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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



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Solicitud de patente de invención titulada: "VACUNA DE PROTEÍNA DE STREPTOCOCCUS PNEUMONIAE"

Solicitante: SMITHKLINE BEECHAM BIOLOGICALS S.A.

RESPUESTA AL OFICIO No. 12038 NOTIFICADO POR FIJACIÓN EN LISTA No. 359

DE FECHA 28 DE SEPTIEMBRE DE 2007

(EXAMEN DE FONDO)

CARLOS R. OLARTE, mayor de edad y vecino de Bogotá, identificado tal como aparece al pie de mi firma, en mi carácter de apoderado de la sociedad solicitante, me permito manifestar lo siguiente ante el auto emanado por ese Despacho, dentro del plazo legal de prórroga previsto en el artículo 45 de la Decisión 486, en los siguientes términos:

1. En primer lugar y con el ánimo de brindar mayor claridad a la materia reivindicada, me permito presentar un **Capítulo Reivindicatorio Modificado**, que reemplaza en su totalidad al Capítulo Reivindicatorio radicado al momento de radicar esta solicitud. Para tal efecto, adjunto el recibo de pago que cubre la tasa de modificación correspondiente.

Así entonces, el nuevo Capítulo Reivindicatorio contiene las siguientes modificaciones:

Reivindicación original	Modificaciones
1	Se limita a una composición inmunogénica que comprende PhtD y neumolisina.
2-5	Reemplazadas por las nuevas reivindicaciones 2-7

Valga anotar que las modificaciones efectuadas al Capítulo Reivindicatorio cumplen a cabalidad con lo estipulado en el Artículo 34 de la Decisión 486, ya que éstas de ninguna manera implican una ampliación de la protección correspondiente a la divulgación contenida en la solicitud inicial, y se encuentran enteramente soportadas por la descripción así:

Reivindicación modificada	Soporte descriptivo
2	Basada en la divulgación de la página 11 líneas 22 a 27
3	Basada en la divulgación de la página 9 línea 30 y página 10 líneas 1 a 4
4	Basada en la divulgación de la página 10 líneas 4 a 5
5	Basada en la divulgación de la página 13 líneas 15 a 22
6	Basada en la divulgación de la página 13 líneas 15 a 22
7	Basada en la divulgación de la página 14 líneas 3 a 13
9	Basada en la divulgación de la página 16 líneas 20 a 21

2. a- El Examinador argumenta que el Capítulo Descriptivo adolece de suficiencia al no presentar de manera completa la invención, ni incluir las ventajas técnicas de la misma sobre el estado arte que brinden soporte para todas las posibles realizaciones reclamadas. Adicionalmente, el Examinador establece que las reivindicaciones reclaman un sinnúmero de composiciones que resultan de la combinación de un número ilimitado de proteínas de *S. pneumoniae*, que pueden comprender más de un concepto común inventivo y que incluso pueden existir en el estado de la técnica.

b- i) La Reivindicación independiente 1 del Capítulo Reivindicatorio Modificado se limita a reclamar composiciones de vacunas en las que se combinan exclusivamente los antígenos PhtD y neumolisina. Con esto, se superan los defectos observados por el Examinador respecto a amplitud (falta de claridad) de las reivindicaciones y de la posible falta de Unidad de Invención.

ii) La composición reclamada en el Capítulo Reivindicatorio Modificado, está soportada en los ejemplos de la presente solicitud, a partir de los cuales queda completamente claro que tanto la neumolisina como PhTD son excelentes candidatos para la preparación de una vacuna neumocócica combinada.

En el ejemplo 2 se midieron las respuestas de anticuerpos que se desarrollaron de manera natural frente a distintas proteínas neumocócicas, incluyendo PhtD y neumolisina en tres tipos de pacientes: infantes, adultos de 20 años y adultos de 60 años, expuestos naturalmente a *S.*

pneumoniae. Así, los resultados de la Tabla 1 indican que la enfermedad neumocócica provoca una respuesta inmune excelente contra PhtD con un GMC de 19µg/ml, y adicionalmente muestra que se generan respuestas aproximadamente similares frente a NR1xNR2, 5p91 y Ply, las cuales presentan lecturas de GMC de 2 a 3.5µg/ml. Los resultados de los estudios de serología efectuados en los tres grupos anteriores por edades, se muestran en las figuras 1 a 6 y allí se evidencia que PhtD y Ply son buenos inmunógenos, frente a los cuales se genera una respuesta inmune por exposición a *S. pneumoniae*. A su vez, la Figura 1 revela una seropositividad del 95 al 100% frente a PhtD y una de 65-80% frente a neumolisina en niños, mientras que la Figura 4 muestra seropositividad del 100% frente a los dos antígenos (PhtD y neumolisina) en adultos jóvenes y la Figura 6 revela 100% de seropositividad para PhtD y 95% para neumolisina en adultos mayores.

De lo anterior se concluye que PhtD y neumolisina constituyen antígenos frente a los cuales el cuerpo desarrolla una respuesta inmune al defenderse de la infección por *S. pneumoninae*, independientemente del grupo por edad al que pertenezca el paciente, correspondiente a la vacuna desarrollada en la presente invención que contiene PhtD y neumolisina.

iii) Para reforzar lo anterior, me permito suministrar datos adicionales que demuestran que la vacuna combinada reclamada en la Reivindicación 1 presenta resultados sorprendentes frente a las vacunas que comprenden los antígenos neumococcicos por separado. En este ensayo se inmunizan ratones con dosis inferiores a las óptimas de PhtD, neumolisina (dPly) y de una combinación de PhTD-neumolisina (dPly). Después de la inmunización se analizó la carga nasofaríngea del ratón en donde se encontró que tras la inmunización con PhtD ó neumolisina (dPly) por separado, entre el 45 al 50% de los ratones no presentaban carga nasofaríngea; en contraste, cuando la inmunización se efectuaba con la combinación de los dos antígenos, el 80% de los ratones proporcionaron resultados negativos para carga nasofaríngea.¹ Es decir, la vacuna combinada presenta un nivel de protección mayor, en aproximadamente un 30%.

Vale la pena aclarar, que el incremento en la inmunidad no se presenta siempre cuando las proteínas se encuentran combinadas en una vacuna. Frecuentemente, el nivel de protección se mantiene a pesar de la adición de una proteína extra. Por ejemplo, las enseñanzas de Ogunniyi et al. 2000 (D1 en el reporte de búsqueda internacional)² muestran que no todas las combinaciones de proteínas neumocócicas resultan en una respuesta inmune aumentada. La Figura 2B de dicho documento muestra los tiempos de supervivencia tras la inmunización con PdB, PsaA, PSpA o combinaciones de los anteriores. Aún cuando la combinación de PdB (neumolisina) con PspA

¹ ANEXO 1

² ANEXO 2

91
4

resultaron en un aumento del tiempo de supervivencia, la combinación de PdB(pneumolisina) con PsaA o de PsaA con PspA no presenta tal mejora.

3. a El Examinador argumenta que las Reivindicaciones 8, 9, 10 y 11 reclaman materia no patentable por reclamar métodos de tratamiento y usos.

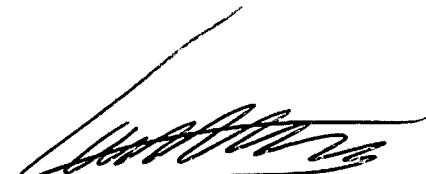
b- Considero superada esta objeción en tanto que el Capítulo Reivindicatorio Modificado eliminó las Reivindicaciones originales 8 a 11.

6. Por todo lo anterior, considero que las objeciones presentadas por el Examinador en su examen de fondo No. 802 han sido resueltas y por consiguiente solicito respetuosamente que se emita un segundo examen de fondo donde se pronuncie sobre los requisitos de patentabilidad, en particular respecto de la novedad y el nivel inventivo. De lo contrario, ruego se tengan por cumplidos tales requisitos, en tanto no se logró desvirtuar ninguno de ellos por parte del Despacho, y se proceda con la concesión de la patente.

7. Finalmente, deseo anotar que para el concepto inventivo comprendido en la presente solicitud, ha sido otorgado el privilegio de patente en Australia, Nueva Zelanda y Sur África.

Si bien es cierto que las concesiones de patentes en oficinas de otros países no son vinculantes en el sentido que obliguen a la SIC a tomar una decisión en el mismo sentido, sí considero que dichas concesiones sugieren el cumplimiento de los requisitos de patentabilidad de la materia reivindicada, ya que los criterios de evaluación y estudio aplicados para concluir la aceptación de dichas patentes resultan equivalentes a los que la SIC debe emplear. Tan cierto es lo anterior, que el Manual Andino de Patentes, al comentar el Artículo 46 de la Decisión 486, establece que una Oficina nacional competente podrá reconocer los resultados de exámenes de novedad o de patentabilidad efectuados respecto a la solicitud extranjera equivalente, como suficientes para acreditar el cumplimiento de las condiciones de patentabilidad.

Atentamente,



CARLOS R. OLARTE

C.C. No. 79.782.747 de Bogotá

T.P. 74.295

Ánexas:

- Capítulo Reivindicatorio Modificado.
- Recibo de pago correspondiente a la tasa de modificación.
- ANEXO 1: Resultados de estudios de protección inducida mediante inmunización con neumolisina (Ply) y PhtD
- ANEXO 2: Ogunniyi et al. Infection and Immunity, May 2000. p3028-3033. Immunization of mice with combinations of pneumococcal virulence proteins elicit enhanced protection against challenge with *Streptococcus pneumoniae*.

CAPÍTULO REIVINDICATORIO MODIFICADO

1. Una composición inmunogénica que comprende al menos 2 proteínas de *S.pneumoniae* en donde una de las proteínas es PhtD y la otra es neumolisina (Ply).
2. La composición inmunogénica de la reivindicación 1 que además comprende NR1xR2 o R1xR2 de CbpA o PspC.
3. La composición inmunogénica de 1-2 donde la neumolisina ha sido detoxificada químicamente.
4. La composición inmunogénica de 1-2 donde la neumolisina ha sido detoxificada genéticamente.
5. La composición inmunogénica de las reivindicaciones 1-4 que comprende adicionalmente otro antígeno seleccionado del grupo que consiste de antígeno de superficie de Hepatitis B(HBsAg), antígenos del virus de polio, proteínas de membrana externas de *Moraxella catarrhalis*, proteínas de *Haemophilus influenzae* no tipificable y proteínas de membranas externa de *N. meningitidis* B.
6. La composición de la reivindicación 5 donde el antígeno adicional es una proteína de *Haemophilus influenzae* no tipificable.
7. La composición inmunogénica de la reivindicación 6 en donde el antígeno adicional es proteína D de *Haemophilus influenzae* no tipificable.
8. La composición inmunogénica de las reivindicaciones 1-7 que comprende adicionalmente un adyuvante.

9. La composición inmunogénica de la reivindicación 8 en donde el adyuvante es inductor preferencial de la respuesta tipoTH1.
10. Una vacuna que comprende la composición inmunogénica de las reivindicaciones 1-9.
11. Un método para fabricar una vacuna según se reivindica en la reivindicación 10 que comprende los pasos de aislar dos proteínas de *S. pneumoniae* diferentes y mezclar dichas proteínas junto con un vehículo farmacéuticamente aceptable.

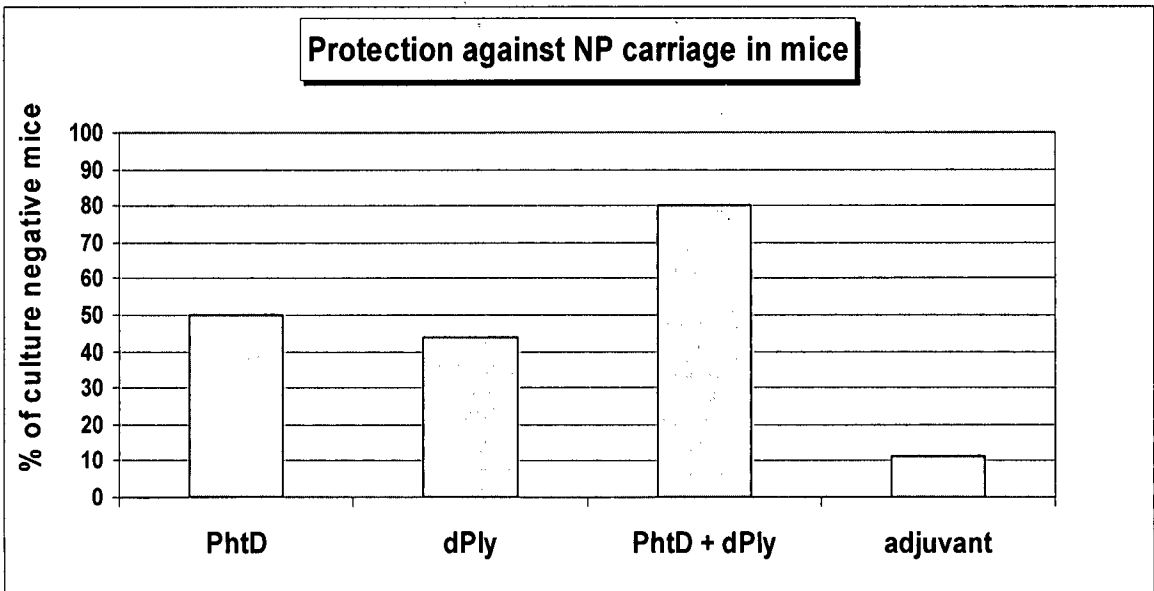
Protección inducida mediante inmunización con neumolisina (Ply) y PhtD

El objetivo del presente experimento es evaluar la protección conferida contra la colonización nasofaríngea de *S. pneumoniae*, por la inmunización con dosis inferiores a las óptimas de PhtD, dPly y la combinación de las dos proteínas.

Grupos de 10 ratones hembras Balb/c (4 semanas) fueron inmunizados intranasalmente a los días 0,14,y 28 con 10 µl de bien sea PhtD (0.6 µg), dPly (0.2 µg), PhtD (0.6 µg) + dPly (0.2 µg) o PBS. Las formulaciones de vacunas administradas los días 0 y 14 fueron suplementadas con 0.2 µg la toxina lábil de *E. coli* (LT) usada como adyuvante. No se adicionó LT a las vacunas administradas el día 28.

Los ratones fueron desafiados el día 42 con 10E5 ufc de neumococo tipo 2(cepa D39) Se recolectaron los lavados nasales el día 6 post-desafío y fueron cultivados en medio de cultivo para la detección de neumococo. Los resultados se expresan como el porcentaje de ratones cuyos cultivos fueron negativos, analizados mediante el test estadístico de Fisher.

La combinación PhtD y Ply indujo una protección significativa ($p<0.003$) y mejorada en comparación con cada proteína individual.



Immunization of Mice with Combinations of Pneumococcal Virulence Proteins Elicits Enhanced Protection against Challenge with *Streptococcus pneumoniae*

A. DAVID OGUNNIYI,¹ REBEKAH L. FOLLAND,¹ DAVID E. BRILES,²
SUSAN K. HOLLINGSHEAD,² AND JAMES C. PATON^{1*}

¹Molecular Microbiology Unit, Women's and Children's Hospital, North Adelaide, SA 5006, Australia,
²and Department of Microbiology, University of Alabama at Birmingham, Birmingham, Alabama²

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The vaccine potential of a combination of three pneumococcal virulence proteins was evaluated in an active-immunization-intraperitoneal-challenge model in BALB/c mice, using very high challenge doses of *Streptococcus pneumoniae*. The proteins evaluated were a genetic toxoid derivative of pneumolysin (PdB), pneumococcal surface protein A (PspA), and a 37-kDa metal-binding lipoprotein referred to as PsaA. Mice immunized with individual proteins or combinations thereof were challenged with high doses of virulent type 2 or type 4 pneumococci. The median survival times for mice immunized with combinations of proteins, particularly PdB and PspA, were significantly longer than those for mice immunized with any of the antigens alone. A similar effect was seen in a passive protection model. Thus, combinations of pneumococcal proteins may provide the best non-serotype-dependent protection against *S. pneumoniae*.

Streptococcus pneumoniae continues to be a major cause of life-threatening invasive diseases such as pneumonia, meningitis, and bacteremia, as well as other highly prevalent albeit less serious infections, such as otitis media and sinusitis (3, 16, 41). Pneumococcal infections are prevalent throughout the world, and children under the age of 5 years, the elderly, and immunocompromised individuals are particularly susceptible (3, 16, 24). Mortality from pneumococcal disease is particularly high in developing countries, where pneumococcal pneumonia has been estimated to account for 20 to 25% of all deaths in children under the age of 5 years (46). Global management of pneumococcal disease is also being complicated by the alarming rate at which this organism is acquiring resistance to multiple antimicrobials (19).

The limitations of the currently available polyvalent vaccine formulations comprising purified pneumococcal capsular polysaccharide (PS) are well documented. These include the facts that the PS vaccines confer strictly serotype-specific protection and that the present formulation contain only 23 of the 90 known serotypes. PS are also T-cell-independent antigens and are poorly immunogenic in children under 2 years of age (15, 20). Protein-PS conjugate vaccines that are currently undergoing clinical trials, although highly immunogenic (21, 23, 36) have more limited serotype coverage. Moreover, they are likely to be expensive, and this may limit their deployment in developing countries where they are needed most. Pneumococcal conjugate vaccines have been shown to be capable of eliminating nasopharyngeal carriage of vaccine serotypes, but there is evidence from some studies that there is a concomitant increase in carriage of non-vaccine serotypes (30). These included types known to be capable of causing invasive disease, and so the actual reduction in the overall incidence of pneumococcal disease achieved by introduction of conjugate vaccines with limited serotype coverage may be less than expected.

The known and potential shortcomings of existing vaccination strategies have necessitated research into development of new cheap and effective vaccines against pneumococcal disease. Studies in our laboratories have been directed towards understanding the mechanism of pathogenesis of *S. pneumoniae* with a view to developing vaccines based on protein antigens common to all serotypes. Such proteins, being T-cell-dependent antigens, are likely to be highly immunogenic in human infants and, moreover, to elicit immunological memory. In addition, they may provide a degree of protection against all serotypes. So far, the three proteins which have shown the greatest promise as vaccine antigens are the thiol-activated toxin pneumolysin (9), pneumococcal surface protein A (PspA) (13, 48), and a 37-kDa metal-binding lipoprotein referred to as PsaA (7, 14). Each of these proteins has been shown to elicit a significant level of protection in animal models against one or more *S. pneumoniae* serotypes (1, 10, 11, 25, 35, 42, 43, 47; E. W. Ades, J. S. Sampson, D. E. Briles, J. D. King, B. De, R. C. Huebner, and G. M. Carlone, Program Abstr. Pneumococcal Vaccines World 1998 Conf., p. 29, session 4, 1998). Comparative sequence analyses indicate that the genes encoding pneumolysin and PsaA are highly conserved among diverse capsular serotypes of *S. pneumoniae* (7, 27, 40), but there is marked heterogeneity in the region encoding the amino-terminal portion of PspA (13, 48, 49). Nevertheless, PspA contains conserved epitopes which result in protection against diverse capsular and PspA types after immunization with a single PspA antigen (10, 11, 25, 43).

All available evidence suggests that pneumolysin, PspA, and PsaA contribute to the virulence of *S. pneumoniae* (4–8, 14, 26, 29, 32, 34) but act at different stages of the pathogenic process (31, 33, 39). Thus, immunization with a combination of these proteins may provide a higher degree of protection than immunization with any of the antigens alone. This possibility was examined in the present study. For this study we used high challenge doses in an effort to enhance our ability to detect additive protective effects of immunizations with combinations of pneumolysin, PspA, and PsaA over that achieved with any of these antigens alone.

* Corresponding author. Mailing address: Molecular Microbiology Unit, Women's and Children's Hospital, 72 King William Rd., North Adelaide, SA 5006, Australia. Phone: 61 8 8204 6302. Fax: 61 8 8204 6051. E-mail: patonj@wch.sa.gov.au.

TABLE 1. Antibody titers obtained from mice immunized with PdB, PspA, and PsaA

Immunization group	Antibody titer (ELISA) ^a against:		
	PdB	PsaA	PspA
Placebo	— ^b	—	—
PdB	28,000	—	—
PsaA	—	18,500	—
PspA	—	—	150,000
PdB-PsaA	40,000	18,500	—
PdB-PspA	50,000	—	62,000
PsaA-PspA	—	30,500	140,000
PdB-PsaA-PspA	45,000	38,000	50,000

^a ELISA titers were determined as the reciprocal of the dilution of serum giving 50% of the highest absorbance reading above the background at 405 nm.

^b —, <200.

Preparation of antigens. Pneumococcal antigens were purified from recombinant *Escherichia coli* expressing the respective cloned gene. The original source of the gene in each case was a capsular type 2 strain, D39 (2). For pneumolysin, a mutated gene encoding a derivative with a Trp-433→Phe substitution was used. This genetic toxoid, designated PdB, has only 0.1% residual cytotoxic activity relative to the native toxin but retains full immunogenicity, and it was purified as previously described (1, 36). PsaA was expressed as a His₆-tagged fusion protein and purified by Ni-nitrilotriacetic acid affinity chromatography (37). A 43-kDa N-terminal portion of PspA was also expressed as a His₆-tagged fusion protein and purified by Ni-nitrilotriacetic acid affinity chromatography. This truncated PspA has been shown to elicit cross-protective immunity against pneumococcal challenge in mice (43, 49). All antigens were >95% pure as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and staining with Coomassie brilliant blue R250.

Immunization of mice and antibody responses. For each experiment, eight groups of 5- to 6-week-old male BALB/c mice (14 or 15 per group) were immunized intraperitoneally with either PdB alone, PsaA alone, PspA alone, PdB plus PsaA, PdB plus PspA, PsaA plus PspA, PdB plus PsaA plus PspA, or a placebo. Each mouse received three doses of 5 µg of each antigen alone or in combination in 50 µg of alum adjuvant (Imject alum no. 77161; Pierce, Rockford, Ill.) at 10- to 12-day intervals. The mice given the placebo received an identical course of saline plus alum.

Sera were collected from mice by retro-orbital bleeding 1 week after the third immunization. The sera were pooled on a group-by-group basis and analyzed by enzyme-linked immunosorbent assay and Western blotting for specific antibodies to each of the purified protein antigens. As shown in Table 1, strong, antigen-specific antibody responses were generated in mice immunized with the pneumolysin toxoid (PdB), PsaA, and PspA when administered alone. Furthermore, there was no obvious diminution in antigen-specific antibody titer when the antigens were administered in combination with others, indicating that there was no detectable antagonistic effect of combining the antigens. Western blot analysis of the purified proteins also demonstrated specific antibody responses to each of the antigens (Fig. 1). Similar results were obtained with whole-cell lysates of D39, with the exception that anti-PspA reacted with the full-length PspA of approximately 86 kDa (data not shown).

Challenge. Intraperitoneal-challenge experiments were carried out 2 weeks after the third immunization of mice, and two separate experiments were performed in parallel using two *S.*

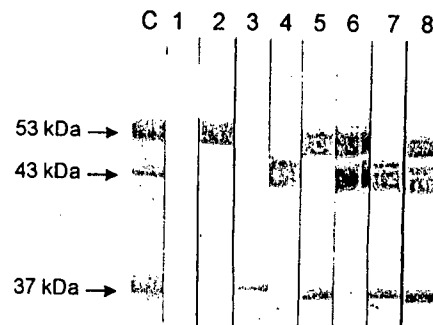


FIG. 1. Western blot of purified PdB (53 kDa), truncated PspA (43 kDa), and PsaA (37 kDa), showing specificity of antibody responses to the various protein antigens. The proteins were separated by sodium dodecyl sulfate-10% polyacrylamide gel electrophoresis and electroblotted onto nitrocellulose. They were then reacted with specific antisera generated from mice immunized with the various protein combinations. Lane C, Coomassie blue-stained gel showing the relative positions of the proteins. Lanes 1 to 8, nitrocellulose membrane reacted with normal mouse serum (lane 1), anti-PdB (lane 2), anti-PsaA (lane 3), anti-PspA (lane 4), anti-PdB-PsaA (lane 5), anti-PdB-PspA (lane 6), anti-PsaA-PspA (lane 7), and anti-PdB-PsaA-PspA (lane 8).

pneumoniae strains. These were D39, a virulent type 2 strain (2), and WCH43, a virulent type 4 clinical isolate from the Women's and Children's Hospital, North Adelaide, South Australia, Australia.

Before challenge, the bacteria were grown at 37°C overnight on blood agar and then inoculated into serum broth consisting of 10% (vol/vol) horse serum in meat extract broth. They were then grown statically for 3 h at 37°C to give approximately 10⁸ CFU/ml. Serotype-specific capsule production was confirmed by quellung reaction using antisera obtained from Statens Seruminstitut, Copenhagen, Denmark. Each immunized BALB/c mouse was then infected with approximately 10⁷ CFU of either the capsular type 2 strain (D39) or the type 4 strain (WCH43). This dose was equivalent to approximately 10⁵ times the 50% lethal dose (LD₅₀) of both strains for BALB/c mice.

The survival of the intraperitoneally challenged mice was closely monitored for 21 days. Differences in median survival time between groups were analyzed by the Mann-Whitney *U* test (one tailed). Differences in the overall survival rate between groups were analyzed by the Fisher exact test.

Figure 2A shows the results obtained when the mice were challenged with the highly virulent capsular type 2 strain D39. Given the high challenge dose, it is not surprising that the median survival time for mice that received the alum placebo was less than 1 day and that all of the mice in this group died in just over 3 days. Although mice that received either PdB, PsaA, or PspA alone survived longer than those in the placebo group, the median survival times of 1.7, 1.8, and 2 days, respectively, were not significantly different from that of the placebo group (Table 2). However, all groups of mice that received combinations of the antigens had significantly longer median survival times than the placebo group. Interestingly, mice that received a combination of PdB and PsaA or PsaA and PspA did not survive significantly longer than those that received the single antigens alone. In contrast, mice that were immunized with PdB plus PspA survived significantly longer than those that were immunized with PdB alone (*P* = 0.01), PsaA alone (*P* < 0.025), or PspA alone (*P* < 0.01). The level of protection obtained with the PdB-PspA combination was very similar to that obtained with the PdB-PsaA-PspA combination. However, mice that received the PdB-PsaA-PspA combination survived significantly longer than those that received the alum placebo (*P* < 0.001), PdB alone (*P* < 0.01), PsaA

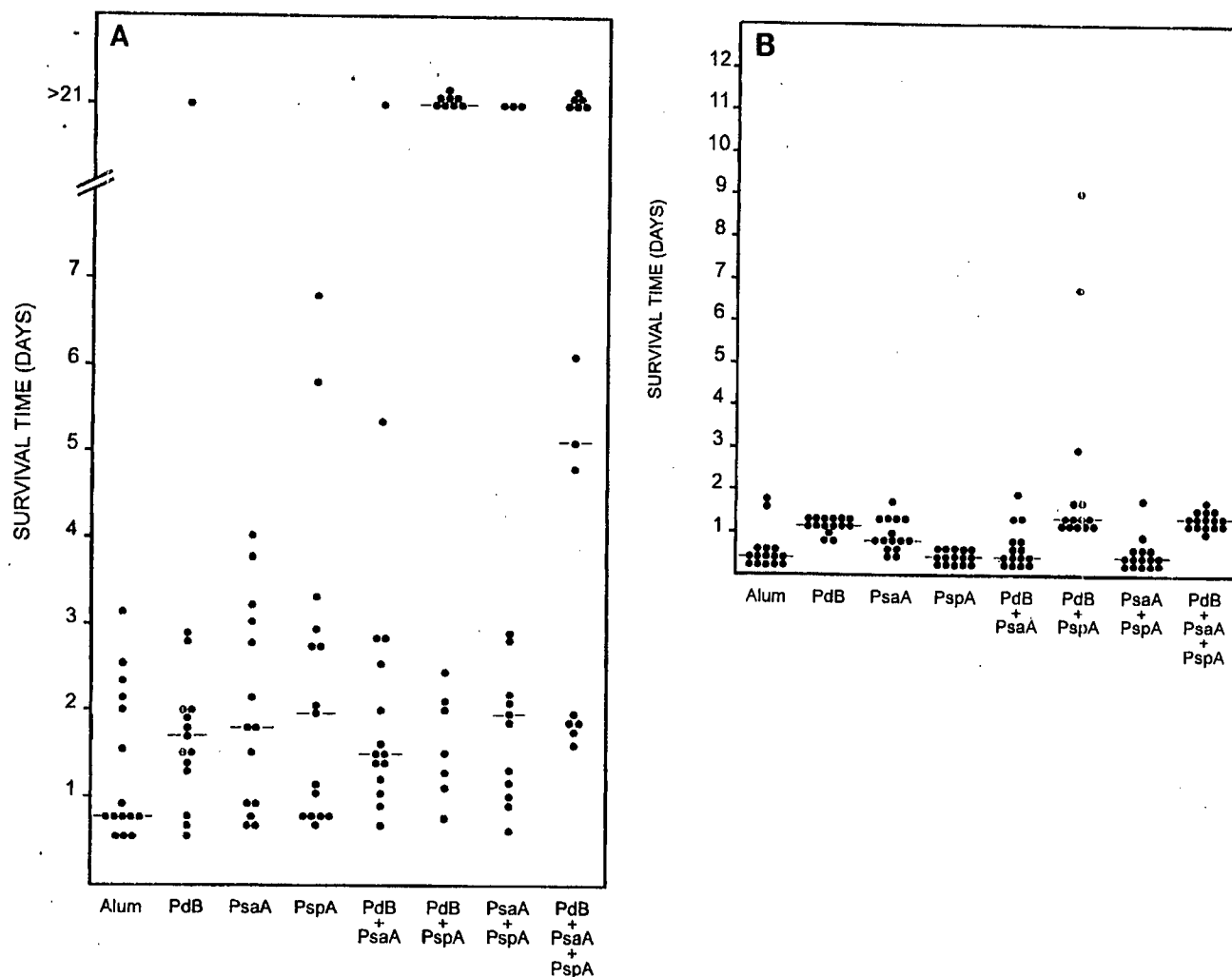


FIG. 2. Survival times for mice after intraperitoneal challenge. Groups of 14 or 15 BALB/c mice were immunized with the indicated antigens and challenged 2 weeks after the third immunization with approximately 10^7 CFU of D39 (type 2) (A) or WCH43 (type 4) (B). The broken lines indicate the median survival time for each group.

alone ($P < 0.01$), PspA alone ($P < 0.01$), PdB-PsaA ($P < 0.01$), or PsaA-PspA ($P < 0.05$). In addition, mice that received PspA in combination with PsaA and/or PdB survived longer than mice that were immunized with a combination of PdB and PsaA, suggesting that PspA may provide slightly superior protection than PdB or PsaA within the context of the challenge strain and dosage used.

The overall survival rates for mice immunized with PdB-PspA and PdB-PsaA-PspA combinations were significantly greater than the survival rates for mice in the placebo group ($P < 0.005$ and $P < 0.01$, respectively). Similarly, there were significant differences in the survival rates for mice that received PdB-PspA and PdB-PsaA-PspA antigens versus those that were immunized with either PdB alone or PdB-PsaA ($P <$

TABLE 2. Statistical comparison of median survival times^a

Type 4 challenge immunization	P value for comparison with the following type 2 challenge immunization							
	Alum	PdB	PsaA	PspA	PdB-PsaA	PdB-PspA	PsaA-PspA	PdB-PsaA-PspA
Alum		NS ^b	NS	NS	<0.05	=0.001	<0.025	<0.001
PdB	<<0.001		NS	NS	NS	=0.01	NS	<0.01
PsaA	<<0.01	<0.05		NS	NS	<0.025	NS	<0.01
PspA	NS	<<0.001	<<0.001		NS	<0.01	NS	<0.01
PdB-PsaA	NS	<<0.001	<0.025	<0.025		<0.01	NS	<0.01
PdB-PspA	<<0.001	<0.01	<0.001	<<0.001	<<0.001		NS	NS
PsaA-PspA	NS	<<0.001	<0.001	NS	NS	<<0.001		<0.05
PdB-PsaA-PspA	<<0.001	NS	<<0.01	<<0.001	<<0.001	NS	<<0.001	

^a Groups of immunized mice were challenged either by a capsular type 2 strain (D39) or by a capsular type 4 strain (WCH43). Differences were analyzed using the Mann-Whitney *U* test (one tailed).

^b NS, not significant ($P \geq 0.05$).

99

12

0.01 and $P < 0.05$, respectively). However, there was no significant difference in the survival rates between mice immunized with the placebo and those that were immunized with PdB alone, PsaA alone, PspA alone, PdB plus PsaA, or PsaA plus PspA.

In the second challenge experiment, mice immunized with the various antigen combinations were challenged intraperitoneally with the type 4 strain WCH43. This strain is as virulent for BALB/c mice as D39, with the challenge inoculum of 10^7 CFU corresponding to approximately 10^5 times the LD_{50} . In this experiment, the median survival time for mice in the placebo group was also less than 1 day (Fig. 2B). Mice that received PspA alone were not significantly protected compared with those that received the alum placebo (Table 2). However, mice immunized with either PdB alone or PsaA alone had significantly longer median survival times than the group that received either the placebo ($P < 0.001$ and $P < 0.01$, respectively) or PspA ($P < 0.001$ in both cases). It was somewhat surprising that the median survival time for mice immunized with a combination of either PdB and PsaA or PsaA and PspA was not significantly different from that for mice in the placebo group. As a corollary, mice immunized with PsaA alone were significantly better protected against the type 4 challenge than mice that received a combination of either PdB and PsaA ($P < 0.025$) or PsaA and PspA ($P < 0.001$). However, the median survival time for mice that received a combination of PdB and PspA was significantly longer than that for those that received PdB alone ($P < 0.01$), PsaA alone ($P < 0.001$), or PspA alone ($P < 0.001$). With the exception of mice immunized with PdB alone and PdB-PspA, the median survival time for mice immunized with PdB-PsaA-PspA was significantly longer than the median survival time for mice that received the placebo ($P < 0.001$), PsaA alone ($P < 0.01$), PspA alone ($P < 0.001$), PdB-PsaA ($P < 0.001$), or PsaA-PspA ($P < 0.001$). Collectively, the results from the two challenge experiments support the hypothesis that immunization with a combination of pneumococcal proteins may give superior protection over immunization with a single antigen alone.

Passive-immunization studies. Passive-immunization-intraperitoneal-challenge experiments were conducted to determine whether the protection afforded by immunization of mice with the various protein antigen combinations was antibody mediated. One hundred microliters of pooled sera from mice immunized with either PdB alone, PspA alone, a combination of PdB and PspA, or an alum placebo was administered by intraperitoneal injection into groups of 15 naive mice. Before injection, anti-PdB and anti-PspA sera were concentrated and/or adjusted to a titer of 50,000. This was followed 1 h later by intraperitoneal challenge with 10^6 CFU of D39 or 10^5 CFU of WCH43. The inoculum was approximately 10^4 and 10^3 LD_{50} s of strain D39 and strain WCH43, respectively, for BALB/c mice. In the D39 challenge, the median survival times for mice that received either anti-PdB, anti-PspA, or anti-PdB-PspA sera were significantly longer than that for mice that received sera from the placebo group ($P < 0.001$, $P < 0.025$, and $P < 0.001$, respectively) (Fig. 3A). Moreover, the median survival time for mice that received anti-PdB-PspA serum was significantly longer than that for mice that received only anti-PdB serum ($P < 0.05$) or that for mice that received only anti-PspA serum ($P < 0.001$). In the WCH43 challenge, the median survival time for mice that received either anti-PdB or anti-PdB-PspA serum was significantly longer than that for mice which received placebo serum ($P < 0.05$ and $P < 0.001$, respectively). Interestingly, a significant difference between the anti-PspA group and the placebo group was not

observed (Fig. 3B). However, the median survival time for mice that received anti-PdB-PspA serum was significantly longer than that for mice that received anti-PdB serum alone ($P < 0.025$) or that for mice that received anti-PspA alone ($P < 0.01$), confirming that both PdB and PspA antibodies contribute to protection. Thus, the results with passive immunization are consistent with those obtained in the active-immunization-challenge experiments, confirming that the protection of mice with the various protein antigen combinations is, at least in part, antibody mediated.

Discussion. The widespread impact of pneumococcal disease throughout the world has prompted considerable efforts to develop cheap, effective pneumococcal vaccines. Appreciable levels of success have been achieved with the polyvalent PS vaccines and, most recently, with protein-PS conjugate vaccine formulations. However, the problems of serotype specificity of protection, geographical and temporal variations in serotype distribution, and the cost of conjugate vaccine formulations remain (15, 20, 21, 23, 36). These problems are further exacerbated by the possibility that nasopharyngeal replacement carriage of non-vaccine serotypes in vaccinated individuals will be reflected in increased rates of disease caused by these types (30).

In the present study, we proposed that immunization with a combination of virulence protein antigens of *S. pneumoniae* may give superior protection against a wider variety of strains over immunization with any of the protein antigens alone. Because these proteins appear to function at different stages of the pathogenic process (31, 33, 39), it was anticipated that a combined vaccine would elicit a higher degree of protection than any single antigen alone. Intraperitoneal challenge of actively immunized mice with particularly massive doses of two different challenge strains has provided unequivocal evidence that immunization with a combination of the proteins gives superior protection over immunization with any single antigen. The protection data obtained from the passive-immunization-intraperitoneal-challenge experiments also indicate that the protection is, at least in part, antibody mediated.

The three protein antigens evaluated in this study have been well characterized and shown to contribute to the pathogenesis of *S. pneumoniae* (33). These proteins have also been shown to be protective in different animal models (1, 10, 11, 25, 35, 42, 43, 47; Ades et al., Program Abstr. Pneumococcal Vaccines World 1998 Conf., 1998). Strong, antigen-specific antibody responses were mounted against these antigens, either alone or when administered in combination, indicating that these antigens are immunogenic and that there are no obvious deleterious or antagonistic consequences of combining these antigens for immunization of mice.

The protection observed due to immunization with PdB is presumed to be a consequence of direct neutralization of the pneumolysin toxin, thereby arresting bacteremia and hindering the exponential growth of the organisms in vivo. On the other hand, protection imparted by immunization with PspA is probably a consequence of the blocking of PspA's ability to inhibit complement fixation (28, 44), thereby facilitating clearance of the virulent pneumococci. Thus, immunization with both antigens might be expected to provide additive protection, as was observed in the present study.

In our high-dose intraperitoneal model, little demonstrable benefit could be attributed to anti-PsaA antibodies, since we could not show a significant level of protection in PsaA-immunized mice. However, there have been reports where PsaA has been found to confer significant levels of protection against nasopharyngeal carriage (Ades et al., Program Abstr. Pneumococcal Vaccines World 1998 Conf., 1998). Moreover, Talk-

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102
15

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800176089-2

RECIBO OFICIAL DE CAJA : 08 - 10.537
FECHA : FEBRERO 7 DE 2008

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 03-021874- -00006-0000

Fecha: 2008-02-07 16:36:53 Dep. 2020 NUECREACIONI
Tra. 11 PATENTEIYII Eve: 379 FASENACIONALI
Act. 444 RESPUESTAREQUE Folios: 15

***** CONSIGNACION *****

SITIANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
OLARTE RAISBEK	CONSIGNACION	BANCO DE BOGOTA	062754387	2661942	07/02/2008	4,673,000.00

***** CONCEPTO *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-03	CAMBIO DE MODALIDAD Y MODIFICA	
		12 MARCAS Y LEMAS COMERCIALES	103,000.00
		TOTAL :	\$ 103,000.00

SON: CIENTO TRES MIL PESOS.

RESPONSABLE : _____

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. _____

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISION DE NUEVAS CREACIONES

Radicación No. 03-21874
Clasificación Internacional (IPC): A61K39/09
Concepto Técnico No. 3594

Patente de Invención	☒	Patente de Modelo de Utilidad	☐
Vía Nacional	☐		
Vía PCT (fase nacional)	☒	Cap. I	☐
		Cap. II	☒
No. Sol. Internacional	PCT/03/21874		
Fecha de Presentación Internacional	12/09/2001		

Como resultado del examen definitivo, realizado con fundamento en el artículo 48 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica:

1. Documentos estudiados

<u>Descripción:</u>	Folios: 23 a 54	tal como se radicó inicialmente
	Folio: 95	tal como se modificó el 07/02/2008
<u>Reivindicaciones:</u>	Folios: 55 a 56	tal como se radicó inicialmente
	Folios: 93 a 94	tal como se modificó el 07/02/2008
<u>Dibujos:</u>	Folios: 58 a 60	tal como se radicó inicialmente
	Folio: 95	tal como se modificó el 07/02/2008
<u>Prioridad:</u>	Folios: 1	
	Fecha	15/09/2000
	País	GB
	Número	022742.1

Argumentos del solicitante: Folios: 88 a 92

2. Modificaciones (artículo 34 D.486 y art. 22 Tratado PCT)

Se aceptan las modificaciones realizadas por el solicitante toda vez que no amplían el objeto inicial de la solicitud.

3. Objeto de la invención

- Título de la solicitud (*folio 72*) "Vacuna de proteínas de *S. pneumoniae*".
- El anterior producto consiste en una composición inmunogénica que contiene al menos dos proteínas de *S. pneumoniae* en donde una de las proteínas es PhtD y la otra es neumolisina (Ply) y su fabricación en medicina.
- La Clasificación Internacional de Patentes (IPC) para esta solicitud es A61K39/09.

4. De la solicitud de Patente

4.1. Descripción (*artículo 28 D. 486*)

Realizadas las modificaciones a la descripción según los requerimientos realizados en el art. 45, la presente solicitud ya cumple con el requisito de claridad o divulgación completa en la descripción. Por lo anterior, la descripción sí cumple con el requisito de claridad o comprensión de acuerdo al art. 28 de la Decisión 486.

4.2. Reivindicaciones (*artículo 30 D 486*)

Realizadas las modificaciones al capítulo reivindicatorio, este define, es claro y conciso y además está lo suficientemente sustentado por la descripción. Por lo anterior, las reivindicaciones sí cumplen con el art. 30 de la Decisión 486.

5. Unidad de invención (*artículo 25 D 486*)

Realizadas las modificaciones, según los requerimientos realizados en el art. 45, el capítulo reivindicatorio estudiado se refiere a una invención o a un grupo de invenciones relacionadas pero que conforman un único concepto inventivo. Por lo anterior, la presente solicitud cumple con el requisito de unidad de invención de acuerdo al art. 25 de la Decisión 486.

6. Excepción a la patentabilidad (*artículo 20 D486 literal a)-d)*

Estudiado el capítulo reivindicatorio, el objeto de la solicitud no se encuentra dentro del alcance de las excepciones señaladas en la legislación, por lo que el objeto de la solicitud puede ser patentable de acuerdo a lo estipulado en el art. 20 de la Decisión 486.

7. Reivindicaciones relativas a un uso o a un segundo uso (*artículos 14 y 21 D 486*)

Realizadas las modificaciones al capítulo reivindicatorio, según los requerimientos realizados en el art. 45, analizar ya no se encuentran reivindicaciones de uso. El objeto de la solicitud corresponde a un producto y/o un procedimiento. Por lo anterior, la materia contenida en las reivindicaciones son invenciones.

8. Determinación del Estado de la Técnica

- La fecha que se ha tenido en cuenta para determinar el estado de la técnica es 15/09/2000.
- Consultadas las bases de datos con que cuenta la oficina y las otras fuentes utilizadas para determinar el estado de la técnica no se encontró documento alguno relacionado con el objeto de la solicitud.

9. Análisis de patentabilidad (artículo 14 D486)

9.1. Novedad (artículo 16 D486)

- Las reivindicaciones estudiadas corresponden de la número 1 a la 11 contenida en los folios 93 a 94
- De acuerdo con el numeral 8, no se encontró dentro del estado de la técnica, documento alguno que evidenciara la existencia de la invención.
- Por consiguiente se concluye que la presente solicitud es novedosa de acuerdo a lo estipulado en el art. 16 de la Decisión 486 de la Comisión de la Comunidad Andina.

9.2. Nivel inventivo

- Las reivindicaciones estudiadas corresponden de la número 1 a la 11 contenida en los folios 93 a 94
- No se encontró dentro del estado de la técnica, de acuerdo al numeral 8, documento alguno que afecte el nivel inventivo.
- Por consiguiente se concluye que la presente solicitud tiene nivel inventivo de acuerdo a lo estipulado en el art. 18 de la Decisión 486 de la Comisión de la Comunidad Andina.

9.3. Aplicación industrial (artículo 19 D 486)

- Las reivindicaciones estudiadas corresponden de la número 1 a la 11 contenida en los folios 93 a 94
- La presente invención sí tiene Aplicación Industrial ya que su objeto puede ser aplicado en la industria de farmacéuticos

10. Conclusión

En consecuencia y por todo lo expuesto anteriormente, se recomienda **Conceder** las reivindicaciones: 1 a 11 (folios: 93 a 94) con el título modificado "**Vacuna de *Streptococcus pneumoniae* a base de proteínas PhtD y neumolisina (Ply)**".

Bogotá, D.C.,

CONCEPTO TECNICO No. 3594	EXAMINADOR TECNICO Código No. 202089
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