaccine compositions comprising an oil in water emulsion optionally with 3 De-O-acylated monophosphoryl lipid A and QS21. The vaccines' compositions are potent inducers of a range of immune responses.

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Vaccines

The present invention relates to novel vaccine formulations, to methods of their production and to their use in medicine. In particular, the present invention relates to an oil in water emulsion. Such emulsions comprise tocopherol, squalene, Tween 80, Span 85 and Lecithin and have useful adjuvant properties. Vaccines containing QS21, an Hpic purified non-toxic fraction derived from the bark of Quillaja Saponaria Molina, and/or 3 De-O-acylated monophosphoryl lipid A (3 D-MPL), together with such oil in water emulsions also form part of the invention.

3 De-O-acylated monophosphoryl lipid A is known from GB2220 211 (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 International Patent Application No. 92/116556.

QS21 is a Hpic purified non toxic fraction of a saponin from the bark of the South American tree Quillaja Saponaria Molina and its method of its production is disclosed (as QA21) in US patent No. 5,057,540.

Oil in water emulsions per se are known in the art, and have been suggested to be useful as adjuvant compositions (EPO 399843).

The present invention is based on the surprising discovery that an oil in water emulsion of the present invention, which unlike emulsions of the prior art contain tocopherol, as such or in combination with QS21 and/or 3 D-MPL enhance immune responses to a given antigen. Such enhancement available affords better immunological responses than hitherto before.

Additionally the oil in water emulsions of the present invention when formulated with 3 D-MPL and QS21 are preferential stimulators of IgG2a production and TH1 cell response. This is advantageous, because of the known implication of TH_1 response in cell mediated response. Indeed in mice induction of IgG2a is correlated with such an immune response.

For example a vaccine formulation of the HIV antigen gp120 in such a combination results in a powerful synergistic induction of gp120 protein specific immune responses.

claims

1. A vaccine composition comprising an antigen and/or antigenic composition, QS21, 3 De-O-acylated monophosphoryl lipid A (3D-MPL) and an oil in water emulsion wherein the oil in water emulsion has the following composition: a metabolisable oil, such as squalene, alpha tocopherol and tween 80.

2. A vaccine as claimed in claim 1 wherein the ratio of QS21:3D-MPL is from 1:10 to 10:1.

3. A vaccine composition as claimed in claim 1 or 2 capable of invoking a cytolytic T cell response in a mammal to the antigen or antigenic composition.

4. A vaccine composition as claimed in any of claims 1 to 3 capable of stimulating interferon γ production.

5. A vaccine composition as claimed in any of claims 1 to 4 wherein the ratio of QS21:3D-MPL is from 1:1 to 1:2.5.

6. A vaccine composition as claimed herein comprising an antigen or antigenic composition derived from any of Human Immunodeficiency Virus, Feline Immunodeficiency Virus, Herpes Simplex Virus type 1, Herpes Simplex virus type 2, Human cytomegalovirus, Hepatitis A,B,C or E, Respiratory Syncytial virus, human papilloma virus, Influenza virus, Salmonella, Neisseria, Borrelia, Chlamydia, Bordetella, Plasmodium or Toxoplasma.

7. A vaccine as claimed in any of claim 1 to 5 wherein the antigen is a tumour antigen.

8. Use of composition as defined in any of claims 1 to 5 for the manufacture of a vaccine for the prophylatic treatment of viral, bacterial, or parasitic infections.

9. Use of composition as defined in any of claims 1 to 5 for the manufacture of a vaccine for the immunotherapeutic treatment of viral, bacterial, parasitic infections or cancer.

10. A method of treating a mammal suffering from or susceptible to a pathogenic infection comprising the administration of a safe and effective amount of a composition according to any of claims 1 to 5.

11. A method of treating a mammal suffering from cancer comprising the administration of a safe and effective amount of a composition according to any of claims 1 to 5.

12. A process for making a vaccine composition according to claims 1 to 5 comprising admixing QS21, 3D-MPL and the oil in water emulsion as defined in claim 1 with an antigen or antigenic composition.

13. A vaccine composition comprising an antigen or antigenic composition in association with an oil in water emulsion which emulsion comprises: a metabolisable oil , alpha tocopherol, and tween 80.