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Doctor
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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
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Referencia: Expediente No. 07.117.685
Solicitud de Patente de Invención titulada: "Composición conjugada de polisacárido-proteína neumocócica multivalente"

Solicitante: Wyeth

RESPUESTA AL AUTO NOTIFICADO POR FIJACIÓN EN LISTA NO. 286 DEL 30 DE MAYO DE 2012, OFICIO NO. 08755 (EXAMEN DE FONDO)

ÁLVARO CORREA ORDÓÑEZ, mayor de edad y vecino de Bogotá D.C., identificado tal como aparece al pie de mi firma, en mi carácter de apoderado de la sociedad en referencia, doy respuesta al auto emanado por ese Despacho dentro del término de prórroga solicitado en mi memorial 07-117685-00005-0000 del 22 de agosto de 2012:

1. Objeciones del Examinador

1.1 Reivindicaciones.

1.1.1 Su Despacho considera que las reivindicaciones 7, 8, 16, 17, 25 y 26 no son patentables.

1.1.2. Su Despacho considera que la reivindicación no es concisa porque no establece la

proteína portadora, ni el adyuvante. Sugiere incluir las reivindicaciones 2, 6 y 8 dentro de la reivindicación 1. Dice además que dicha reivindicación no es clara porque no establece que cada uno de los trece serotipos se encuentran separadamente conjugados a la proteína portadora.

1.1.3. Dice que la reivindicación 9 no es concisa no soportada en la descripción.

1.1.4. Menciona que la reivindicación 18 no es concisa ni esta soportada en la descripción.

1.1.5. Estima que la descripción no divulga la invención de manera completa porque la persona del oficio no podría reproducirla por cuanto no presenta el certificado de depósito de los serotipos que hacen parte de la invención.

1.2 Novedad

Su Despacho considera que las reivindicaciones 9 y 10 de la presente solicitud no posee novedad debido a las divulgaciones de los documentos del estado de la técnica "The Journal of infectious diseases, 180:1171-1176. Immunogenicity and Impacto n Nasopharyngeal Carriage of a Nonavalent Pneumococcal Conjugate Vaccine. Sept. 8, 1999" (D2) and the document "Clin. Infect. Dis. (2000) 30(1): 100-121. Which Pneumococcal Serogroups Cause the Most Invasive Disease: Implications for Conjugate Vaccine Formulation and Use. Part I." (D4).

Particularmente, el Examinador considera que estos documentos D2 y D4 afectan la novedad de la presente solicitud por cuanto:

"Las características esenciales de la reivindicación 9 y 10 y 18, 19 y 20 habían sido divulgadas previamente en D1, el cual enseña formulaciones de conjugados de polisacáridos capsulares de los serotipos 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F y 23F de Streptococcus pneumoniae con una proteína portadora (Resumen, página 101 columna de la derecho, último párrafo, página 103, columna de la izquierda tercer párrafo, página 111, columna de la derecha segundo párrafo)
En consecuencia, la reivindicación 9 carece de novedad, según lo divulgado en el documento D4).

(...)

Las características esenciales de la composición de la reivindicación 18 habían sido divulgadas previamente en D2, el cual divulga una vacuna liofilizada nonavalente que comprende polisacáridos capsulares de Streptococcus pneumoniae conjugados a CRM_{197} y la inmunización de niños con dicha formulación (Resumen, Pag. 1171, segunda columna de la derecha, Pag. 1173, segunda columna de la derecha, Pag. 1175, tercera columna de la izquierda), la formulación contiene 2 µg de los polisacáridos conjugados de los serotipos 1, 4, 5, 9V, 14, 18C, 19F y 23F y 4 µg del polisacárido 6B.

Las reivindicaciones 19 y 20 no mencionan alguna característica que haga nueva la composición de la reivindicación 18, por lo cual se considera que no son nuevas."

1.3 Nivel Inventivo

Sobre el requisito del nivel inventivo, el Examinador establece que una persona del nivel técnico medio, versada en la materia, ensayaría las soluciones que se proponen en los documentos WO2003051392 (D1) y US5153312 (D3) y llegaría de manera obvia a la solución que se propone en la presente solicitud. Particularmente, el Examinador considera que las reivindicaciones 1 - 6, 111 - 15 y 21 - 24 carecen de nivel inventivo debido a las enseñanzas contenidas en los documentos D1 y D3.

El Examinador establece:

"En consecuencia, la persona del oficio normalmente versada en la materia, incluiría esta característica de D3 (conjugar individualmente todos los polisacáridos capsulares revelados en D1, porque el mismo documento D3 ya sugería que se podía obtener una vacuna multivalente de esta manera. Por lo tanto se considera que la composición de la reivindicación 1 se deriva de las enseñanzas que en conjunto revelan los documentos D1 y D3 y carece de nivel inventivo

En consecuencia, la persona del oficio normalmente versada en la materia, incluiría esta característica de D3 (conjugar individualmente los polisacáridos capsulares de S. pneumoniae con CRM_{197} y mezclar los conjugados producidos) a los polisacáridos capsulares revelados en D1, porque el mismo documento D3 ya sugería que se podía obtener una vacuna multivalente de esta manera. Por lo tanto se considera que la composición de la reivindicación 11 se deriva de las enseñanzas que en conjunto revelan

los documentos D1 y D3 y carece de nivel inventivo."

2. Argumentos a favor de la patentabilidad de la solicitud

2.1 Reivindicaciones

De acuerdo con las observaciones de su Despacho el solicitante presenta un nuevo capítulo reivindicatorio notoriamente mas limitado y siguiendo las sugerencias de su Despacho con el fin de responder a todas las objeciones.

2.2 Descripción

El examinador considera que la invención no describe suficiente y completamente la invención para una persona medianamente versada en la materia técnica en razón a que no se suministra el certificado de depósito de los serotipos 3, 4, 5, 6A, 6B, 7F, 9V, 18C, 19A, 19F y 23F de S. pneumoniae.

El solicitante no esta de acuerdo con la observación planteada por su Despacho en razón a que las cepas de S. pneumoniae de todos los serotipos listados estaban disponibles en la técnica anterior y accesibles para la persona medianamente versada.

Las diferentes cepas de S. pneumoniae de todos los serotipos mencionados se encuentran, por ejemplo, disponibles en el ATCC (American Type Culture Collection).

Adicionalmente, la estructura de los varios polisacáridos era conocida.

Se le recuerda a su Despacho que la obligación de mencionar el certificado de depósito solo aplica para aquellas cepas que son nuevas y hacen parte de la invención que no es del caso en la presente solicitud.

2.3 Novedad

La novedad de una solicitud está legalmente definida en el Artículo 16 de la Decisión 486 así: "*una invención se considerará nueva, cuando no está comprendida en el estado de la*

técnica. 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..."

En virtud de la anterior definición, consideramos que los documentos D2 y D4 no constituyen documentos del arte previo que afecten la novedad de la presente solicitud.

El objeto de la presente invención es una composición inmunogénica multivalente y una formulación líquida estéril que no se han revelado en ninguna de los documentos mencionados. Además, las reivindicaciones objetadas por novedad 9 y 10 y 18, 19 y 20 se han suprimido de tal manera que las objeciones por novedad se consideran como superadas.

2.4 Nivel inventivo

El nivel inventivo de una solicitud se encuentra definido en el Artículo 18 de la Decisión 486 y se establece cuando "*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*".

Contrario a lo que plantea el Examinador, el documento del arte previo citado no afecta el nivel inventivo de la presente solicitud por cuanto no es posible derivar la presente invención a partir de las enseñanzas presentadas en los documentos D1 y D3.

El solicitante desea manifestar que el nuevo capítulo reivindicatorio posee nivel inventivo posee nivel inventivo. No era obvio agregar conjugados utilizando el mismo portador CRM_{197} y obtener una respuesta inmune contra 13 conjugados de polisacárido capsular de 13 diferentes serotipos de S, pneumoniae, por las razones que se exponen a continuación.

Al momento de la presentación de la presente solicitud, la técnica anterior enseñaba que la valencia de las vacunes conjugadas era una tarea difícil.

De hecho, era conocido en la técnica que la combinación de conjugados utilizando la misma proteína portadora en una inyección multivalente única puede resultar en competición entre

los diferentes componentes y puede afectar adversamente la inmunogenicidad de cualquier conjugado individual.

Este fenómeno, conocido como interferencia de la respuesta inmune se debe al hecho de que una gran cantidad de proteína portadora puede conducir a una alta carga epitópica específica de célula T, la supresión mediada por el portador de la respuesta del anticuerpo y se vuelve así un factor limitante para la adición de conjugados individuales.

Fattom *et. al.* (Vaccine (1999) página 126-133, Vol 17, anexa a la presente) por ejemplo, observó una reducción de respuestas de anticuerpo a varios serotipos en ratones cuando la inmunogenicidad de las vacunas de conjugado multivalente se compararon con la inmunogenicidad de cada vacuna monovalente evaluado de manera separada.

Este fenómeno también se observó en Dagan *e. al.* (Infection and Immunity, 1998, p.2093-2098; anexo a la presente) que concluyó que:

"es deseable la restricción del contenido del portador de la proteína en la vacuna conjugada, como lo es el desarrollo de la otra proteína o portadores no proteínicos para vacunas de sacárido" (ver página 2097, columna derecha, última frase del segundo párrafo).

Las soluciones a estos problemas sugeridas en la técnica anterior son:

- el uso simultáneo de dos o mas portadores, con algunos serotipos de polisacárido sobre uno y algunos sobre otros (ver Fattom *et. al.* y Wuorimaa et al. The paediatric Infectious Disease Journal, Volume 20(3), 2001, pp 272-277, anexo a la presente); **Notamos que el documento D1 citado como la técnica anterior mas cercana también enseña el uso de dos o mas portadores;**

- otra solución sugerida por la técnica anterior fue mezclar dosis divididas en dos del mismo polisacárido sobre dos portadores, que, de manera similar, dividen en dos las dosis del portador individual para cualquiera de las dosis PS totales dadas (Anderson P *et al.*, Vaccine, 2003; 21(13-14):1554-9; anexo a la presente.).

La vacuna de la presente invención difiere de la de la técnica anterior por el uso de muchos

mas conjugados utilizando el mismo portador CRM_{197}. Esto significa un incremento en el número de conjugados, y un incremento en la dosis total del portador.

D1 citado por el examinador como la técnica anterior mas cercana esta utilizando **al menos dos diferentes proteínas portadoras**.

D3, citado por el examinador, se relaciona con la vacuna conjugada de **oligo**sacárido en donde la presente solicitud menciona una vacuna de **poli**sacárido que es mucho mas difícil de obtener.

D3 es simplemente listar los serotipos en la columna 1 y 14. Notamos que el serotipo 6A no es parte de la lista.

D3 solo ejemplifica una formulación de oligosacárido tetravalente (4 conjugados).

Esto es, por supuesto, mucho menos que la vacuna conjugada tridecavalente (13) de la presente invención.

La persona versada en la materia técnica conociendo del fenómeno de interferencia en la respuesta inmune, que limita potencialmente el número de conjugados **utilizando la misma proteína portadora no habría agregado mas conjugados usando el mismo portador CRM_{197}**.

Sin embargo, los inventores de manera sorprendente encontraron que la adición de polisacáridos derivados de los serotipos 3, 6A, 7F y 19A conjugados a CRM_{197} permitió generar una poderosa respuesta inmune en una composición inmunogénica multivalente que comprende los antígenos de polisacárido conjugados CRM_{197} de la vacune PCV7 licenciada (Prevnar™).

El solicitante puntualiza a este respecto los altos títulos IgG de suero obtenidos en el ejemplo 16 (ver tabla 3 y 5) y la buena actividad funcional del anticuerpo medida mediante la OPA (ver tabla 4) de la solicitud.

Estos resultados sorprendentes de la presente solicitud fueron confirmados posteriormente

por muchos ensayos clínicos en humanos, por ejemplo por Yeh S et al., Pediatrics 2010;126;e493-e505, anexa a la presente. Yeh et al. establece que: "*este estudio apoya que el PCV13 será tan efectivo como el PCV7 en evitar la enfermedad causada por los serotipos comunes a ambas vacunas. El PCV13 tiene un perfil de seguridad comparable al PCV7. Además, el PCV13 debe mediar protección contra los 6 serotipos adicionales, todos los cuales son una causa importante en todo el mundo de enfermedad neumocócica severa.*" (ver página e504, sección de conclusiones).

Contrario a lo que se habría esperado de la técnica anterior, no se observó la temida interferencia inmune entre el componente de la vacuna. Adicionalmente, no se observó la interferencia en inmunogenicidad de otras vacunas para niños concomitantemente administradas.

En contraste, otros grupos buscan suministrar cubrimiento mas allá de la vacuna PCV7 licenciada (Prevnar™) siguió una senda que es fundamentalmente diferente de la presente invención, buscando en su lugar evitar interferencia a través del uso de portadores de tipos múltiples.

Por ejemplo, los investigadores en GlaxoSmithKline (solicitante de D1) han publicado reportes con relación al estudio de la inmunogenicidad de una vacuna neumocócica undecavalente (11) donde el portador fue la proteína D (Prymula *et al.*, Expert Review of vaccines, 2009, Vol. 8, No. 11, Páginas 1479-1500, ver en particular la página 1480 columna derecha, sección "Por qué seleccionar una nueva proteína portadora ?").

La formulación final (conocida como Synflorix™) es muy diferente de aquella previamente probada en ensayos clínicos y se hicieron tres cambios claves durante los ensayos clínicos: la proteína portadora para el serotipo 18C se cambió a toxoide de tétano, la proteína portadora para el serotipo 19F se cambió a toxoide de difteria y el serotipo 3 se removió de la vacuna en razón a que la eficacia contra el serotipo 3 neumocócico no se podía demostrar (ver página 1481, columna izquierda, sección "Cambios a la primera formulación").

Se observó que el serotipo 3 conjugado a la proteína D tuvo que ser removida como efecto no cebado para el serotipo 3 en niños que habían recibido tres dosis tres dosis de vacuna seguía por una dosis reforzada de la misma vacuna o una vacuna a de polisacárido

neumocócico (ver Nurkka *et al.*, aquí anexa; por ejemplo, página 1012 columna derecha, penúltimo párrafo). En otro estudio, los resultados del ensayo opsonofagocítico (OPA) en niños que habían recibido dosis de la vacuna undecavalente (11) no mostró las respuestas de anticuerpo para el serotipo 3 a niveles comparables con otros serotipos probados (ver Gatchalian *et al.*, 17 reunión anual de la Eur. Soc. Paed. Inf. Dis (ESPID), número de cartel 4, P1A Poster Session 1, Estambul, Turquía, Mar. 27, 2001, aquí incluido). En aún otro estudio que examinó la eficacia de la vacuna undecavalente en la prevención de la otitis media aguda, la vacuna no suministran protección contra episodios causados por el serotipo 3 (Prymula., (2006) Lancet, 367: 740-748, aquí anexo; ver, por ejemplo, página 744 columna derecha, segundo párrafo y 747, columna derecha, penúltimo y último párrafos).

Así, los resultados suministrados en la presente solicitud de patente fueron inesperados y el nuevo capítulo reivindicatorio que ahora se refiere exclusivamente a la composición inmunogénica que comprende 13 diferentes polisacáridos conjugados a la proteína portadora CRM_{197} es inventiva.

En conclusión, la persona medianamente versada en la materia no habría contemplado la composición inmunogénica de la reivindicación 1 porque se hubiera temido que la adición de muchos mas conjugados utilizando la misma proteína portadora daría como resultado la interferencia de la respuesta inmune que afecta la inmunogenicidad de la vacuna. Los inventores de manera sorprendente tuvieron éxito con esta aproximación, utilizando el portador CRM_{197}, obtener buenas respuestas al antígeno donde otros han fallado.

De acuerdo con lo anterior la invención claramente posee nivel inventivo.

El solicitante presenta, además, una solicitud divisional. El soporte para las reivindicaciones de la solicitud divisional es el siguiente:

- La reivindicación 1 encuentra soporte en la página 17, líneas 23 - 25 y página 18 líneas 1-7 en conjuntó con página 19 líneas 5 -8.

- La reivindicación 2 y 3: característica adicional encuentra soporte en la página 18 líneas 7 - 10.

- La reivindicación 4 está relacionada con la formulación y dosis (cada dosis de 0.5mL es formulada para contener: 2.2.µg de cada sacárido, excepto por 6B a 4.4 µg; aproximadamente 29 µg CRM197 proteína portadora; 0.125 mg de aluminio elemental (0.5 mg de fosfato de alumino) adyuvante; y cloruro de sodio y búfer de succinato de sodio como excipiente.

Esta formulación encuentra soporte en página 25 líneas 15-25 y página 26 línea 1-6 en relación con página 25 líneas 1-6 y página 81 líneas 1 - 4

La dosis 2.2 µg de cada sacárido, excepto por 6B a 4.4 µg es soportado por la solicitud (como se mencionó anteriormente, esta fue de hecho la dosis utilizada en el estudio descrito en la página 81, y en vista de lo divulgado en la página 25 líneas 1-6 sería inmediatamente evidente para una persona experta en la materia que dicha dosis es una modalidad de la invención).

- La reivindicación 5 y la 6 encuentran soporte en la página 25 líneas 15-25. Para la reivindicación 5 (dosis 2.2 µg de cada sacárido, excepto para 6B a 4.4 µg; por favor ver arriba).
- La reivindicación 7 encuentra soporte por ejemplo en la página 24 líneas 14-20;
- La reivindicación 8: encuentra soporte por ejemplo en la página 18 líneas 13-26 y página 19 líneas 1 a 8 en conjunción con página 21 líneas 6-9 y página 17, líneas 23 - 25 y página 18 líneas 1-7;
- La reivindicación 9 es soporte de reivindicación 11 anteriormente así como en página 19 líneas 5 -8.
- La reivindicación 10 encuentra soporte por ejemplo en la página 21 líneas 6-9 y página 17, líneas 23 - 25

3. Solicitudes concedidas en otros países para esta misma invención.

A manera informativa, pongo en conocimiento del Examinador que las patentes EP1868645B1, europea; JP4472770 (B2), japonesa y AU2006235013 B2, australiana, equivalentes a la presente solicitud colombiana ha sido otorgadas. Aún cuando entiendo que estas decisiones de ninguna manera obligan al Despacho para decidir de igual forma, considero que por tratarse de una pregunta fáctica la determinación de la existencia de novedad y nivel inventivo, las decisiones de otras oficinas de patentes constituyen un antecedente persuasivo en la materia.

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En virtud de lo anterior, solicito que se acepten las razones expuestas en el presente documento y en consecuencia, se otorgue el privilegio de patente de invención para el invento que se tramita en este expediente.

Atentamente,

ÁLVARO CORREA ORDÓÑEZ
C.C.No.79.143.366 de Usaquén
T.P.20.281

Anexos:
- Capítulo reivindicatorio modificado
- Solicitud divisional
- Recibo por presentación de solicitud divisional
- Documentos:
  1. Fattom et. al. (Vaccine (1999)) página 126-133, Vol 17)
  2. Fattom *et. al.* y Wuorimaa et al. The paediatric Infectious Disease 3. Journal, Volume 20(3), 2001, pp 272-277
  3. Dagan et. al. (Infection and Immunity, 1998, p.2093-2098
  4. Anderson P et al, Vaccine, 2003; 21(13-14):1554-9
  5. Yeh S et al., Pediatrics 2010;126;e493-e505
  6. Nurkka et al. página 1012 columna derecha, penúltimo párrafo.
  7. Gatchalian et al., 17 reunión anual de la Eur. Soc. Paed. Inf. Dis (ESPID), número de cartel 4, P1A Poster Session 1, Estambul, Turquía, Mar. 27, 2001
  8. Prymula et al (2006) Lancet, 367: 740-748
  9. Prevnar13

REIVINDICACIONES

1. Una composición inmunogénica multivalente, que comprende: 13 diferentes conjugados de polisacárido-proteína, junto con un vehículo fisiológicamente aceptable, en donde cada uno de los conjugados comprende un polisacárido capsular de un serotipo diferente de *Streptococcus pneumoniae* conjugado a una proteína portadora, y los polisacáridos capsulares se preparan de los serotipos 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F y 23F, en donde la proteína portadora es CRM197; que comprende, además, un adyuvante en donde el adyuvante el fosfato de aluminio.

2. Una formulación líquida estéril de polisacáridos capsulares neumocócicos de los serotipos 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F y 23F individualmente conjugados al CRM197 para uso como una vacuna.

3. La formulación líquida estéril de la reivindicación 2, en donde cada dosis de 0,5 mL se formula para contener: 2 µg de cada sacárido, excepto para 6B a 4 µg; aproximadamente 29 µg de proteína portadora CRM_{197}; 0,125 mg de adyuvante de aluminio elemental (0,5 mg de fosfato de aluminio); y cloruro de sodio y succinato de sodio como excipientes.

PERGAMON

Vaccine 17 (1999) 126–133

Vaccine

Epitopic overload at the site of injection may result in suppression of the immune response to combined capsular polysaccharide conjugate vaccines

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Abstract

Capsular polysaccharide (CP) conjugate vaccines targeting a variety of bacterial infections are currently under development and clinical evaluation. The inclusion of multiple CP serotypes combined in a single injection is an important maneuver being evaluated. The combination of CP conjugate vaccines into a single multivalent injection may result in competition among the different components and adversely affect the immunogenicity of any individual conjugate. We observed a reduction of 30–90% in antibody responses to several serotypes in mice when immunogenicity of a 12-valent *Escherichia coli* (*E. coli*) lipopolysaccharide (LPS) conjugate vaccine was compared to the immunogenicity of each monovalent vaccine evaluated separately. A reduction of 30% was observed in the *Staphylococcus aureus* (*S. aureus*) type 8 CP antibodies when a type 8-rEPA conjugate was combined with a type 5-rEPA conjugate. *S. aureus* types 5 and 8-rEPA conjugates were combined with 100 μg of either rEPA (homologous) or diphtheria toxoid (DT) (heterologous) carrier proteins, and evaluated in rEPA or DT primed mice. The addition of the homologous protein resulted in a 64% reduction in type 5 CP antibodies. The heterologous protein did not affect the immunogenicity of the type 5. We postulate that the free protein competed with the conjugate and recruited most of the rEPA primed T cells. In the case of the DT conjugates, the DT targeted different populations of the T cells, thus interference was not observed. These data suggested that the epitopic load rather than the antigenic load at the site of injection caused reduced immunogenicity of the conjugates. We theorize that individual components of multivalent CP vaccines conjugated to the same carrier proteins would compete for a limited number of specific carrier protein primed T cells. This would result in one or more components being unavailable in eliciting a sufficient immune response. The use of multiple carrier proteins should be considered as an approach to reduce interference when multivalent conjugate vaccines are to be formulated into a single injection. © 1998 Elsevier Science Ltd. All rights reserved.

Keywords: Combination vaccines; Conjugate vaccines; Interference; Immune response

1. Introduction

Bacterial capsular polysaccharides (CPs) are virulence factors and protective antigens [1–3]. By covering the surface of these pathogens, CPs shield the microorganisms and enable them to evade opsonophagocytosis, the major clearance mechanism of bacterial pathogens. Antibodies elicited by these CPs can opsonize bacteria for phagocytosis and killing by polymorphonuclear leukocytes (PMNs) in *in vitro* assays, and can protect animals against the effects of bacterial challenges in experimental (*in vivo*) animal models [4–6]. Mono- and multivalent CP based vaccines have been formulated, and shown to be efficacious in inducing protective immunity in humans. Such vaccines include the 23-valent pneumococcal and the tetravalent meningococcal vaccine, as well as the highly successful *Haemophilus influenzae* type b conjugate vaccine [7–10].

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Purified CPs, with a few exceptions, are poor and age-related immunogens, and T cell independent antigens. When immunized with CPs vaccines, Children less than 2 years old and elderly mounted a poor immune response which resulted in lower efficacy of these vaccines [10,11]. The development of conjugate vaccines comprising CPs chemically-linked to protein carriers has been pursued to overcome these limitations. Covalently binding CPs to carrier proteins has improved their immunogenicity, conferred T cell dependent immunologic response patterns, and overcome the age-related behavior of immune responsiveness [12].

Multivalent conjugate vaccines and combination vaccine formulations have been introduced to permit protection against multiple pathogen serotypes within a simple immunization schedule [9,13,14]. While the combination of purified capsular polysaccharide vaccines has not altered the immunogenicity of the individual polysaccharides, a reduced immunogenicity of individual conjugate vaccines when presented in multivalent formulations has been observed [14–17]. The reduced immunogenicity of conjugate vaccines in combined formulations has been attributed largely to the epitopic suppression phenomenon, and in some cases, to non-epitope-specific suppression [18,19]. We investigated the phenomenon of interference in combined polysaccharide-conjugate vaccines in animals. Our theory is that interference is due to competition between CPs bound to homologous carrier proteins for a limited number of carrier-specific primed helper T cells, such competition would result in a diminished T cell help and consequently in a reduced immune response to one or more polysaccharide components of the combined vaccine.

2. Methods and materials

2.1. Materials and methods

2.1.1. Vaccines

S. aureus capsular polysaccharides type 5 and type 8 conjugated to recombinant, nontoxic exotoxin A derived from *Pseudomonas aeruginosa*, expressed in *E. coli* (rEPA) (T5-rEPA and T8-rEPA, respectively) were used as monovalent vaccines [20]. Their preparation and characterization were described [21]. The *S. aureus* type 8 CP conjugated to diphtheria toxoid (T8-DT) was prepared using the SPDP method as described [22]. The *E. coli O*-polysaccharides (PS) used in preparation of these vaccines were prepared by acid hydrolysis of purified lipopolysaccharide (LPS), activated by cyanogen bromide, derivatized with adipic acid dihydrazide, and conjugated to rEPA through activation with carbodiimide as described [23–25]. The 12-valent vaccine contained the following LPS serotypes: O1, O4, O6, O7, O8, O11, O15, O16, O18, O22, O25, and O75.

2.1.2. Immunization

Groups of 10 outbred, general purpose, strain ICR female mice (Harlan Sprague Dawley, Indianapolis, IN), 6–8 weeks old, were immunized subcutaneously with 0.1 ml (2.5 μg CP or CP-conjugate) containing 2.5 μg of the appropriate conjugate vaccine. The multivalent combination vaccine volumes did not exceed 0.5 ml and contained 2.5 μg (based on polysaccharide content) of each individual conjugate. All immunizations in the priming or secondary series were delivered at two week intervals and terminal exsanguination was carried out one week after the last immunization [12,22].

2.2. Serological analysis

Mouse sera were assayed for the presence of *S. aureus* T5 and T8 CP IgG antibodies using an avidin–biotin ELISA method [24]. The *E. coli O*-specific polysaccharide IgG antibodies from animals immunized with either the mono or multivalent vaccines were assayed in ELISA using the appropriate LPS to coat the ELISA plates [25]. Sera were evaluated using the Titer Max Calculation Program (Dynatech Software) and quantitated by comparison with a pooled hyperimmune mouse reference serum using a parallel line analysis [26,27].

2.3. Statistical evaluation

The geometric means (GM) were calculated from the anti-log of the average of the natural log of each sample value. Student *t*-tests were performed via Sigma Statistics (Jandel Scientific Software) for comparing groups.

3. Results

3.1. Effect of previous immunizations on the immune response to S. aureus T8 antibodies

Table 1 shows that while immunization with non-conjugated CP did not elicit antibodies, $< 1\ \mu g\ ml^{-1}$, mice immunized once with conjugate vaccine elicited a weak immune response, $7.5\ \mu g\ ml^{-1}$, compared to $149.3\ \mu g\ ml^{-1}$ maximum response achieved by two injections of the T8-rEPA conjugate vaccine. When T8-rEPA conjugate immunized mice were injected with T8 CP alone, homologous (rEPA), heterologous (DT) carrier protein, or the T8-DT conjugate, these animals did not elicit significant increases in the T8 antibody titers. Mice previously injected once or more with a

Table 1. The effect of antigen booster injection on the *S. aureus* T8 antibody response in mice with and without priming

Priming		Vaccination	GM antibody (μg ml^{-1})
Immunogen	Doses	Immunogen	1st dose (range)
None	0	T8-rEPA	7.5^a (3.8–44.7)
S. aureus T8-rEPA	1	rEPA	10.3^a (4.8–16.1)
S. aureus T8-rEPA	1	DT	9.1^a (3.8–26.6)
S. aureus T8-rEPA	1	T8 Ps	11.8^a (4.8–18.9)
S. aureus T8-rEPA	1	T8-DT	16.9^a (7.2–68.7)
S. aureus T8-rEPA	1	T-8-rEPA	149.3^b (71.5–249)
E. coli 075-DT	1	T8-DT	157.6* (40.5–727.8)
E. coli 075-DT	3	T8-DT	237.5* (40.0–2079.7)
T8 CP	1	T8 CP	< 1
Saline	1	Saline	< 1

^{a} vs ^{b}, $p < 0.01$; *not significant $p > 0.05$.

nonrelated CP conjugated with a homologous carrier protein (DT) responded to T8-DT with a booster response in T8 antibodies, 157.6 and 237.5 μg ml^{-1}. This large response was related to the carrier moiety, since it was seen when T8-DT was administered following immunization with the *E. coli*–DT conjugate, and was not observed when mice were previously immunized with T8-rEPA.

Mice are naive to both carrier proteins used in this study, i.e. rEPA and DT. Therefore, the first immunization of the naive animals elicited a mild immune response to type 5 polysaccharide. Following the second injection, a booster response was observed (> 30-fold increase) ($p < 0.01$). The third immunization gave rise to another boost in the antibody levels of type 5 ($p < 0.02$) (Table 2). The number of previous immunizations with conjugate vaccines affected the immune response to *S. aureus* CP only when the animals were previously exposed to the homologous carrier protein. Upon priming with a different CP conjugated to the same carrier protein, one injection of the vaccine elicited an immune response comparable to that elicited by two injections of the same conjugate (29.2 vs 57.3, $p > 0.05$). Animals immunized previously with T8-rEPA conjugate vaccine did not show a priming effect or booster response when injected once with T8-DT conjugate (Table 2). The immune response to the T8-DT conjugate in these animals was similar to immunization of naive animals i.e. a booster response following the second and the third immunization.

3.2. Effect of combination of vaccines into multivalent formulations on the immune response to single components

Since the first immunization of mice with conjugate vaccines did not elicit significant amounts of CP antibodies, animals' immune response to the second and third immunization with the conjugates will be analysed. Table 3(a) shows that the combination of *S. aureus* T5-rEPA with T8-rEPA or T8-DT conjugates to produce a bivalent vaccine did not affect the immune response to T5 CP following the second and the third immunization. The levels achieved were comparable to the immunization with the monovalent vaccine. However, a 64% reduction in the antibody levels to T8CP was observed when T8-rEPA was combined with T5-rEPA conjugate ($p < 0.05$) [Table 3(b)]. No reduction in T8 immunogenicity was observed when T8-DT conjugate was combined with T5-rEPA. The reduced immunogenicity of T8-rEPA was largely corrected following the second booster injection of the vaccine [Table 3(b)] ($P < 0.01$).

Results depicted in Fig. 1 demonstrate a dramatic effect of combining conjugate vaccines into a multivalent formulation. All *E. coli* O–PS conjugates elicited a significant immune response following three

Table 2. The effect of previous immunization of mice on the *S. aureus* T5 CP antibody response

Priming		Booster vaccination	GM antibody μg ml^{-1}		
Immunogen	# Doses	Immunogen	1st dose (range)	2nd dose (range)	3rd dose (range)
None	0	T5-rEPA	1.6^a (0.1–5.0)	57.3^b (3.0–454.9)	184.2^c (92.8–757.5)
S. aureus T8-rEPA	3	T5-rEPA	29.2^d (7.0–144.0)	28.3^e (9.0–152.9)	164.7^f (13.5–3751.8)
S. aureus T8-DT	3	T5-rEPA	4.7^g (1.0–57.9)	104.6^h (26.6–379.9)	262.4^i (21.9–671.8

^{a} vs ^{b}, $p < 0.01$; ^{b} vs ^{c}, $p < 0.02$; ^{d} vs ^{b,e}, $p > 0.05$ (not significant); ^{e} vs ^{f}, $p < 0.01$; ^{e} vs ^{c}, $p < 0.01$; ^{g} vs ^{h}, $p < 0.01$; ^{g} vs ^{d}, $p < 0.01$.

Table 3. The effect of multiple conjugate combinations administered in a single injection on the immune response to *S. aureus* CPs

(a)Anti CP5 antibodies		
Immunization	CP antibody	GM ($\mu g\ ml^{-1}$)
Vaccine	2nd dose (range)	3rd dose (range)
T5-rEPA	54.7[a] (35.5–136.5)	494.2[b] (190.0–1090.0)
T5-rEPA/T8-rEPA	36.7[a] (18.7–78.5)	508[b] (325.0–1230.0)
T5-rEPA/T8-DT	77.3[a] (36.5–131.0)	307.3[b] (213.0–595.0)
(b)Anti CP8 antibodies		
T8-rEPA	149.3[a] (71.5–249.0)	306.1[b] (137.0–939.0)
T5-rEPA/T8-rEPA	54.3[c] (34.0–84.0)	218.8[b] (60.0–863.0)
T5-rEPA/T8-DT	129.7[a] (56.0–383.0)	851.5[b] (394–1877)

[a] vs [b], $p < 0.01$; [a] vs [c], $p < 0.01$.

immunizations with monovalent formulation. The number of responders, i.e. >fourfold increase of antibodies titers, was >90% for 9/12 serotypes. The rest of the serotypes, 3/12, achieved <60% (range 40–60%). A dramatic reduced immunogenicity was observed when a combination 12-valent vaccine was administered in one single injection. A significant ($p < 0.05$) decrease in the immune response to 6/12 PS was observed ranging from 25–95% of the response achieved when the same conjugates were administered as monovalent. A similar reduction was observed in the number of responders to each vaccine. Only 4/12 serotypes achieved >90% responders and the rest, 8/12 serotypes, achieved <75% response rate (range 40–75%). Three serotypes, O7, O11, and O75, were poor immunogens in monovalent as well as polyvalent formulation. A slight, but not significant, increase in antibody titers was observed with four serotypes in the multivalent formulation ($p > 0.3$).

3.3. Effect of overload of homologous or heterologous carrier protein on the immunogenicity of S. aureus conjugates

Data presented in Table 4 show that the addition of 10 μg homologous carrier resulted in an insignificant decrease in the antibody titers against both T5 and T8 CPs. However, a dramatic (63%) decrease in the T5 antibody titers was observed when 100 μg of the carrier was added to the vaccine ($p < 0.02$). Addition of the same amount of the heterologous DT protein did not affect the immune response to T5-rEPA conjugate. As type 8, on the other hand, had already reduced immunogenicity in the bivalent, no further inhibition was observed in any of the combinations and the immune response resembled that obtained with one injection of the vaccine (Table 1).

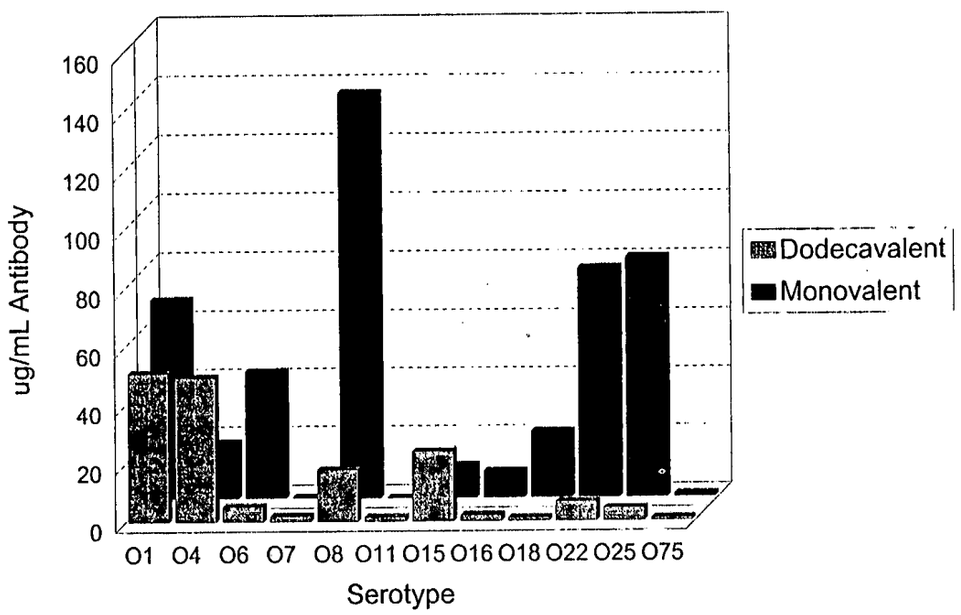

Fig. 1. Immunogenicity in mice of *E. coli O*-specific polysaccharide conjugates injected separately as monovalent or combined as dodecavalent vaccines. Mice in groups of ten were immunized three times, 2 weeks apart, and exsanguinated 1 week after the last injection. The dose was 2.5 μg as a monovalent or dodecavalent conjugated PS in a volume not exceeding 0.5 ml. Mouse sera were evaluated in ELISA against a high titered reference. Results are expressed as geometric mean μg IgG ml^{-1} and the ranges of the antibody concentration in each group.

Table 4. The effect of overload with homologous and heterologous free carrier protein (rEPA or DT) on the T5 and T8 CP antibody response

Group	Priming		Vaccination		T5 response (GM (μg ml^{-1}) (range)	T8 response GM (μg ml^{-1}) (range)
	Immunogen (2.5 μg/0.1 ml)	Dose	Immunogen (2.5 μg/0.1 ml)	Dose		
1	T5/T8rEPA	1	T5/T8-rEPA	1	40.9[a] (22.0–88.0)	17.9 (4.0–65.0)
2	rEPA	1	T5/T8-rEPA	1	28.8[b] (12.0–148.0)	6.8 (3.0–13.0)
3	rEPA	1	T5/T8-rEPA + rEPA (10 mg)	1	24.3[c] (4.0–78.0)	10.4 (4.0–31.0)
4	rEPA	1	T5/T8-rEPA + rEPA (100 mg)	1	10.6[d] (6.0–76.0)	5.7 (0.0–9.7)
5	rEPA	1	T5/T8-rEPA + DT (100 mg)	1	30.6[c] (7.0–156.0)	10.6 (5.0–22.0)

[a] vs [c]; [b], NS; [b] vs [d], $p < 0.02$; [c] vs [d], $p < 0.02$.

4. Discussion

Polysaccharides are poor immunogens, as they elicit less than optimal levels of antibodies in infants, children under the age of two years, adults with immunodeficiencies, and the elderly. Their immunogenicity is T cell independent and they elicit no booster response upon reinjection [28,29]. To overcome the restricted immunogenicity of polysaccharide vaccines, a new generation of semisynthetic vaccines has been explored. These new vaccines are based on the conjugation (covalent coupling) of polysaccharides to protein carriers. In contrast to the restricted and otherwise poor immunogenicity of polysaccharides, these polysaccharide–protein conjugates were shown to elicit high levels of polysaccharide-specific antibodies [30,31]. Conjugate vaccines were also shown to induce a boostable antibody response. Furthermore, the conjugate vaccines elicited high levels of polysaccharide-specific antibodies in infants during the early years of life when they are at high risk of contracting bacterial infection. The increased immunogenicity of these conjugate vaccines is due to the induction of carrier-specific T lymphocytes which possess the ability to induce proliferation and differentiation of B lymphocytes with specificity for the polysaccharide portion of the conjugate [2,3].

The immune response to conjugate vaccines can be influenced by either the carrier protein or the polysaccharide portion of the vaccine. Preexisting antibodies to the CP portion have been found to negatively affect the level of vaccine-induced CP antibodies. Barrington *et al.* reported that the immunization of human adults with Hib-TT previously immunized with Hib-DT, or vice versa, did not elicit an additional increase in the immune response to Hib [19]. It was also shown that reinjection of the same conjugate did not elicit a booster response in adult humans [30,31]. This suggests that the first injection may behave like a booster as adults have likely been previously exposed to this antigen. Furthermore, there may be a maximum allowable antibody response that is internally regulated by immune mechanisms. This 'response ceiling' may be the limit which the immune response cannot exceed. Another explanation for this lack of response may involve the formation of immunocomplexes between preexisting antibodies and injected vaccines. These immunocomplexes are taken up by macrophages and processed in a manner that would inhibit the latter's ability to properly present the antigen to T and B lymphocytes [32].

Conflicting results have been observed concerning the effect of carrier protein on the immune response to the polysaccharide portion of conjugate vaccines in humans. In infants immunized with Hib-DT, a better immune response was observed when the vaccine was administered one month following their first routine immunization with the DPT vaccine than when the two vaccines were given concomitantly [33,34]. This may be due to the existence of maternally acquired DT antibodies at the time of injection in the latter group. For those immunized one month later, these pre-existing antibody levels may have decreased while the population of carrier primed T cells was given time to expand. This hypothesis is supported by another study wherein active immunization with TT one month prior to administering the Hib-TT conjugate resulted in enhanced HibCP antibody levels [35]. Our data show that one, two, or three immunizations of mice with conjugates containing the homologous carrier equally enhanced the immune response to a subsequent immunization with conjugate containing a different CP. Conversely, prevaccination of adult volunteers with homologous carrier protein, however, reduced the subsequent increase in the total HibCP antibodies when Hib-DT or Hib-TT was administered [19]. A recent report on the suppression of the immune response to polysaccharide conjugate by carrier priming in mice showed that suppression was abrogated and the immune response even enhanced when the amount of carrier protein used for priming was reduced to levels causing a low antibody response to carrier [36]. Similar results were achieved when the B-cell epitopes were removed from the carrier [37]. These varying data indicate that there may be several factors involved in the enhancement/suppression issues. An expanded population of primed T cells will enhance the response to the second exposure to antigen, but the presence of too much antibody

may suppress a subsequent immune response through immunocomplexing or the 'response ceiling' phenomenon. This provides an interesting problem for researchers who are attempting to design vaccines, their formulation, and immunization schedule in such a way as to predict response.

The development of effective vaccines against bacterial pathogens necessitates the combination of several serotype conjugates into one injection. Previous experience with the Hib conjugate/DPT combination and the subsequent reduced immune response to Hib is worrisome [38,39]. We utilized *S. aureus* conjugate vaccines as a tool to explore the effect of combining vaccines into one injection and of carrier protein overload at the site of injection. Our theory was that the injection of a multivalent vaccine using the same carrier will result in competition between carrier-specific primed T lymphocytes. This would result in a less than optimal response to one or more components of the multivalent vaccine. If this theory is valid, then an overload with free carrier protein at the site of injection will result in reduced immunogenicity of the conjugates. Since mice do not possess pre-existing antibodies to rEPA, they were primed with either rEPA or conjugate and the immune response to the second injection was evaluated. The data show that with the bivalent T5-rEPA/T8-rEPA conjugate, the type 5 CP antibodies were not affected but the type 8 CP antibodies were significantly reduced compared to the monovalent vaccine ($p < 0.01$). The third injection of the combined vaccine resulted in correction of the interference observed with the second injection. A combination of T5-rEPA with T8-DT did not result in any reduction of antibodies to either CP.

A similar inhibitory effect was observed when conjugates were supplemented with free homologous carrier protein. The data suggest that the extent of interference is related to the concentration of the carrier protein. Only a trend of suppression was observed when rEPA was added to the combined vaccine at a 10 μg/dose. A significant suppression, however, was observed when the amount was increased to 100 μg ($p < 0.05$). Addition of the same amount of heterologous protein (DT) did not result in suppression of the immune response to either CP.

The more serotypes included in the vaccine, the greater the likelihood that interference will be an issue. Data presented here demonstrate the effect of combining 12 *E. coli* conjugates into a single injection. A significant suppression of the immune response to seven out of the twelve conjugates compared to the response when these conjugates were injected as monovalents. Previous studies evaluating a pentavalent pneumococcal vaccine in infants and toddlers showed that, in infants, the vaccine was less immunogenic than in toddlers. Using 1 μg ml^{-1} antibodies as an endpoint for response, only one of five serotypes (18C) achieved this level. Following the third immunization, however, another two serotypes (14 and 19F) achieved the 1 μg ml^{-1} IgG level [40]. A follow up study in toddlers that the response rate ($\geq$1 μg ml^{-1}) was <75% following the second immunization for three of the five pneumococcal serotypes included in the vaccine [41]. Similar results observed in elderly volunteers showed a lack of improvement in the immune response to combined conjugate vaccines for serotypes 6B and 14 [42].

It seems that interference is a multifaceted immunological phenomenon. The existence of a limited number of carrier-specific Th lymphocytes at the site of injection will likely result in competition among the different conjugates through their protein portion for the limited specific helper T cell population. The outcome would be less than optimal help to one or more of the vaccine components thus resulting in reduced immunogenicity. The severe interference observed in the multivalent *E. coli* conjugate and the reduced immunogenicity of type 5-rEPA conjugate when combined with free rEPA are good examples of this phenomenon. This epitopic load suppression phenomenon is different from the epitopic suppression where antibodies to the carrier protein from previous exposure facilitates the clearance of the injected vaccine causing reduced immunogenicity similar to that induced by maternally acquired antibodies [35]. Prior exposure to carrier protein may, under certain circumstances, induce proliferation of the carrier-specific helper T cell population, thus increasing their availability for the majority of the multivalent components. This would result in reduced competition and an enhanced immune response.

In conclusion, the data presented in this manuscript support the suggestion that, in addition to epitopic suppression induced by pre-existing carrier protein antibodies, the epitopic load at the site of injection might contribute to the severity of the suppression. We recommend that special attention should be given to the possibility of epitopic load at the site of injection when designing a multivalent conjugate vaccine. Epitopic suppression may be abrogated by choosing a conjugate methodology that blocks B-cell epitopes on the carrier protein or by diversification of the carrier protein used for these vaccines.

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Tolerability and immunogenicity of an eleven-valent pneumococcal conjugat vaccine in healthy toddlers

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Abstract

Background. A need to increase the serotype coverage of pneumococcal conjugate vaccines exists. The use of a single carrier protein may cause overload of the carrier and decrease the immune response by not providing sufficient carrier-specific T helper cell support. A vaccine composed of a mixture of tetanus- and diphtheria-conjugated polysaccharides (PS) is a potential solution to this issue.

Objectives. The aim of this study was to assess the tolerability and immunogenicity in healthy toddlers of an 11-valent pneumococcal conjugate vaccine that uses both tetanus and diphtheria toxoids as carriers. We explored the effect of an aluminum adjuvant on safety and immunogenicity by comparing the vaccine with and without adjuvant.

Methods. Twenty Finnish and 23 Israeli toddlers received the conjugate vaccine with or without aluminum adjuvant. Safety data were recorded for 5 days after vaccination. Sera were obtained before and 28 days after the immunization. IgG antibodies to the 11 vaccine-type PSs were determined by enzyme immunoassay.

Results. No serious adverse events occurred. The formulation with the adjuvant tended to induce fewer local but more systemic reactions than the non-adjuvant-containing formulation. Both vaccine formulations induced significant IgG increases for the vaccine-specific PSs. Types 3 and 7F were the most immunogenic; antibodies reached a concentration of 1 μg/ml in all individuals. Conjugates of types 6B, 14 and 23F were the weakest immunogens; antibodies reached the concentration of 1 μg/ml in 36, 27 and 32% of the individuals in the nonadjuvant group and in 53, 38 and 53% in the adjuvant group, respectively.

Conclusions. An 11-valent mixed carrier pneumococcal conjugate vaccine is safe

and immunogenic in toddlers. The use of an adjuvant do not seem to offer any significant benefit.

INTRODUCTION

Pneumococcal polysaccharide (PS) vaccine is immunogenic in children older than 2 years of age, but in young infants its efficacy is limited. 1 The immunogenic limitations of PS vaccines were overcome by the development of conjugate vaccines. Several mono- to nanovalent pneumococcal conjugate vaccines with *Neisseria meningitidis* outer-membrane protein complex, CRM_{197} protein (nontoxic variant of diphtheria toxin), tetanus or diphtheria proteins as carriers have been safe and immunogenic in toddlers 2-10 and infants. 7, 11-19 The secondary immune responses to pneumococcal conjugates in toddlers have T cell-dependent characteristics. 20-22 Recently a seven-valent conjugate vaccine (PncCRM) was efficacious in preventing vaccine-type pneumococcal invasive disease (97.4% efficacy, 95% confidence interval 82.7 to 99.9%) 23 and an acute otitis media (57% efficacy, 95% confidence interval 44 to 67%). 24 There is now a need to increase the valency of pneumococcal conjugate vaccines for larger coverage of pneumococcal diseases in different populations.

Increasing the valence of conjugate vaccines is more difficult than for PS vaccines, because the protein component of multivalent conjugate vaccines probably competes for a limited number of carrier-specific T cells. 25 A large amount of carrier protein may lead to high T cell-specific epitopic load, carrier-mediated suppression of the antibody response, especially after repeated injections, 26-27 and thus become a limiting factor for addition of further conjugates. In addition the amount of carrier protein might influence the immunogenicity of other conjugate vaccines sharing the same carrier when simultaneously administered. This appeared with simultaneously given *Haemophilus influenzae type b* (Hib) and pneumococcal tetanus-conjugated vaccines. 28 Anti-Hib PS response was suppressed with increasing doses of tetanus toxoid in the pneumococcal vaccine. Furthermore the increasing tetanus toxoid content seemed to be associated with lower priming capacity of the pneumococcal conjugate vaccine. 29 For these reasons it is desirable to optimize the carrier protein content. The use of a mixed carrier vaccine reduces the load of an individual protein and might reduce interference in immunogenicity when multivalent conjugate vaccine is formulated into a single dose. 25

Immunogenicity of pneumococcal PS conjugates varies depending on the carrier and the serotype. In an octavalent conjugate vaccine PS from types 3, 9V and 14 evoked a better response in infants when conjugated to diphtheria toxoid, and PS from type 4 evoked better response when conjugated to tetanus toxoid. 30 No carrier-specific differences existed in immunogenicity for types 6B, 18C, 19F and 23F.

Eleven of the most common serotypes cause ~80% of the all pneumococcal invasive diseases worldwide. 31-33 The newest experimental multivalent pneumococcal

conjugate vaccine consists of capsular PS from these 11 most common serotypes conjugated to either tetanus or diphtheria toxoid. This vaccine uses optimal carriers for each PS with minimized protein content to circumvent the overload of single carrier protein. 30 Previously the vaccine was well-tolerated and immunogenic in healthy adults. 34 The objective of this study was to assess in healthy toddlers the tolerability and immunogenicity of this 11-valent pneumococcal conjugate vaccine.

MATERIALS AND METHODS

Vaccinees

Twenty Finnish (mean age, 23.2 months; range, 21.1 to 24.8) and 23 Israeli toddlers (mean age, 18.5 months; range, 13 to 24.5) were recruited. History of documented invasive pneumococcal disease, prior vaccination with any other pneumococcal vaccine or recent (<14 days) immunization with any other vaccines or administration of immunoglobulin and corticosteroid therapy prohibited inclusion into the trial. The Finnish toddlers had previously received 3 doses of diphtheria-tetanus-pertussis (DTP) and three doses of Hib vaccine (HbOC) conjugated to mutant diphtheria toxoid (CRM_{197}) in infancy, before the study vaccine was given. The Israeli toddlers had previously received 3 doses of a combined DTP and inactivated poliovirus vaccine, 1 dose of DTP and 4 doses of Hib PS conjugated to tetanus toxoid in infancy. The Ethics Committee of the National Public Health Institute, Helsinki, Finland, and the Israeli National Ethics Committee reviewed the protocol. Written informed consent was obtained from the parents before enrollment. The International Conference on Harmonisation Guidelines for Good Clinical Practice were followed in the study.

Vaccines

The study vaccine (manufactured by Aventis Pasteur) was a mixture of 11 polysaccharide-protein conjugates. Pneumococcal PSs of types 3, 6B, 14 and 18C were conjugated to diphtheria toxoid, and PSs of types 1, 4, 5, 7F, 9V, 19F and 23F were conjugated to tetanus toxoid protein. The vaccines contained 1 μg/dose of type 1, 4, 5, 7F, 9V, 19F and 23F PSs; 3 μg/dose of type 3, 14 and 18C PSs; and 10 μg/dose of type 6B PS. It was given with aluminum adjuvant (F3 alum, adjuvant-containing formulation) or without it (F3, non-adjuvant-containing formulation).

Vaccination and sampling

The study was an open, nonrandomized trial. For safety reasons the first 10 participants in Finland and the first 5 in Israel received the F3 formulation. The next 10 in Finland and 5 in Israel received the F3 alum formulation. The next 7 in Israel received the F3 formulation, and the last 6 received the F3 alum formulation. Altogether 10 Finnish and 12 Israeli toddlers received the F3 formulation, and 10 Finnish and 11 Israeli toddlers received the F3 alum formulation. The vaccines were administered by deep intramuscular injections into the right upper thigh. Blood samples were obtained before and 28 days after the vaccination. The serum samples were stored at -20°C in Finland and at -70°C in Israel.

Safety data

Each participant was observed for 30 min after the vaccination for immediate allergic reactions. The parents were requested to follow adverse events for 5 days after vaccination and report these on a diary card. Events followed were local reactions (redness, swelling, induration and pain) at the injection site and systemic reactions (including rectal temperature >38.5°C, drowsiness, irritability, anorexia, vomiting, diarrhea or insomnia). Toddlers were followed for 1 month for serious adverse events.

Serology

An enzyme-linked immunosorbent assay was used to measure IgG antibodies to the 11 pneumococcal PSs in serum samples taken at Days 0 and 28. 7 Antibodies to cell wall PSs were neutralized before measurements. The reference serum with officially assigned concentrations for each PS used was 89-SF (Food and Drug Administration, Bethesda, MD). 35

Statistical methods

Adjusted geometric mean concentrations (GMC) of antibodies were used to allow pooling of the data from the two study centers. 36 Adjusted GMCs were calculated as the antilog of least squares means adjusted by center factor. A paired *t* test was used to calculate the statistical significance of intragroup antibody responses.

RESULTS

The 11-valent pneumococcal conjugate vaccine was safe, and no serious adverse events occurred. The F3 formulation induced more local reactions, although the F3 alum formulation tended to induce more pain of moderate or severe intensity (Table 1). Swelling and/or redness over 4 cm appeared in 2 of 21 cases in the F3 alum group and in 4 of 22 cases in the F3 group. Prominent local reactions appeared in 2 individuals in the F3 group and 1 in the F3 alum group. These prominent local adverse effects were experienced by Finnish toddlers, and vaccination by a more strictly deep intramuscular route with an emphasis not to administer it superficially in Israeli toddlers appeared to resolve the issue. The F3 alum induced more systemic reactions than the F3 (Table 1). Irritability was the most common systemic reaction. High fever (>38.5°C) was rare in both groups. No statistically significant differences occurred between the groups regarding to any adverse event.

TABLE 1. Adverse effects within 5 days after vaccination with (F3 alum) or without aluminum hydroxide (F3)*

Vaccine	% of Individuals	
	Fe	F3 Alum
N	22	21
Redness >2.0 cm	32	10
Swelling >2.0 cm	14	10
Induration >2.0 cm	14	5
Pain		
Mild	32	24
Moderate	9	14
Severe	5	24
Fever >38.5°C	9	19
Irritability	36	57
Anorexia	14	38

* No statistically significant differences occurred between the groups regarding any adverse reaction.

Table 1. Adverse effects within 5 days after vaccination with (F3 alum) or without aluminum hydroxide (F3)* No statistically significant differences occurred between the groups regarding any adverse reaction.

Prevaccination antibody concentrations to pneumococcal PSs were low and similar in the F3 and F3 alum groups. All conjugates were immunogenic, and both vaccines induced a statistically significant ($P < 0.05$) antibody increases to all vaccine-specific serotypes (Table 2). Almost all children acquired antibody concentration higher than 0.15 µg/ml against all vaccine types. The proportion of individuals not gaining this level was 13.6, 27.3 and 9.1% in the F3 group against types 6B, 14 and 23F, respectively, and 9.5, 9.5, 4.8 and 4.8% in the F3 alum group against types 6B, 14, 18C and 23F, respectively. Adjusted geometric mean concentrations reached 1 µg/ml for all serotypes with both formulations, except for types 6B (0.7 µg/ml with F3), 14 (0.5 µg/ml with F3 and 0.8 µg/ml with F3 alum) and 23F (0.8 µg/ml with F3). The comparison of adjusted GMCs and the percentage of individuals with IgG antibody concentrations higher than 1 µg/ml showed that PSs 6B, 14 and 23F were more immunogenic in the adjuvant-containing F3 alum formulation than in the non-adjuvant-containing F3 formulation. The opposite was true for PSs 9V and 18C. For the other serotypes geometric mean concentrations were similar between the groups. Overall no significant differences occurred between the vaccines regarding immunogenicity.

TABLE 2. Adjusted GMC with 95% CI before and after vaccination of IgG antibodies to pneumococcal polysaccharides*

Type	Carrier	Days	F3 (N = 224)		F3 Alum (N = 211)		% of individuals with IgG Antibodies >1.0 µg/ml after Immunization	
			GMC (µg/ml)	95% CI	GMC (µg/ml)	95% CI	F3 >1.0 µg/ml	F3 alum >1.0 µg/ml
1	T	0	0.2	0.18–0.24	0.2	0.15–0.27		
		28	2.1	1.87–2.43	2.2	1.85–2.66	77	86
3	D	0	0.2	0.13–0.45	0.3	0.16–0.58		
		28	4.6	3.41–6.73	5.5	3.66–8.33	100	100
4	T	0	0.2	0.13–0.24	0.2	0.13–0.26		
		28	7.1	4.91–10.94	6.9	4.46–10.69	91	95
5	T	0	0.3	0.22–0.44	0.3	0.22–0.46		
		28	2.5	1.94–3.21	2.0	1.58–3.51	91	81
6B	D	0	0.1	0.10–0.15	0.2	0.13–0.28		
		28	0.7	0.37–1.27	1.5	0.68–3.58	36	63
7F	T	0	0.3	0.15–0.44	0.2	0.12–0.32		
		28	8.2	5.92–11.92	10.6	8.02–13.69	100	100
9V	T	0	0.3	0.17–0.44	0.3	0.18–0.41		
		28	3.5	2.39–4.74	2.5	1.45–4.17	95	73
14	D	0	0.2	0.16–0.30	0.3	0.20–0.38		
		28	0.5	0.33–0.83	0.8	0.44–1.31	27	38
18C	D	0	0.1	0.09–0.20	0.1	0.09–0.21		
		28	2.4	1.40–4.14	1.7	0.96–2.94	82	67
19F	T	0	0.4	0.26–0.53	0.5	0.38–0.77		
		28	2.4	1.43–3.99	2.2	1.27–3.91	77	71
23F	T	0	0.2	0.15–0.30	0.2	0.14–0.33		
		28	0.8	0.57–1.21	1.0	0.62–1.74	32	53

* No significant differences occurred between the groups.
95% CI, 95% confidence interval.

Table 2. Adjusted GMC with 95% CI before and after vaccination of IgG antibodies to pneumococcal polysaccharides* No significant differences occurred between the groups.95% CI, 95% confidence interval.

DISCUSSION

The 11-valent pneumococcal conjugate that uses both diphtheria and tetanus toxoids as carriers appeared safe and well-tolerated in toddlers. Local reactions were infrequent and small, with the exception of few larger local reactions in 3 individuals. Previously the vaccine has been immunogenic and acceptable in reactogenicity in healthy adults, although the formulation without adjuvant induced frequently local reactions.[34] The frequency of local adverse events was lower in toddlers than in adults, and the frequency in infants appears even lower. 37 This might be explained by the fact that adults have been primed for pneumococci via natural carriage of the bacteria in the nasopharynx and for tetanus and diphtheria toxoids via vaccinations and therefore have higher concentrations of preexisting antibodies. 38 Systemic reactions, of which irritability was the most frequent in toddlers, appeared more commonly than local reactions. The F3 alum formulation induced more irritability and anorexia than did F3. Previously in infants Hib conjugate vaccine with aluminum adjuvant was better tolerated than the nonadjuvant formulation, 39 and interestingly no systemic reactions occurred. The distribution of all adverse events was similar to that reported in the previous adult study. In general the F3 formulation induced more local reactions, and the F3 alum formulation induced more pain of severe modality and systemic reactions. The lower rate of local reactions after F3 alum may have resulted from a slower release of antigens or a blockage of epitopic sites of antigens by the adjuvant. On the other hand aluminum hydroxide itself may be a systemic irritant because of its absorbable properties. 40

Both conjugate formulations were immunogenic in toddlers and were able to induce significant antibody increases to all vaccine-specific PS serotypes. Conjugates of types 6B, 14 and 23F were the weakest immunogens. Interestingly in adults type 6B and 14 conjugates were among the best immunogens in this vaccine.[34] The poor

immunogenicity of 6B and 23F in toddlers has previously been observed in many studies as well and might be the result of inherent properties of individual PS. 7, 9, 10, 41 However, protective antibody concentrations to pneumococcal PSs are not known, and they may differ between serotypes and for different types of disease. Although IgG concentrations to PS 6B are usually low in children, their functional capacity is high. 42

No significant differences in the immunogenicity of the PS-protein conjugates appeared between the two formulations. However, types 6B, 14 and 23F were more immunogenic in the F3 alum vaccine than in the F3 vaccine. The opposite was true for types 9V and 18C. For types 6B, 9V and 14 the pattern of immunogenicity in toddlers was similar to that seen previously in adults. The adjuvant effect, although slight, for types 6B, 14 and 23F might be caused by increased uptake of antigens by antigen-presenting cells. Reasons for the tendency of types 9V and 18C to be less immunogenic in the F3 alum than in the F3 vaccine are unknown.

The increase of valency may decrease immunogenicity because of carrier-mediated influence. 26-27 In this study such an effect was not observed; the antibody concentrations are similar to those with a monovalent, 4-valent and 11-valent tetanus and/or diphtheria conjugate vaccines in toddlers. 3, 10, 22 The study vaccine is designed to circumvent carrier related problems by using 2 carriers with optimized concentrations. This approach seems to work for 11 serotypes, but additional optimization might be needed if the valency is further increased.

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Key words: Tolerability; immunogenicity; pneumococcal conjugate vaccine; toddlers

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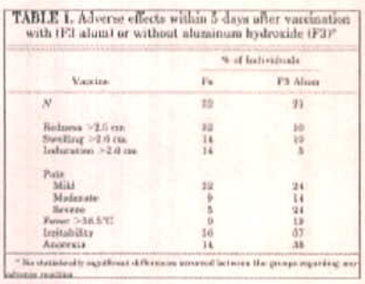

TABLE 1. Adverse effects within 5 days after vaccination with (F3 alum) or without aluminum hydroxide (F3)*

Vaccine	% of Individuals	
	Fs	F3 Alum
N	22	21
Redness >2.5 cm	32	10
Swelling >2.0 cm	14	10
Induration >2.0 cm	14	5
Pain		
Mild	32	24
Moderate	9	14
Severe	5	24
Fever >38.5°C	0	19
Irritability	36	57
Anorexia	14	38

* No statistically significant differences observed between the groups regarding any adverse reaction

Table 1

Table 2

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Reduced Response to Multiple Vaccines Sharing Common Protein Epitopes That Are Administered Simultaneously to Infants

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The plethora of newly discovered vaccines implies that, in the future, many vaccines will have to be administered simultaneously to infants. We examined the potential interference with the immune response of several coadministered vaccines containing the same protein component, namely, tetanus toxoid (TT). Infants simultaneously receiving a tetravalent pneumococcal vaccine conjugated to TT (PncT) and a diphtheria-tetanus-pertussis–poliovirus–*Haemophilus influenzae* type b-tetanus conjugate vaccine showed significantly lower anti-*H. influenzae* type b polysaccharide (polyribosylribitol phosphate [PRP]) antibody concentrations than those receiving either a tetravalent pneumococcal vaccine conjugated to diphtheria toxoid or placebo. A dose range study showed that anti-PRP antibody concentrations were inversely related to the TT content of the PncT vaccines administered in infancy. Postimmunization antitetanus antibody concentrations were also affected adversely as the TT content of the coadministered vaccines was increased. This phenomenon, which we believe derives from interference by a common protein carrier, should be taken into account when the introduction of an immunization program including multiple conjugate vaccines is considered.

Recently licensed vaccines include the *Haemophilus influenzae* type b (Hib) conjugate, varicella-zoster, acellular pertussis, and hepatitis A vaccines. Many additional vaccines are being tested in clinical studies. For practical reasons, if included in childhood vaccination programs, they ought to be administered simultaneously at separate sites or as combined vaccines (6). Possible interactions between the vaccines thus become important from both theoretical and practical points of view (7).

The first conjugate vaccines were those against Hib, in which a polysaccharide or oligosaccharide derived from the Hib capsule (polyribosylribitol phosphate [PRP]) was covalently conjugated to a protein carrier (24, 29). The same technology is now used to widen the range of conjugate vaccines against invasive organisms such as pneumococci and other encapsulated organisms (28). Multiple vaccines based on the same protein carrier and thus having common antigenic epitopes might be available soon, and the possibility of their interactions must be considered. The simultaneous administration of several conjugate vaccines sharing the same protein carrier and the carrier itself may be associated with the suppression of the response to polysaccharides through various mechanisms. Examples of such theoretical mechanisms are competition for antigen capture and presentation between B cells with surface immunoglobulins specific for epitopes on the carrier and B cells specific for the polysaccharide; prevention of the binding of the conjugate vaccines to polysaccharide-specific B cells by the free protein carrier; and suppression of the response to polysaccharides by expansion of the number of carrier-specific B cells induced by previous injection of the carrier, thus directing the conjugate away from polysaccharide-specific B cells (17).

We recently studied the immunogenicity of two newly developed tetravalent pneumococcal conjugate vaccines (2, 8, 9). Both of these vaccines contained polysaccharide antigens of four pneumococcal serotypes (6B, 14, 19F, and 23F) conjugated either to tetanus toxoid (TT) (PncT vaccine) or to diphtheria toxoid (PncD vaccine). These pneumococcal vaccines were administered simultaneously with two other vaccines, diphtheria-tetanus-pertussis (DTP) and Hib polysaccharide-TT conjugate (PRP-T). The purpose of this study was to examine if the simultaneous administration of PncT adversely affects the immunologic response to the two other vaccines also containing TT, namely, DTP and PRP-T.

MATERIALS AND METHODS

Study design. Two parallel studies on the safety and immunogenicity of new tetravalent pneumococcal conjugate vaccines were conducted, one in Israel and one in Finland. Both studies were double blinded, randomized, and controlled. Each study was approved by the relevant ethics committees, and written informed consent was obtained from the parents or legal guardians before enrollment at both study sites.

Vaccines. (i) Pneumococcal conjugate vaccines and placebo. PncT vaccine (manufactured by Pasteur Mérieux Connaught, Lyon, France; lot S2840) was a mixture of four purified capsular polysaccharides from *Streptococcus pneumoniae* serotypes 6B, 14, 19F, and 23F conjugated to TT. The ratios of TT to polysaccharide in the bulk (individual batches) were 1.6 for type 6B, 2.2 for type 14, 1.4 for type 19F, and 2.2 for type 23F. PncD vaccine (manufactured by Pasteur Mérieux Connaught, Swiftwater, Pa.; lot 930095) was a mixture of the same four pneumococcal polysaccharides conjugated to diphtheria toxoid. The respective ratios of diphtheria toxoid to polysaccharide were 2.0, 2.7, 3.1, and 2.8. The placebo used in the study consisted of phosphate-buffered saline.

All of these vaccines were contained in single-dose, ready-to-use glass syringes indistinguishable in appearance. The vaccines were administered as a 0.5-ml intramuscular injection into the upper part of the anterolateral thigh.

(ii) Other vaccines. In Israel, the DTP, PRP-T, and trivalent inactivated poliovirus (IPV) vaccines were administered as a single dose after lyophilized PRP-T was reconstituted with 0.5 ml of liquid DTP-IPV to form a pentavalent DTP–IPV–PRP-T vaccine (Pasteur Mérieux Connaught, Lyon, France). This mixture contained 10 flocculation units (Lf) (approximately 30 μg) of TT, 25 Lf of diphtheria toxoid, and 10 μg of PRP conjugated to 24 μg of TT.

In Finland, DTP was produced locally (National Public Health Institute, Helsinki, Finland). The vaccine contained 5 Lf (approximately 15 μg) of purified TT and 19 Lf of purified diphtheria toxoid. PRP-T (ActHIB; Pasteur Mérieux Connaught, Lyon, France) was reconstituted with 0.5 ml of liquid DTP to form DTP–PRP-T.

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TABLE 1. Study designs in Israel (75 infants) and Finland (200 infants)

Country and parameter	Regimen at mo:					
	2	4	6	7	12	13
Israel						
Study vaccines	+[a]	+[a]	+[a]		+[b]	
Other simultaneously administered vaccines	DTP–IPV–PRP-T	DTP–IPV–PRP-T, OPV	DTP–IPV–PRP-T, OPV		DTP–IPV–PRP-T, OPV	
Blood samples	+	+	+	+	+	+
Finland						
Study vaccines	+[c]	+[c]	+[c]			
Other simultaneously administered vaccines	DTP–PRP-T	DTP–PRP-T	DTP–PRP-T			
Blood samples	+	+	+	+		

[a] PncT (n = 25), PncD (n = 25), or placebo (n = 25). PncT and PncD were given at a dose of 3 μg of polysaccharide per conjugate.
[b] 23-valent pneumococcal nonconjugate vaccine.
[c] PncT (n = 75), PncD (n = 75), or placebo (n = 50). For PncT and PncD, each group of 75 was further divided into three groups receiving 1 μg of each component ($PncT_{01}$; n = 25), 3 μg of each component ($PncT_{03}$; n = 25), or 10 μg of each component ($PncT_{10}$; n = 25).

Trivalent oral live poliovirus (OPV) vaccine (Pasteur Mérieux Connaught, Lyon, France) was administered in Israel at 4, 6, and 12 months of age.

In addition, Israeli children received an intramuscular dose of a 23-valent pneumococcal nonconjugate vaccine (Pneumo-23; Pasteur Mérieux Connaught, Lyon, France) as a booster vaccination at 12 months of age.

Vaccination and sampling schedule. Infants were enrolled in the study at the age of 2 months (range, 6 to 10 weeks). They were examined by a physician at enrollment and by a physician or a public health nurse before each immunization.

In Israel, healthy infants were recruited from six Mother and Child Health Centers located in the cities of Beer-Sheva and Arad, in southern Israel. The total number of recruited infants was 75 (25 in the PncT group, 25 in the PncD group, and 25 in the placebo group). PncT (3 μg of polysaccharide for each serotype [$PncT_{03}$]), PncD (3 μg of polysaccharide for each serotype), or placebo was administered at 2, 4, and 6 months of age (Table 1). At each visit, all infants received a combined DTP–IPV–PRP-T injection at the contralateral site. To comply with national vaccination program requirements, OPV vaccine also was administered at 4 and 6 months. At 12 months of age, all children received an injection of pneumococcal polysaccharide vaccine simultaneously with the fourth dose of DTP–IPV–PRP-T and a third dose of OPV vaccine.

In the Finnish study, children were recruited from Child Health Centers in the towns of Joensuu, Kerava, and Tampere. They were divided into three groups: 75 received PncT vaccine (25 received 1 μg of polysaccharide of each serotype per vaccine dose [$PncT_{01}$], 25 received $PncT_{03}$, and 25 received a 10-μg dose of each polysaccharide [$PncT_{10}$]), 75 received PncD vaccine (25 each received 1, 3, or 10 μg of polysaccharide of each serotype), and 50 received placebo (since both PncD and placebo did not contain TT protein, they were both considered $PncT_0$, implying that no TT content except that in DTP was present). These vaccines were administered at 2, 4, and 6 months of age. At each visit, all infants received a combined DTP–PRP-T injection at the contralateral site (Table 1). In Finland, no PRP-T was given simultaneously with the booster dose of pneumococcal vaccine; therefore, the follow-up period for the present analysis ended by the age of 7 months.

The total TT content administered at each visit to infants in the various regimens in Finland and Israel is presented in Table 2.

Venous blood was obtained from all children at 2, 4, 6, and 7 months; additional samples in Israel were taken at 12 and 13 months. Sera were stored at −20°C until analyzed.

Serologic assays. Type-specific pneumococcal polysaccharide immunoglobulin G was assessed for each of the four serotypes in the conjugate vaccines. Detailed data are presented elsewhere (1, 8, 9). The anti-PRP antibody concentration was measured by a radioimmunoassay (25) in two different laboratories. The sera of the Israeli infants were tested at Pasteur Mérieux Connaught in France, and the sera of the Finnish infants were tested at Pasteur Mérieux Connaught in the United States.

Methods to measure TT and diphtheria toxoid antibody concentrations differed in the two studies. In Israel, tetanus antibody concentration was measured by a solid-phase enzyme-linked immunosorbent assay (EIA) with 0.11 Lf of purified TT per ml and compared to the international standard for tetanus immunoglobulins (TE.3; Statens Seruminstitut). The lower limit of quantification was 0.006 IU/ml. Diphtheria antibody concentration was measured by a solid-phase EIA with 0.13 Lf of purified diphtheria toxoid per well and compared to the equine international standard for diphtheria antitoxin (Statens Seruminstitut).

In Finland, a modified version of an indirect alkaline phosphatase EIA was used to assay the tetanus and diphtheria antitoxin immunoglobulin G concentrations (21). Duplicates of four serum dilutions, starting with the 1:100 dilution and progressing by dilution factors of five, were incubated in EIA plate wells coated with TT (0.5 Lf/ml) or diphtheria toxoid (2.5 Lf/ml) from the Vaccine Department at the National Public Health Institute. The A_{405} was measured with a Multiscan MS EIA reader (Labsystems, Helsinki, Finland), and the antibody concentrations were calculated with a reference line method (Unitcalc; Unisys, Stockholm, Sweden). An immunoglobulin was used as an in-house standard and was calibrated against the World Health Organization international standards for tetanus and diphtheria antitoxins. Tetanus and diphtheria antitoxin concentrations were thus expressed in international units per milliliter. The quantification limit was 0.03 IU/ml for both types of antibodies.

The assays were done in a randomized manner, and the laboratory technicians were blinded to the group to which the subject belonged.

Statistical analysis. In Israel, the anti-PRP response was analyzed 1 month after the third dose by use of analysis of variance (ANOVA) after logarithmic transformation for comparison of the three groups, and Dunnett's t test was used for the comparisons of PncT versus placebo and PncD versus placebo (11). In the Finnish study, the response was analyzed by use of ANOVA for the seven groups. Duncan's multiple-range procedure was used to classify the seven groups, in particular, PncT, PncD at 3 μg, and placebo (10).

P values of 0.05 were considered significant. Correlations between antibody response to PRP and TT or diphtheria toxoid were calculated with the Pearson coefficient.

RESULTS

Of the 75 infants recruited in Israel, 39 (52%) were males. The mean age at enrollment was 2.1 months; the mean ages at second and third injections were 4.0 and 6.1 months, respec-

TABLE 2. TT content administered at 2, 4, 6, and 12 months in Israel and 2, 4, and 6 months in Finland[a]

Regimen	TT content (μg) of vaccines administered at each encounter at mo:	
	2, 4, and 6	12
Israel		
$PncT_0$ (placebo and PncD groups)	54	54
$PncT_{03}$	78	54
Finland		
$PncT_0$ (placebo and PncD groups)	39	NR
$PncT_{01}$	48	NR
$PncT_{03}$	63	NR
$PncT_{10}$	111	NR

[a] The TT contents in individual vaccines were as follows: DTP in Israel, 30 μg; DTP in Finland, 15 μg; PRP-T, 24 μg; $PncT_{01}$, 9 μg; $PncT_{03}$, 24 μg; and $PncT_{10}$, 72 μg. NR, not relevant.

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TABLE 3. Anti-PRP and antitetanus antibody responses of infants receiving PncT, PncD, or placebo at 2, 4, and 6 months of age[a]

Country and mo (time related to dose)	Anti-PRP antibodies						Antitetanus antibodies					
	GMC (μg/ml)			% with concn ≥1.0 μg/ml			GMC (mIU/ml)			% with concn ≥1.0 μg/ml		
	PncT	PncD	Placebo	PncT	PncD	Placebo	PncT	PncD	Placebo	PncT	PncD	Placebo
Israel												
2 (pre)	0.17	0.36	0.20	13	28	8	0.08	0.12	0.21	0	13	4
4 (post 1)	0.18	0.23	0.30	8	14	26	0.06	0.07	0.09	5	0	0
6 (post 2)	0.86[b]	1.19[b]	2.95[b]	44	64	74	0.26	0.24	0.25	16	10	13
7 (post 3)	2.81[c]	4.62[c]	6.62[c]	83	91	96	1.85	2.08	1.75	61	74	71
Finland												
2 (pre)	0.07	0.08	0.12	0	4	4	0.65	0.60	0.54	33	28	22
4 (post 1)	0.09	0.09	0.11	0	4	6	0.31	0.29	0.26	8	8	1
6 (post 2)	0.93	1.64	1.51	56	68	67	0.60	0.67	0.50	32	28	22
7 (post 3)	7.18	12.50	11.0	92	100	96	3.49	3.99	4.27	96	96	96
Booster (Israel)												
12 (preboooster)	0.55[d]	1.44[d]	1.37[d]	24	64	71	0.36	0.42	0.24	12	9.5	0
13 (postbooster)	10.95[e]	20.13[e]	20.84[e]	96	100	100	4.37	6.94	4.95	100	100	96

[a] Each group contained 25 subjects, except for the Finnish placebo group, which contained 50 subjects.
[b] $P = 0.0085$ among the three groups.
[c] $P = 0.0218$ among the three groups.
[d] $P = 0.0052$ among the three groups.
[e] $P = 0.076$ among the three groups.

tively. Seventy-four of the 75 enrolled subjects (99%) completed the follow-up at 1 month after the third dose; 70 (93%) received the vaccination at 12 months of age and completed the follow-up at 13 months of age according to the protocol.

Two hundred infants were recruited in Finland; 102 (51%) of them were males. The mean age at enrollment was 2.5 months; the mean ages at second and third injections were 4.3 and 6.1 months, respectively. Ninety-nine percent (197 of 200) of the enrolled subjects completed the follow-up at 1 month after the third dose.

Effect of PncT on anti-PRP antibody responses. The anti-PRP antibody response was significantly impaired when PRP-T was administered simultaneously with DTP and the tetravalent PncT (Table 3). In Israel, the geometric mean concentrations (GMC) of anti-PRP antibodies in the PncT group were 0.86 μg/ml after the second dose and 2.81 μg/ml after the third dose, versus 1.19 and 4.62 μg/ml in the PncD group and 2.95 and 6.62 μg/ml in the placebo group, respectively (ANOVA for the three groups: $P = 0.0085$ after the second dose and $P = 0.0218$ after the third dose). Dunnett's t test revealed significant differences between PncT and placebo but not between PncD and placebo or between PncD and PncT.

The same trend was observed in Finland with the 3-μg dose. After the second and third doses, the GMC of anti-PRP antibodies were 0.93 and 7.18 μg/ml, respectively, in the PncT group, versus 1.64 and 12.50 μg/ml in the PncD group and 1.51 and 11.0 μg/ml, respectively, in the placebo group (nonetheless, the differences between the groups did not reach statistical significance).

When the proportions of infants with anti-PRP antibody levels of ≥1.0 μg/ml were compared, the same tendency toward a lower proportion in the PncT group (83% in Israel and 92% in Finland after the third dose) than in the PncD group (91 and 100%, respectively) or the placebo group (96% in both sites) was found.

In Israel, infants were vaccinated with DTP–IPV–PRP-T and pneumococcal polysaccharide vaccine at 12 months of age. Before this booster vaccination, both the GMC and the proportion of children having antibody levels of ≥1.0 μg/ml were significantly lower in the PncT group than in the PncD or placebo group (Table 3). One month after the booster, although all children but one had anti-PRP antibody concentrations of ≥1.0 μg/ml, the GMC in the PncT group was still only about half (10.95 μg/ml) that in the PncD (20.13 μg/ml) or placebo (20.84 μg/ml) group (ANOVA for the three groups: $P = 0.076$).

Dose effect of pneumococcal conjugate vaccines on anti-PRP antibody responses. To examine whether the dosage of PncT could influence the responses to PRP and TT, we compared the antibody responses of four groups in Finland: $PncT_0$ (placebo), $PncT_{01}$ (1 μg of polysaccharide of each of the four serotypes included in the tetravalent vaccine), $PncT_{03}$ (3 μg of polysaccharide of each serotype), and $PncT_{10}$ (10 μg of polysaccharide of each serotype). Thus, the total TT protein loads for the vaccines administered at 2, 4, and 6 months (simultaneously with DPT–PRP-T) were 39, 48, 63, and 111 μg for $PncT_0$ (placebo), $PncT_{01}$, $PncT_{03}$, and $PncT_{10}$, respectively (Table 2).

The concentrations of the anti-PRP antibodies elicited by these vaccines gradually decreased with increasing TT load (Fig. 1). After the third dose, the GMC of anti-PRP antibodies were 11.0, 10.1, 7.2, and 4.1 μg/ml for $PncT_0$, $PncT_{01}$, $PncT_{03}$, and $PncT_{10}$, respectively (ANOVA for the four groups: $P = 0.0121$). The same trend was seen even after the second dose (GMC of 1.5, 1.2, 0.9, and 0.6 μg/ml for $PncT_0$, $PncT_{01}$, $PncT_{03}$, and $PncT_{10}$, respectively; data not shown). In contrast, anti-PRP antibody concentrations did not decrease significantly with increasing PncD concentration. After the third dose, the GMC of anti-PRP antibodies were 11.0, 11.5, 12.5, and 7.8 μg/ml for $PncD_0$, $PncD_{01}$, $PncD_{03}$, and $PncD_{10}$, respectively.

Similarly, a trend toward reduced proportions of infants with anti-PRP antibody levels of ≥1.0 μg/ml was seen with increasing PncT concentration (96, 96, 92, and 80% for $PncT_0$, $PncT_{01}$, $PncT_{03}$, and $PncT_{10}$, respectively). Such a trend was not seen with PncD (with which the respective proportions were 96, 92, 100, and 92%).

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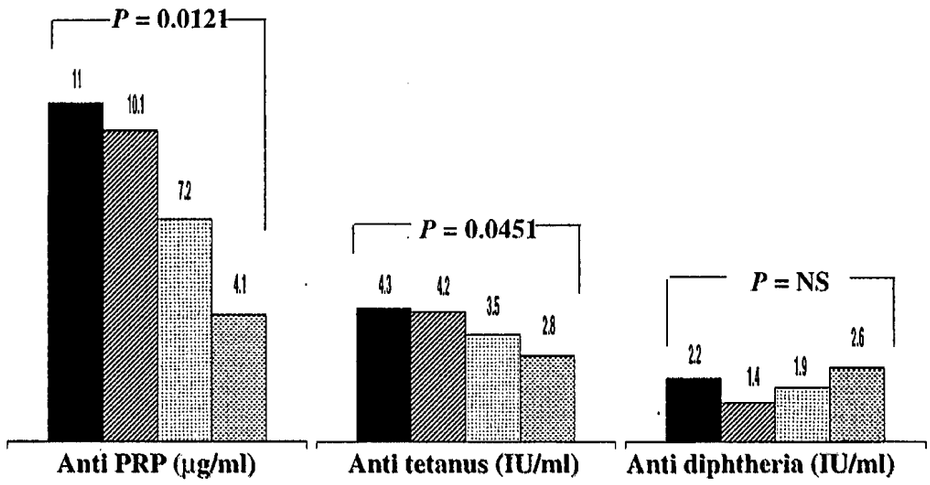

FIG. 1. Anti-PRP, antitetanus, and anti-diphtheria toxoid antibodies after the third injection of one of the various doses of PncT: placebo ($PncT_0$) (■); tetravalent PncT vaccine, 1 μg of each polysaccharide ($PncT_{01}$) (▨); tetravalent PncT vaccine, 3 μg of each polysaccharide ($PncT_{03}$) (▣); and tetravalent PncT vaccine, 10 μg of each polysaccharide ($PncT_{10}$) (▩). NS, not significant.

Effect of PncT on antitetanus antibody responses. Although there were no significant differences in antitetanus antibody concentrations among the three study groups with regard to primary response or response to booster in either country when the 3-μg dose was used (Table 3), a significant trend toward a decrease was found with increasing doses of PncT in Finland (GMC of 4.3, 4.2, 3.5, and 2.8 IU for $PncT_0$, $PncT_{01}$, $PncT_{03}$, and $PncT_{10}$, respectively; $P = 0.045$, as determined by ANOVA for the four groups) (Fig. 1).

Since an interference effect of PncT was found for anti-PRP antibodies in infants immunized with PRP-T and DTP and a similar trend was found for antitetanus antibodies, we examined whether a correlation between anti-PRP and antitetanus antibody responses was present in the 200 Finnish vaccine recipients. A correlation was indeed found between anti-PRP and antitetanus antibody concentrations in each conjugate group (Table 4). In other words, the subjects who had low anti-PRP antibody concentrations tended to be the same ones who also had low antitetanus antibody concentrations. This linkage was not found with the anti-PRP antibody response and the anti-diphtheria toxoid antibody response.

DISCUSSION

New vaccines against encapsulated bacteria are based on the chemical coupling of capsular oligo- or polysaccharides to carrier proteins. For practical reasons, the future of vaccination schedules will require the simultaneous administration of these various conjugates. So far, only a few protein carriers have been used for the preparation of these vaccines. Therefore, the addition to the routinely used Hib vaccine of conjugate vaccines against pathogens such as *Neisseria meningitidis* and *S. pneumoniae* could require the simultaneous administration of several polysaccharides conjugated to the same protein carrier. It is thus of primary importance to analyze possible interference between conjugates that share the same carrier moiety. In the present study, such an issue was addressed for infants who received either the DTP or the PRP-T vaccine alone or together with a vaccine containing pneumococcal polysaccharide antigens conjugated to TT or diphtheria toxoid. Our results clearly showed that the anti-PRP antibody response decreased significantly when the PRP-T conjugate was administered together with DTP and the tetravalent PncT conjugates. This decrease was dose dependent and also affected the antitetanus antibody response. Notably, this suppression was carrier specific, since it was more accentuated with increasing load of TT and was not observed after simultaneous administration of PRP-T with pneumococcal polysaccharide conjugated to diphtheria toxoid. This last finding excludes the possibility that the suppression was due to some immunosuppressive properties of the pneumococcal polysaccharides.

Additional support for the suggestion that TT overload interferes with the anti-PRP antibody response can be derived from the finding that when $PncT_{03}$ (tetravalent PncT vaccine with 3 μg of polysaccharide of each serotype) was administered to Israeli and Finnish infants, the interference with the anti-PRP antibody response was more accentuated in the Israeli group (Israeli DTP contains twice the concentration of TT as Finnish DTP).

Several mechanisms can be proposed. First, the decrease in the anti-PRP antibody response seems to be related to the load of TT injected as free or conjugate proteins: the largest decrease in the anti-PRP antibody response was observed with the highest dose of PncT conjugates. The load of TT injected seems to affect the antitetanus antibody response as well, since

TABLE 4. Correlation between antibody responses to PRP and TT or diphtheria toxoid among Finnish infants receiving PncT, PncD, or placebo

Correlation of:	PncT group (n = 75)		PncD group (n = 75)		Placebo group (n = 50)	
	r	P	r	P	r	P
Anti-PRP and antitetanus antibodies	0.395	0.0005	0.638	≤0.0001	0.608	≤0.0001
Anti-PRP and anti-diphtheria toxoid antibodies	−0.015	0.897	0.231	0.046	0.137	0.347

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a correlation between anti-PRP antibody and antitetanus antibody concentrations was found (in other words, vaccinees who tended to have lower anti-PRP antibody levels also had lower antitetanus antibody levels).

Second, previous studies indicated that the development of an immune response against the carrier can interfere with the response to saccharide-protein vaccines (3–5, 13, 23). Preimmunization with the protein carrier can reduce antibody responses both to the carrier and to Hib polysaccharide epitopes (5). Such a phenomenon may be related to the epitopic suppression initially described with 2,4,6-trinitrobenzenesulfonic acid hapten (TNP) or 2,4,6-dinitrophenyl hapten (DNP) (15, 16) or peptide epitopes (26). This suppression is mediated by the clonal dominance of B cells specific for carrier epitopes (20, 27). Although these conclusions were obtained with carrier-primed animals, we can now propose that similar mechanisms control the responses to simultaneously administered conjugates.

Whereas optimal doses of TT will rapidly stimulate the activation and proliferation of carrier-specific B-cell clones that will then efficiently take up the conjugate and present it to T cells, polysaccharide-specific B-cell clones are certainly much less frequent and will never reach the same frequency as carrier-specific B-cell clones. Thus, the extent of the decrease in the response against the polysaccharide will be proportional to the intensity of the immune response to the carrier (i.e., dependent upon its immunogenicity and on the dose) and will be more pronounced with saccharide epitopes for which a low frequency of B-cell precursors exists. This reasoning could explain the results of a recent study where an overload of homologous protein carrier at the site of injection reduced the immunogenicity in mice of two experimental conjugates (12). Indeed, a 12-valent conjugate *Escherichia coli* vaccine and an 8-valent *Staphylococcus aureus* vaccine consisting of polysaccharide conjugated to protein induced significantly lower antibody concentrations than each monovalent conjugate administered separately. A similar reduction in immunogenicity was seen when free protein carrier was injected together with the conjugate vaccine.

Further support for the theory of carrier suppression can be found in a recently presented work by Greenberg et al. (14). The authors coadministered to infants a heptavalent pneumococcal conjugate vaccine (which was conjugated to an outer membrane protein of *N. meningitidis* group B [OMPC]) with either PRP conjugated to OMPC (PRP-OMPC) or PRP conjugated to a mutant diphtheria toxin polypeptide (PRP-CRM) at 2, 4, and 6 months. After the primary immunization series (at 7 months), anti-pneumococcal polysaccharide antibody concentrations to some serotypes were significantly lower in infants receiving PRP-OMPC (sharing the same carrier as the heptavalent pneumococcal vaccine) than in those receiving PRP-CRM.

We cannot rule out the possibility that the mechanism of epitopic suppression based on the dominance of carrier-specific B cells played at least in part a role in the currently described phenomenon. We believe, however, that its role, if any, is only minor, based on the finding that the decrease in the anti-PRP antibody response was linked to the decrease in the antitetanus antibody response.

Third, an elevated concentration of antibodies to the protein carrier could also interfere with conjugate immunogenicity through antigen capture or modulation of antigen presentation. Barington et al. (3) showed that maternal antibodies to TT inhibited the response of human infants to the polysaccharide moiety of Hib conjugate vaccine. However, such an effect was not seen by others (19).

Fourth, the finding that the injection of high doses of PncT affected both anti-carrier and anti-PRP antibody responses may reflect inadequate T-helper-cell activity. Several recent studies have indicated that the attachment of oligosaccharides to peptides profoundly affects binding to major histocompatibility complex class II molecules and peptide immunogenicity (22). Moreover, it has been shown that T cells specifically recognize synthetic glycopeptides (18). It could therefore be suggested that TT modified by conjugation to Pnc stimulates a T-cell repertoire that does not cross-react with TT-specific T cells. Since both PncT and PRP-T conjugates would be processed and presented by TT-specific B cells, the processing of these two antigens could lead to different glycopeptides that may compete for major histocompatibility complex class II binding.

Although the present study indicates that the simultaneous administration of PncT and PRP-T conjugates may decrease the anti-PRP antibody response, the levels of anti-PRP antibodies induced by such combined vaccines still exceeded the concentrations needed for protection against invasive Hib disease. In addition, clear evidence for the induction of memory toward the polysaccharide antigens was obtained. While the present study analyzed the interference between PRP-T and a tetravalent pneumococcal conjugate vaccine, the licensed pneumococcal vaccine will most probably contain more than seven pneumococcal serotypes. This increase in the amount of TT used for immunization could potentially reduce the anti-PRP or even the antitetanus antibody response to an unacceptable level. Thus, although the safety and immunogenicity of TT make it suitable as a protein carrier for human vaccines, increasing its quantity in multicomponent vaccines might affect the efficiency of such vaccines. Restriction of the content of the protein carrier in conjugate vaccines is desirable, as is the development of other protein or nonprotein carriers for saccharide conjugate vaccines.

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Non-interference between two protein carriers when used with the same polysaccharide for pneumococcal conjugate vaccines in 2-year-old children

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Abstract

The carriers tetanus toxoid (T) and diphtheria CRM-197 (C) were compared in conjugate vaccines using identical coupling chemistry and polysaccharide (PS) loading, for safety and immunogenicity in 2-year-old children. Also tested were a mixture of halved doses of both carriers bearing the same PS serotypes. For this study, PS types 6A, 14, 19F, and 23F (separately) were coupled to T or C by reductive amination at PS/protein ratios of 0.50 ± 0.18. With each carrier the four PS types were combined, giving the tetravalent vaccines "T-6, -14, -19, -23" or "C-6, -14, -19, -23" containing 50 μg of the carrier and roughly 20 μg total PS per ml of saline (no adjuvant). The children received primary (1′) injections of 50 μg (protein) of either vaccine or a mixture of 25 μg of both; identical secondary (2′) injections were given 2 months afterwards. Sera were taken before the 1′ and 2′ and 1 month post-2′, and serum IgG responses to the four PS were determined by ELISA.

For geometric mean (GM) post-1′ antibody, "C-6, -14, -19, -23" exceeded "T-6, -14, -19, -23" for type 19F; for 2′ antibody, "T-6, -14, -19, -23" exceeded "C-6, -14, -19, -23" for type 14, but "C-6, -14, -19, -23" and the T/C mixture exceeded "T-6, -14, -19, -23" for the type 23F response. No other differences were significant. Analyzed by individual fold-rises, "C-6, -14, -19, -23" and the T/C mixture exceeded "T-6, -14, -19, -23" for types 19F and 23F. Thus, there was no consistent difference between the T and C carriers; rather, the results differed by serotype.

When a mixture of halved doses of "T-6, -14, -19, -23" and "C-6, -14, -19, -23" was injected, neither negative nor positive interference with the PS antibody responses was found. Anticipating multivalent PS conjugate vaccines of the future to be used in infancy, this strategy would have two hypothetical advantages worth further investigation—avoiding "carrier epitopic overload" by reducing each carrier dosage and recruiting T-helper activity by both carriers for each PS.
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Keywords: Pneumococcal conjugate vaccines; Protein carriers; Vaccine interference; Carrier epitopic overload

1. Introduction

Antibody (Ab) to the capsular polysaccharide (PS) is protective against some invasive bacterial species, but PS vaccines generally fail to immunize the youngest and most susceptible subjects. Protein–PS conjugate vaccines were licensed in the early 1990s for prevention of infections in infancy by *Haemophilus influenzae* type b (Hib). Several constructs differing in carrier proteins, sizes and ratios of PS component, and coupling chemistries were used, on a somewhat arbitrary basis, but there was little systematic study of the effect of these variables on human immunogenicity [1]. The commonly-used carrier proteins, tetanus toxoid (T) and the diphtheria toxin mutant protein CRM-197 (C), were never compared using identical Hib PS coupling. It seemed opportune to make such a comparison with pneumococcal PS, since at the time of this study no conjugate vaccine had yet been licensed.

A heptavalent C-based pneumococcal conjugate was approved in February 2000. This construct showed no apparent negative interference among the seven PSs presented on the carrier. However, additional PS serotypes are likely to be used in future pneumococcal conjugates [2], and these might be combined with Hib and/or meningococcal conjugate vaccines; thus interference must be watched for as the number of components is increased. One possibility already observed is that if the same carrier is used for too many/much PS haptens, there may be interference by carrier "epitopic

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overload" [3–5]. One proposed solution is the simultaneous use of two or more carriers, with some PS serotypes on one and some on another [5,6]. In the present study, along with our comparison of T- and C-based conjugates, we have explored the alternative of mixing halved doses of the same PSs on both carriers, which likewise halves the individual carrier dosage for any given total PS dosage. (This approach was also described in a recent abstract; [7]). The strategy has an additional hypothetical advantage: if carrier-specific T-cell help is the limiting factor for the response to a particular PS epitope, then presentation of the epitope on more than one carrier might increase the available T-helper activity. Thus, there are two mechanisms that might produce positive interference, i.e. PS Ab responses greater than from the same PS on a single carrier. However, by mechanisms unknown, there might instead be negative interference, a possibility that should be explored before testing in infancy.

For the present study, PS serotypes 6A, 14, 19F, and 23F were coupled (separately) to T and to C carriers by periodate activation and reductive amination, at PS/protein mass ratios of 0.5 ± 0.18. Within each carrier the four serotypes were combined, giving the tetravalent vaccines "T-6, -14, -19, -23" and "C-6, -14, -19, -23". After preliminary testing in mice and adult humans, the vaccines were tested for safety and immunogenicity in 2-year-old children—given separately for comparison or given as a mixture of halved doses, mainly to test for negative interference. To examine where on the dose–response curve the experiment was being conducted, a four-fold range in dosage was examined with a trivalent vaccine made with the C carrier "C-6, -14, -19".

2. Materials and methods

Tetanus toxoid, lot LP501P, from the Massachusetts Public Health Department was further purified by ion exchange chromatography on DEAE Trisacryl (LKB, Inc.) as described [8]; it eluted on Sepharose CL-4B (Pharmacia, Inc.) in a peak with K_d 0.63. CRM-197, a gift of Lederle–Praxis Biologicals, was prepared as described [9]; its peak K_d on Sepharose CL-4B was 0.75.

Pneumococcal PSs for the vaccine preparations were produced by Eli Lilly & Co. Typing was confirmed by Ouchterlony gel diffusion. The PSs were assayed for hexose content by the anthrone reaction [10] standardized by mixtures of D-hexoses corresponding to the known structures of the PSs [11]. For each PS the amount of $NaIO_4$ required to generate a specified proportion of reducing groups was empirically determined under the conditions described below.

For preparation of vaccines, sterile pyrogen-free water was used for all procedures. To generate reducing groups the PSs were dissolved in water at 10 mg/ml, and $NaIO_4$ as specified (Table 1) was added in a negligible volume of water. The reaction mixtures were held at 37 °C in the dark for 100 min then dialyzed for 2 days at 4 °C versus 15 mM NaCl. Recovery of PS was determined by hexose assay and the generation of aldehyde groups was determined by the increase in reducing activity as assayed by the Park–Johnson method [10] standardized by D-glucose. The molecular size profiles of small samples of the oxidized PSs were determined on Sepharose CL-4B.

Table 1
Pneumococcal polysaccharides (PS) activated by periodate oxidation: molecular size before and after coupling to protein carriers

Serotype	$NaIO_4$ used per mg of PS (μmol)	Reducing activity per μmol of hexose (μmol)	K_d on Sepharose CL-4B	
			PS	Coupled PS
6A	0.19	0.036	0.25	0.10
14	0.48	0.072	0.50	0.20
19F	0.37	0.030	0.75	0.28
23F	0.20	0.034	0.50	0.20

For coupling, the proteins and the oxidized PS solutions were adjusted to pH 8.0 with K_2HPO_4, mixed at concentrations of 1 mg/ml each and incubated 18 h at 25 °C; 1/200 volume of sodium cyanoborohydride dissolved in water at 8 mmol/ml was then added, and the reaction mixtures were incubated 48 h at 37 °C. To "cap" any unreacted aldehyde groups, sodium borohydride was added at 2 μmol/ml, and the incubation at 37 °C was continued 1 h. To separate conjugates from uncoupled protein and PS and from borohydride, the solutions were applied to Sepharose CL-4B columns and eluted with 0.15 M NaCl; fractions were assayed for hexose and protein, the latter by the Lowry method [10] standardized with bovine albumin. Early-eluting fractions (Table 1) containing hexose and protein were pooled, diluted with 0.15 M NaCl to a concentration of 125 μg protein/ml and drawn through 0.2-μm sterilizing filters (GV type, Millipore Corp.) into sterile evacuated containers. Samples of the individual conjugates were combined and diluted with 0.15 M NaCl as necessary to give tetravalent or trivalent solutions (Table 2). Filter-sterilized U.S.P. thimerosal was added at 0.1 mg/ml, and aliquots were dispensed into type 1 borosilicate glass injection vials, which were stored upright at −80 °C. Tests for sterility, safety and identity were as specified by the US Food and Drug Administration (FDA) [12]; identity tests were by ELISA-inhibition, showing that the specified tetanus, diphtheria, and pneumococcal antigenicities were present.

Pneumococcal PS types 14, 19F, and 23F for ELISA were obtained from the American Type Culture Collection; Eli Lilly and Co. PS was used for the type 6A. ELISA for human serum IgG Ab to the PS was as described [13]. ELISA

Table 2
Composition (μg/ml) of the three multivalent conjugate vaccines

Vaccine	Total protein	Total hexose	Hexose of specific serotype			
			6A	14	19F	23F
T-6, -14, -19, -23	50.3	24.1	6.8	6.9	5.3	5.1
C-6, -14, -19, -23	52.8	22.4	6.7	5.2	5.0	5.5
C-6, -14, -19	101	41.0	15.8	12.7	12.5	None

for mouse serum Ab was done by the same method except for the use of an anti-murine Ig conjugate as the secondary reagent. Murine immunogenicity was tested in weanling female Swiss–Webster (outbred) animals given primary (1′) i.p. injections of saline—0.01% thimerosal diluent, 1 μg of the four unconjugated Eli Lilly and Co. PSs, or tetravalent conjugates at a dose supplying about 1 μg each of the four coupled PSs. Identical secondary (2′) injections were given after 4 weeks, and sera were taken for Ab assay after 6 weeks. Types 6A, 19F, and 23F PSs gave titers no higher than diluent alone, while type 14 PS gave a modest rise; in contrast, both tetravalent conjugates gave titers substantially above the diluent and PS controls for all four serotypes.

Safety and immunogenicity was tested in healthy, university-affiliated adult volunteers who had had no previous pneumococcal vaccine, no tetanus nor diphtheria vaccines within 5 years, nor history of severe allergic reactions to injected biologicals. After written informed consent in accord with the Research Subjects Review Board (RSRB), groups of six volunteers received single intramuscular injections of "T-6, -14, -19, -23"; "C-6, -14, -19, -23"; or "C-6, -14, -19"; the doses were 50 μg protein and about 20 μg total PS (cf. Table 2). They were instructed to record temperatures and any adverse signs or symptoms within 3 days of injection. Sera for Ab assay were taken before and 4 weeks after injection. Mild or moderate local inflammation was reported by about half the subjects and were the expected reactions from an injection of protein toxoids in adults [14]. PS Ab to all four serotypes was measurable in all subjects before immunization, and there were rises to all the serotypes contained in the vaccines. Details of the signs and symptoms and Ab responses were reported to the RSRB and FDA along with the plan described below to test the vaccines in children.

Healthy 2-year-old children were recruited from a private pediatric practice (Elmwood Pediatric Group, Rochester, NY). A parent gave written informed consent in accord with the RSRB. The children had received all scheduled routine immunizations with no severe reactions but had had no experimental vaccines nor pneumococcal vaccine. They were assigned in order of enrollment to one of five experimental groups to receive injections as follows: (a) "T-6, -14, -19, -23"; 50 μg total protein in 1 ml, (b) "C-6, -14, -19, -23"; 50 μg in 1 ml, or (c) a mixture of the T and C conjugates, 25 μg each in 1 ml total. (These groups thus compared the two carriers at a single moderate dosage with a mixture of half doses of both). Group d received 25 μg of "C-6, -14, -19" in 0.25 ml and Group e received 100 μg in 1 ml. (Limitation of materials unfortunately restricted this dosage titration to three serotypes with the C carrier). Injections were given intramuscularly in the thigh. The 1′ vaccination was done at age 24 ± 1 months and 2′ approximately 2 months afterwards. Parents were instructed to record rectal temperatures and adverse signs and symptoms within 4 days of each injection on furnished report forms; compliance was reinforced by daily telephone contact. Sera were taken before both injections and 1 month after the 2′; 11–16 children per group began and 10–13 completed the protocol.

Natural logarithms of ELISA values were used for statistical calculations. A 95% confidence interval (CI) for the geometric mean (GM) was calculated as the interval between $\exp(x - ts/n)$ and $\exp(x + ts/n)$, where x is the mean and s is the standard deviation of the logs of the values and t is the Student's t corresponding to $P = 0.05$ for n values (e.g. 2.303 for $n = 10$).

3. Results

In the children, in 4 days of monitoring (Table 3), erythema of any degree was observed in 19%, any swelling in 14%, and any pain in 46% of the 122 injection sites, all of mild or moderate and none of severe degree. Maximum rectal temperatures of ≥101 °F were reported after 9% of injections; the highest was 103.6 °F, found in one observation in a child experiencing an intercurrent respiratory infection. Any form of unusual behavior indicating upset (excessive sleeping, fretfulness, crying, anorexia, vomiting, or screaming) was reported after 43% of injections; in no child was the behavior severe or sufficiently intense to cause the parents to contact their pediatrician. There was no significant difference in reactogenicity among the conjugate constructs nor between 1′ and 2′ injections. With the trivalent mixture "C-6, -14, -19", the 100-μg dose trended toward more local inflammation than the 25-μg dose, which may be due in part to the larger volume (1 ml versus 0.25 ml). Details of the signs and symptoms were reported to RSRB and FDA.

Geometric means of IgG Ab to PSs in the children are shown in Table 4. In all the groups there were rises in the GM of Abs to all the component PSs between the prevaccination and the post-1′ sera. In respect to differences among post-1′ sera, the GM for serotype 19F of the "C-6, -14, -19, -23" group exceeded that of "T-6, -14, -19, -23". No other GM differences among the groups were significant. There were larger rises between post-1′ and the post-2′ sera. Among the groups, the 2′ GM for serotype 14 of the "T-6, -14, -19, -23" group exceeded that of "C-6, -14, -19, -23", while the GM for serotype 23F of the "C-6, -14, -19, -23" and the T/C mixture groups exceeded that of "T-6, -14, -19, -23". The dose–response relation of three of the C conjugates was examined: only for serotype 14 did the 2′ GM produced by the 100-μg dose of "C-6, -14, -19" exceed that of the 25-μg dose; thus for serotypes 6A and 19F the studies above were being conducted on a dose–response plateau.

Individual rises of ≥4× are shown in Table 5. In 1′ rises, "C-6, -14, -19, -23" and the T/C mixture exceeded "T-6, -14, -19, -23" for serotype 19F, and "C-6, -14, -19, -23" exceeded "T-6, -14, -19, -23" for serotype 23F. In ≥4× rises produced by the combined 1′ and 2′ vaccinations, "C-6, -14, -19, -23" and the T/C mixture exceeded "T-6, -14, -19, -23" for serotype 23F. No other differences in the proportion of ≥4× rises were significant.

Table 3
Adverse signs and symptoms in children within 4 days of vaccination

Vaccine injected[a]	Injection[b]	Number observed/number of injections				Upset behavior[c]
		Erythema	Swelling	Pain	T_{max} >101 °F[d]	
T-6, -14, -19, -23	1′	2/12	1/12	4/12	0/12	7/12
	2′	2/10	1/10	5/10	2/10	5/10
C-6, -14, -19, -23	1′	0/11	0/11	5/11	0/11	6/11
	2′	2/9	0/9	5/9	2/9	4/9
T/C mixture[e]	1′	4/14	2/14	7/14	2/14	4/14
	2′	1/10	0/10	4/10	0/10	3/10
C-6, -14, -19[f]	1′	3/11	2/11	4/11	1/11	7/11
	2′	1/8	1/8	4/8	1/8	4/8
C-6, -14, -19[g]	1′	5/14	6/14	9/14	1/14	6/14
	2′	2/12	4/12	7/12	2/12	7/12

[a] 50 μg total protein in 1 ml saline except for T/C mixture and "C-6, -14, -19".
[b] 1′, primary; 2′, secondary.
[c] As observed by parents—any unusual degree of sleep, fretfulness, crying anorexia, vomiting, or screaming.
[d] Maximum rectal temperature observed in three daily measurements.
[e] 25 μg of the two previously described vaccines, combined just before injection.
[f] 25 μg in 0.25 ml.
[g] 100 μg in 1 ml.

Table 4
Geometric means of IgG antibodies to pneumococcal polysaccharides (μg/ml) in 2-year-old children before (Pre) and after primary (1′) and secondary (2′) injections

Vaccine injected[a]	N	Serotype 6A			Serotype 14			Serotype 19F			Serotype 23F		
		Pre	1′	2′	Pre	1′	2′	Pre	1′	2′	Pre	1′	2′
T-6, -14, -19, -23	10	0.5	1.2	4.3	0.8	1.9	16[b]	1.2	2.5[b]	8.6	0.5	1.0	1.2[b]
C-6, -14, -19, -23	11	0.9	1.7	2.9	0.4	1.4	5.3[b]	0.4	5.4[b]	11	0.3	1.9	4.8[b]
T/C mixture[c]	13	0.4	1.6	4.8	0.6	2.3	12	0.6	5.0	10	0.4	1.5	3.0[b]
C-6, -14, -19[d]	11	0.3	0.9	2.6	0.4	1.6	5.8[b]	0.9	7.2	16	0.4[e]	0.9[e]	0.9
C-6, -14, -19[f]	11	0.3	1.0	3.0	0.5	1.9	12[b]	0.3	4.7	10	0.3[e]	0.4[e]	0.5[e]

[a] 50 μg total protein in 1 ml except for "C-6, -14, -19".
[b] Pairs different by Student's t-test at $P < 0.05$.
[c] 25 μg of the two previously described vaccines, combined immediately before injection.
[d] 25 μg in 0.25 ml.
[e] Sequential rises not significant (no 23F antigen in the vaccine).
[f] 100 μg in 1 ml.

Table 5
Proportions of children with antibody rises of ≥4× to the primary (1′) or the combined primary plus secondary (1′ + 2′) vaccinations

Vaccine injected[a]	Serotype 6A		Serotype 14		Serotype 19F		Serotype 23F	
	1′	1′ + 2′	1′	1′ + 2′	1′	1′ + 2′	1′	1′ + 2′
T-6, -14, -19, -23	4/10	7/10	4/10	10/10	2/10[b]	6/10	2/10[b]	3/10[b]
C-6, -14, -19, -23	8/12	9/11	4/12	11/11	10/12[b]	11/11	9/12[b]	11/11[b]
T/C mixture[c]	7/13	12/13	7/13	11/13	12/13[b]	12/13	8/13	12/13[b]
C-6, -14, -19[d]	3/11	8/11	4/11	8/11	8/11	8/11	1/11[e]	2/11[e]
C-6, -14, -19[f]	5/13	9/11	4/13	11/11	10/13	11/11	1/13[e]	1/11[e]

[a] 50 μg total protein in 1 ml saline except for "C-6, -14, -19".
[b] Differences significant at $P < 0.05$ by χ^2-test with Yates correction.
[c] 25 μg each of the two previous vaccines mixed immediately before injection.
[d] 25 μg in 0.25 ml.
[e] No 23F antigen in the vaccine.
[f] 100 μg in 1 ml.

4. Discussion

Infants are the major target population for pneumococcal conjugate vaccines. For safety, our RSRB requires preliminary safety testing in adults and then children, which we have done here. All the adults had Ab responses to all the PS components; however, such responses are influenced by extensive but varied natural priming experience and thus are not detailed here. In the 2-year-old subjects, both our T- and C-based conjugates induced modest primary and larger secondary responses to all four pneumococcal PSs. Within the limitation of the number of subjects studied, there were no consistent differences in potency between the T and C carriers; rather, there were some serotype-specific differences, i.e. C exceeded T for serotypes 19F and 23F, T exceeded C for serotype 14, and there was no significant difference for serotype 6A. The similarity in immunogenicity may in part be due to the fact that the children had already received four injections of tetanus and diphtheria toxoids and thus were well-primed for both carriers.

Interestingly, however, similar results in infants as well were reported in an abstract: tetanus and conventional diphtheria toxoid were more, less, or equally potent as carriers depending upon the pneumococcal serotype [15].

The mixture of half-doses of our T and C conjugates was studied primarily to rule out negative interference. Indeed, with none of the PS serotypes was the mixture of halved doses less effective than the full dose of either conjugate given alone. Positive interference (a response significantly greater than of both conjugates given alone) was also not observed; but, with the limitations of this study, positive interference was not expected, for two reasons. Halving the dosage of each carrier hypothetically would improve the PS responses if carrier overload were operative; however, at carrier dosages of only 50 μg, overload might not be expected [4]. Hypothetically also, if the limiting factor for a particular anti-PS Ab response were carrier-specific T-helper cell activity, presentation of the PS simultaneously on two carriers might give an additive response. As mentioned however, the 2-year-old subjects are already highly primed for both carriers; moreover, they have had an unknown and variable priming from possible carriage of pneumococci. They thus are of little value for testing the hypothesis. We would like to test the strategy in 2-month-old infants in which carrier-specific T-helper activity is more likely to be limiting. The observed lack of negative interference increases our confidence that mixtures of our conjugates could be ethically studied in infancy. A study of this kind in infants, using T and conventional diphtheria toxoid as carriers, was described in a recent abstract [7]: there appeared no negative nor positive effect of the mixture in respect to mean titers for the four studied pneumococcal serotypes; however, statistical details of the responses were not given. Nonetheless, the strategy may be worth further evaluation. If antigen-presenting cells for a particular PS could elicit T-helper activity specific for both carriers, two measures of the benefit might be found: in a population, the mean PS Ab response might be additive compared to the PS being presented singly on either carrier; but, demonstrating a significant effect would require a very large study, ideally conducted at the dose–response maxima for each of the components. However, among individuals heterogeneous in T-helper cell responsiveness, an infant with a poor response to one carrier might respond well to the other; thus a benefit of the mixing strategy might be to produce a more consistent response, which would be seen as lower variance or a higher proportion of infants above a protective titer.

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Immunogenicity and Safety of 13-Valent Pneumococcal Conjugate Vaccine in Infants and Toddlers

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KEY WORDS
safety, immune response, pneumococcal conjugate vaccine, infants, toddlers

ABBREVIATIONS
PCV7—7-valent pneumococcal conjugate vaccine
IPD—invasive pneumococcal disease
WHO—World Health Organization
PCV13—13-valent pneumococcal conjugate vaccine
AE—adverse event
IgG—immunoglobulin G
ELISA—enzyme-linked immunosorbent assay
OPA—opsonophagocytic assay
GMC—geometric mean concentration
GMT—geometric mean titer
CI—confidence interval

This trial has been registered at www.clinicaltrials.gov (identifier NCT00373958).

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WHAT'S KNOWN ON THIS SUBJECT: To expand coverage of PCV7, a 13-valent vaccine with serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F plus 1, 3, 5, 6A, 7F, and 19A has been approved in the United States and elsewhere for routine pediatric immunization.

WHAT THIS STUDY ADDS: These study results reveal that PCV13 was as effective as PCV7 in the prevention of disease caused by the 7 common serotypes and could provide expanded protection against the 6 additional serotypes with a comparable safety profile.

abstract

BACKGROUND: 7-Valent pneumococcal conjugate vaccine (PCV7 [Prevnar, Wyeth Pharmaceuticals Inc, Philadelphia, PA], serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F) is effective in preventing vaccine-serotype pneumococcal disease. 13-Valent pneumococcal conjugate vaccine (PCV13) (PCV7 serotypes plus 1, 3, 5, 6A, 7F, and 19A) was designed to provide broader pneumococcal disease coverage. We evaluated the immunogenicity and safety of PCV13 compared with PCV7.

METHODS: Infants received PCV13 or PCV7 at ages 2, 4, 6, and 12 to 15 months with routine pediatric vaccinations. Pneumococcal anticapsular polysaccharide-binding immunoglobulin G responses and functional antipneumococcal opsonophagocytic activity were assessed 1 month after dose 3, before the toddler dose, and 1 month after the toddler dose. Safety and tolerability were also assessed.

RESULTS: For the 7 common serotypes, PCV13-elicited immunoglobulin G titers were noninferior to those elicited by PCV7, although PCV13 responses were generally somewhat lower. PCV13 also elicited functional opsonophagocytic activity comparable with that elicited by PCV7. For the 6 additional serotypes in PCV13, PCV13 elicited binding and functional antibody levels notably greater than those in PCV7 recipients. After PCV13 immunization, concordance between antipolysaccharide and opsonophagocytic responses was noted for all 13 serotypes. The PCV13 toddler dose resulted in higher immune responses compared with infant-series doses. Safety and tolerability were comparable; reactogenicity was generally mild.

CONCLUSIONS: PCV13 will be as effective as PCV7 in the prevention of pneumococcal disease caused by the 7 common serotypes and could provide expanded protection against the 6 additional serotypes. The PCV13 safety profile was comparable to that of PCV7. *Pediatrics* 2010; 126:e493–e505

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Approximately 14.5 million episodes of serious disease caused by *Streptococcus pneumoniae* were estimated to occur globally in children younger than 5 in 2000.[1] An estimated 1 million deaths annually are attributed to this organism in this age group worldwide.[2] In the United States, use of 7-valent pneumococcal conjugate vaccine (PCV7) has dramatically reduced the incidence of vaccine serotype-specific invasive pneumococcal disease (IPD).[3–6] In addition to direct benefits in vaccinated children, PCV7 administration has indirectly reduced IPD incidence in unvaccinated adults.[3,7]

The World Health Organization (WHO) has stated it should be a priority to include PCV7 in national immunization programs.[2] PCV7 serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F were responsible for ~80% to 90% of IPD in the United States before the introduction of PCV7.[8] Data from the Active Bacterial Core Surveillance Network revealed an overall 77% reduction in the incidence of IPD in children younger than 5, a 98% decline in vaccine-serotype IPD, and a small but significant increase in IPD because of nonvaccine serotypes, particularly type 19A.[4]

The serotypes in PCV13, which include PCV7 serotypes plus types 1, 3, 5, 6A, 7F, and 19A, are the most common serotypes that cause IPD globally, accounting for 80% to 92% of IPD in children younger than 5 worldwide.[9] Serotypes 6A and 19A are important additions, because both mediate a substantial proportion of pneumococcal disease.[4,10–12] Surveillance data have shown that PCV7 provides some protection against 6A disease, but not to the same extent as that seen against types actually contained in the vaccine.[13,14] PCV7 provides no protection against 19A disease.[2,15,16] Serotypes 1 and 5 are particularly important causes of pneumococcal disease in the developing world.[9] Finally, serotype 3 has been associated with a broad spectrum of disease, including IPD, necrotizing pneumonia, and acute otitis media.[17,18]

The current study represents the pivotal US study that compares the safety, tolerability, and immunogenicity of PCV13 with that of PCV7, administered as a 4-dose series with routine pediatric vaccinations, according to the US-recommended infant vaccination schedule. The results of another pivotal study conducted in Germany have been published elsewhere.[19]

PATIENTS AND METHODS

Study Design

This phase 3, randomized, double-blind, active-controlled, multicenter trial compared PCV7 with PCV13 given to healthy infants aged 2, 4, 6, and 12 to 15 months concomitantly with routine pediatric vaccinations. Two-month-old infants were enrolled at 1 of 38 sites and randomly assigned to receive either PCV7 or PCV13. Blood samples were obtained 1 month after the third infant dose, as well as before the toddler dose and 1 month afterward.

The study was conducted in accordance with the ethical principles that originated in the Declaration of Helsinki. The protocol was reviewed and approved by each institution's review board or delegated authority. Written informed consent was obtained from a subject's parent/guardian before study enrollment.

Eligible subjects were 2 months old (42–98 days) and healthy. Subjects were excluded if they previously were vaccinated with any study vaccines (except hepatitis B vaccine at birth); had any contraindication to vaccination; had a known or suspected immunodeficiency or suppression; had a history of *S pneumoniae* or *Haemophilus influenzae* type b disease or confirmed measles, mumps, rubella, or varicella infection; had a severe chronic disorder including significant congenital malformations; had a history of seizures; or had received blood products or γ-globulin.

Vaccines and Interventions

Each subject received 0.5 mL of either PCV13 or PCV7 at age 2, 4, 6, and 12 to 15 months. Each subject also received Pediarix (GlaxoSmithKline Biologicals, Rixensart, Belgium) and ActHIB (Sanofi Pasteur SA, Lyon, France) at the 2-, 4-, and 6-month visits, as well as ProQuad (Merck and Co, Inc, Whitehouse Station, NJ), PedvaxHIB (Merck and Co, Inc, West Point, PA), and VAQTA (Merck and Co, Inc, Whitehouse Station, NJ) at the 12- to 15-month visit.

Each vaccine was administered intramuscularly, except for ProQuad (injected subcutaneously into the arm). PCV13 and PCV7 were injected into the left thigh, and the other concomitant vaccines were injected into the right thigh.

As with PCV7, each PCV13 polysaccharide is covalently conjugated to a common carrier protein, cross-reactive material 197, a nontoxic variant of diphtheria toxin. PCV13 contains 2.2 μg of each polysaccharide, except for 4.4 μg of type 6B, with 0.125 mg per dose of aluminum as aluminum phosphate. The presentations of PCV13 and PCV7 were identical.

Study Objectives

Our objectives were to compare immune responses of PVC13 with those of PCV7, 1 month after both the infant series and the toddler dose, when coadministered with concomitant vaccines. As an exploratory measure, the functional opsonophagocytic capacity of the elicited antibodies was assessed in a randomly selected subset of 100 subjects per group.

The safety of PCV13 was evaluated by incidence rates of local reactions, sys-

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temic events, and adverse events (AEs). Immune responses to specific concomitant antigens also were determined. These results will be published separately.

Randomization

Eligible subjects were randomly assigned (1:1) to receive PCV13 or PCV7 on the basis of a randomization schedule with a block size of 4 performed through the sponsor's Clinical Operations Randomization Environment system accessible via Internet and telephone 24 hours/day. Participants, study staff, and those who assessed outcomes were blinded to the group assignment.

Analysis Population

Immunogenicity was analyzed only in the evaluable infants who adhered to protocol requirements, had valid and determinate assay results, and had no major protocol violations. The all-available infant immunogenicity population included all subjects who had at least 1 valid and determinate assay result; results were similar to those for the evaluable immunogenicity population (data not shown). The safety population included all subjects who received ≥1 dose of PCV.

Antipneumococcal Immunogenicity Assessments

Serotype-specific immunoglobulin G (IgG) was measured using standard pneumococcal anticapsular polysaccharide enzyme-linked immunosorbent assay (ELISA).[20–23] Functional antibacterial opsonophagocytic activity was measured by opsonophagocytic assay (OPA). The OPA used in this study[24] is a modification of the method of Romero-Steiner et al.[25] Unlike IgG ELISA, OPA has no agreed-on reference sera. Therefore, although antibody concentration values measured by ELISA can be compared across serotypes, OPA titer values cannot be similarly compared. Nonetheless, OPA titers within individual serotypes can be compared.

Statistical Analysis

The study was designed by using the intersection-union procedure described by Wellek[26] (Supplemental Text 1).

End points included immunologic comparisons of pneumococcal responses elicited by PCV13 relative to PCV7 1 month after both the infant series and the toddler dose. Responders after the infant series were defined as the proportion of subjects for whom there was an anticapsular polysaccharide IgG concentration of ≥0.35 μg/mL, a reference concentration for assessment of vaccine efficacy against IPD defined by the WHO.[27,28] Postinfant series and posttoddler dose analyses included comparison of antipolysaccharide IgG geometric mean concentrations (GMCs), comparison of functional antibacterial OPA titer of ≥1:8 as defined by the WHO,[26,27] and a comparison of functional antibacterial OPA geometric mean titers (GMTs).

Within each vaccine group and for each serotype, GMCs (IgG) or GMTs (OPA) and corresponding 2-sided 95% confidence intervals (CIs) were calculated by using logarithmically transformed values and the Student's *t* test distribution for CIs.

After the infant series, the difference in proportion of antipolysaccharide IgG responders elicited by PCV13 relative to PCV7 (PCV13–PCV7) was calculated for each serotype. The exact, unconditional, 2-sided 95% CIs on the difference in proportion of responders were computed using the noninferiority procedure of Chan and Zhang.[29] For the serotypes in common to the 2 vaccines, noninferiority was declared if the lowest limit of the 2-sided 95% CI for the difference in proportion of responders in the 2 groups was more than −0.10.

For the comparison of IgG GMCs elicited by PCV13 relative to PCV7, the GMC ratio (PCV13/PCV7) for each pneumococcal serotype was calculated. Noninferiority of PCV13 relative to PCV7 for the common serotypes was declared if the lower limit of the 2-sided 95% CI for the GMC ratio was >0.5 (twofold criterion). Fold-rises in antibody concentrations from pretoddler dose to posttoddler dose were reported by geometric means and CIs, also computed using the logarithmically transformed assay results.

Sample-size estimation was based on the anticipated proportion of responders in each vaccine group. Data from 2 previous PCV clinical studies (studies 6096A1-003[30] and D140-P001 [unpublished data]) were used to estimate proportion of responders and GMCs for each pneumococcal serotype. The study was powered to show the noninferiority of PCV13 relative to PCV7. After the infant series, it was estimated that 250 evaluable subjects per group provided 91% power to declare noninferiority for each pneumococcal serotype when using a noninferiority criterion of −0.10 and a 2-sided, type I error of 0.05.

Safety Assessments

Local pneumococcal vaccine injection site reactions (tenderness, redness, and swelling), systemic events (rectal temperature, decreased appetite, irritability, increased sleep, and decreased sleep), presences of hives, and use of antipyretic medication to prevent or treat symptoms were monitored by parents or legal guardians and recorded using an e-diary that solicited specific signs and symptoms for 7 days after each vaccination. Tenderness was recorded as none, present, or interfered with limb movement. Redness and swelling were measured

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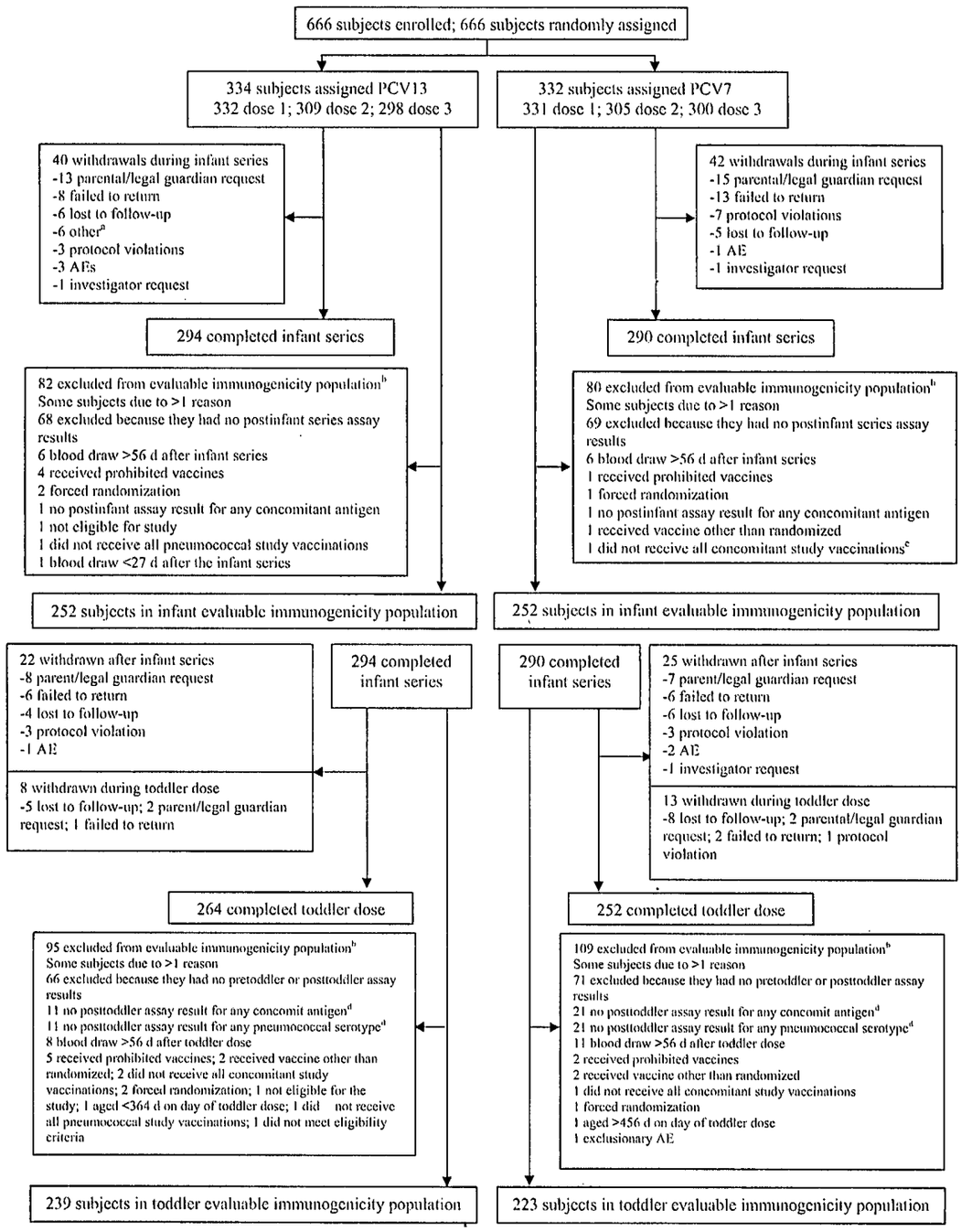

FIGURE 1

Subject disposition. [a]Other reasons were that subjects lost Kaiser coverage (4 subjects), the child was removed from the home and put in protective custody (1 subject), and the subject's parent/legal guardian never provided consent and the child was randomly assigned in error (1 subject). [b]Subjects may have been excluded for >1 reason. [c]Subject was not counted among the subjects who received Pediarix at dose 2 because of an error. [d]Subjects with no posttoddler assay results for any serotype/concomitant vaccine antigen are not included in this category and are classified as "No data received for these subjects."

with a caliper, reported in the diary as caliper units (1 unit = 0.5 cm), and then categorized as absent, mild (0.5–2.0 cm), moderate (2.5–7.0 cm), or severe (>7.0 cm). Temperature was measured each evening and whenever the child felt feverish; the highest temperature was recorded each day. Severity of fever was categorized as ab-

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sent (<38.0°C), mild (≥38.0°C to ≤39.0°C), moderate (>39.0°C to ≤40.0°C), or severe (>40°C). Unsolicited AEs were recorded until 6 months after the last vaccination.

RESULTS

Baseline Characteristics and Disposition of Subjects

A total of 666 subjects were enrolled, consented to participate, and were randomly assigned (see Fig 1) from September 18 to December 14, 2006. The 6-month follow-up was completed on June 2, 2008. The evaluable immunogenicity populations consisted of 504 infants (PCV13: 252; PCV7: 252) and 462 toddlers (PCV13: 239; PCV7: 223).

Demographic characteristics are presented in Table 1. There were no statistically significant differences in baseline characteristics between groups. The safety population included 663 subjects (PCV13: 332; PCV7: 331). Characteristics were similar to those in the evaluable immunogenicity populations.

Immune Responses to the Common Serotypes After the Infant Series

Comparison of immune responses to the 7 common serotypes is shown in Table 2. With regard to the antipolysaccharide responder proportion at 0.35 μg/mL (see "Patients and Methods"), noninferiority was achieved for 5 of the 7 serotypes. The exceptions were serotypes 6B and 9V, for which the values at the lower limit of the 95% CI did not meet noninferiority criteria (−10.9 and −12.4, respectively). The antipolysaccharide GMC noninferiority criterion for each of the common serotypes was met for all 7 serotypes, although the GMCs in the PCV13 group were somewhat lower than those in the PCV7 group.

The proportions of subjects with a serotype-specific OPA antibody titer of ≥1:8 were 90.4% or higher in the PCV13 group and 92.6% or higher in the PCV7 group. PCV13 and PCV7 also elicited comparable functional antibody responses, as noted by comparison of OPA GMTs. For all 7 serotypes and for both vaccines, functional antibody responses were concordant with antipolysaccharide responses (Fig 2).

Immune Responses to the Additional Serotypes After the Infant Series

Immune responses against the 6 additional serotypes are shown in Table 3. For all, PCV13 generally gave rise to anticapsular polysaccharide-binding IgG levels that were notably greater than those induced by PCV7, both in terms of proportion of responders as well as by GMC comparison. In addition, PCV13 elicited notably greater functional antibody activity against the additional serotypes.

OPA responses after PCV7 for the additional serotypes did not correlate with serotype-specific binding IgG concentrations, with the exception of serotype 6A (Fig 2). By contrast, OPA titers after PCV13 correlated with IgG concentrations for all serotypes. For serotype 6A, OPA response was 10-fold greater in the PCV13 group compared with PCV7.

For type 7F, data in Table 3 and Fig 2 show that PCV7 recipients achieved high concentrations of 7F OPA activity despite known ineffectiveness of PCV7 against serotype 7 disease.[27,28] A second OPA titer cutoff of 1:2048 was selected, therefore, as indicative of a type 7F OPA response on the basis of the 95th percentile of observed responses in PCV7 recipients. The proportion of responders above this level was 90.4% (95% CI: 82.6–95.5) in PCV13 recipients and 13.5% (95% CI: 7.2–22.4) in PCV7 recipients.

TABLE 1 Demographic Characteristics: Evaluable Infant and Toddler Immunogenicity Populations

Population	Evaluable Infant Immunogenicity			Evaluable Toddler Immunogenicity		
	PCV13 (*N* = 252)	PCV7 (*N* = 252)	Total (*N* = 504)	PCV13 (*N* = 239)	PCV7 (*N* = 223)	Total (*N* = 462)
Gender, *n* (%)						
Male	129 (51.2)	146 (57.9)	275 (54.6)	124 (52)	134 (60)	258 (56)
Female	123 (48.8)	106 (42.1)	229 (45.4)	115 (48)	89 (40)	204 (44)
Race, *n* (%)						
White	175 (69.4)	179 (71.0)	354 (70.2)	173 (72)	163 (73)	336 (73)
Black	52 (20.6)	46 (18.3)	98 (19.4)	45 (19)	33 (15)	78 (17)
Other	19 (7.5)	21 (8.3)	40 (7.9)	15 (6)	20 (9)	35 (8)
Asian	6 (2.4)	5 (2.0)	11 (2.2)	6 (3)	6 (3)	12 (3)
Native Hawaiian or other Pacific Islander	0 (0)	1 (0.4)	1 (0.2)	0 (0)	1 (0.4)	1 (0.2)
Ethnicity, *n* (%)						
Non-Hispanic and non-Latino	212 (84.1)	206 (81.7)	418 (82.9)	204 (85)	188 (84)	392 (85)
Hispanic or Latino	40 (15.9)	46 (18.3)	86 (17.1)	35 (15)	35 (16)	70 (15)
Weight at enrollment						
n	252	252	504	—	—	—
Mean (SD), lb	11.5 (1.6)	11.7 (1.6)	11.6 (1.6)	—	—	—
Age at toddler dose, mo						
n	—	—	—	239	223	462
Mean (SD)	—	—	—	12.4 (0.4)	12.4 (0.4)	12.4 (0.4)

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TABLE 2 Immune Responses to the 7 Common Serotypes After the Infant Vaccination Series

Immune Measurement	4	6B	9V	14	18C	19F	23F
% of responders according to antipolysaccharide IgG (95% CI)[a]							
PCV13	94.4 (90.9 to 96.9)[b]	87.3 (82.5 to 91.1)	90.5 (86.2 to 93.8)	97.6 (94.9 to 99.1)	96.8 (93.8 to 98.6)	98.0 (95.4 to 99.4)	90.5 (86.2 to 93.8)
PCV7	98.0 (95.4 to 99.4)	92.8 (88.9 to 95.7)	98.4 (96.0 to 99.6)	97.2 (94.4 to 98.9)	98.4 (96.0 to 99.6)	97.6 (94.9 to 99.1)	94.0 (90.4 to 96.6)
Difference[c]	−3.6 (−7.3 to −0.1)[d]	−5.5 (−10.9 to −0.1)	−7.9 (−12.4 to −4.0)	0.4 (−2.7 to 3.5)	−1.6 (−4.7 to 1.2)	0.4 (−2.4 to 3.4)	−3.6 (−8.5 to 1.2)
Antipolysaccharide IgG GMC (95% CI), μg/mL[e]							
PCV13	1.31 (1.19 to 1.45)[f]	2.10 (1.77 to 2.49)	0.98 (0.89 to 1.08)	4.74 (4.18 to 5.39)	1.37 (1.24 to 1.52)	1.85 (1.69 to 2.04)	1.33 (1.17 to 1.51)
PCV7	1.93 (1.75 to 2.13)	3.14 (2.64 to 3.74)	1.40 (1.27 to 1.55)	5.67 (5.02 to 6.40)	1.79 (1.63 to 1.96)	2.24 (2.01 to 2.50)	1.90 (1.68 to 2.15)
Ratio[g]	0.68 (0.59 to 0.78)[h]	0.67 (0.52 to 0.85)	0.70 (0.61 to 0.80)	0.84 (0.70 to 1.00)	0.77 (0.67 to 0.88)	0.83 (0.72 to 0.96)	0.70 (0.59 to 0.84)
OPA titer, % ≥ 1:8 (95% CI)[i]							
PCV13	97.8 (92.4 to 99.7)[j]	98.9 (94.2 to 100)	100 (96.1 to 100)	100 (96.2 to 100)	100 (96.2 to 100)	90.4 (82.6 to 95.5)	98.9 (94.2 to 100)
PCV7	98.9 (94.1 to 100)	100 (96.2 to 100)	98.9 (94.2 to 100)	100 (96.2 to 100)	100 (96.2 to 100)	92.6 (85.3 to 97.0)	98.9 (94.2 to 100)
OPA GMT (95% CI)[i]							
PCV13	359 (276 to 468)[k]	1055 (817 to 1361)	4035 (2933 to 5553)	1240 (935 to 1646)	276 (210 to 361)	54 (40 to 74)	791 (605 to 1034)
PCV7	536 (421 to 681)	1514 (1207 to 1899)	3259 (2288 to 4641)	1481 (1133 to 1934)	376 (292 to 484)	45 (34 to 60)	924 (709 to 1204)

[a] The proportion of subjects with antipolysaccharide IgG concentrations of ≥0.35 μg/mL (see text).
[b] Exact 2-sided CI is based on the observed proportion of subjects.
[c] Difference in proportions, PCV13–PCV7, expressed as a percentage.
[d] Exact 2-sided CI for the difference in proportions, PCV13–PCV7, is expressed as a percentage. Noninferiority was met if the lower limit of the 2-sided CI was greater than −10.0.
[e] GMCs were calculated by using all subjects with available data for the specified blood draw.
[f] CIs are back-transformations of a CI based on the Student's *t* test distribution for the mean logarithm of the concentrations.
[g] Ratio of GMCs: PCV13/PCV7.
[h] Exact 2-sided 95% CI of ratios. Noninferiority was met if the lower limit of the 2-sided CI was >0.5.
[i] Number of subjects with a determinate antibody titer for the specified serotype ranged from 89 to 94.
[j] Exact 2-sided CI is based on the observed proportion of subjects.
[k] CIs are back-transformations of a CI based on the Student's *t* distribution for the mean logarithm of the titers.

Immune Responses Before and After the Toddler Dose

For all vaccine serotypes in the respective vaccines, antipolysaccharide IgG GMCs before the toddler dose had decreased substantially (Table 4). The toddler dose elicited a marked increase in antibody levels. IgG GMCs to the 7 common serotypes elicited by PCV13 after the toddler dose were noninferior to those in PCV7 recipients for all serotypes. For the 6 additional serotypes, GMCs induced by PCV13 were notably higher than those induced by PCV7.

OPA responses after the toddler dose were also higher than after the infant series. For the 7 common serotypes, proportions of subjects who achieved an OPA antibody titer of ≥1:8 were similar across groups, and OPA GMTs were comparable (Table 5). For the 6 additional serotypes, OPA responder rates and OPA GMTs were uniformly higher in PCV13 recipients (Table 5).

Postinfant and Posttoddler Population Immune Responses

Reverse cumulative distributions curves in Fig 3 present the proportion of responders 1 month after the infant series and toddler dose at different antibody concentrations and provide comparisons of the full spectrum of immune response distribution. For the common serotypes, responses elicited by PCV13 were slightly lower than those elicited by PCV7, but in all cases, responses were well above levels considered effective. Population responses after the toddler dose were substantially greater than those after the infant series.

Safety and Tolerability

Local reactions (Table 6) and systemic events (Table 7) were mainly mild and comparable between groups. There were no statistical differences in incidence of solicited injection site local

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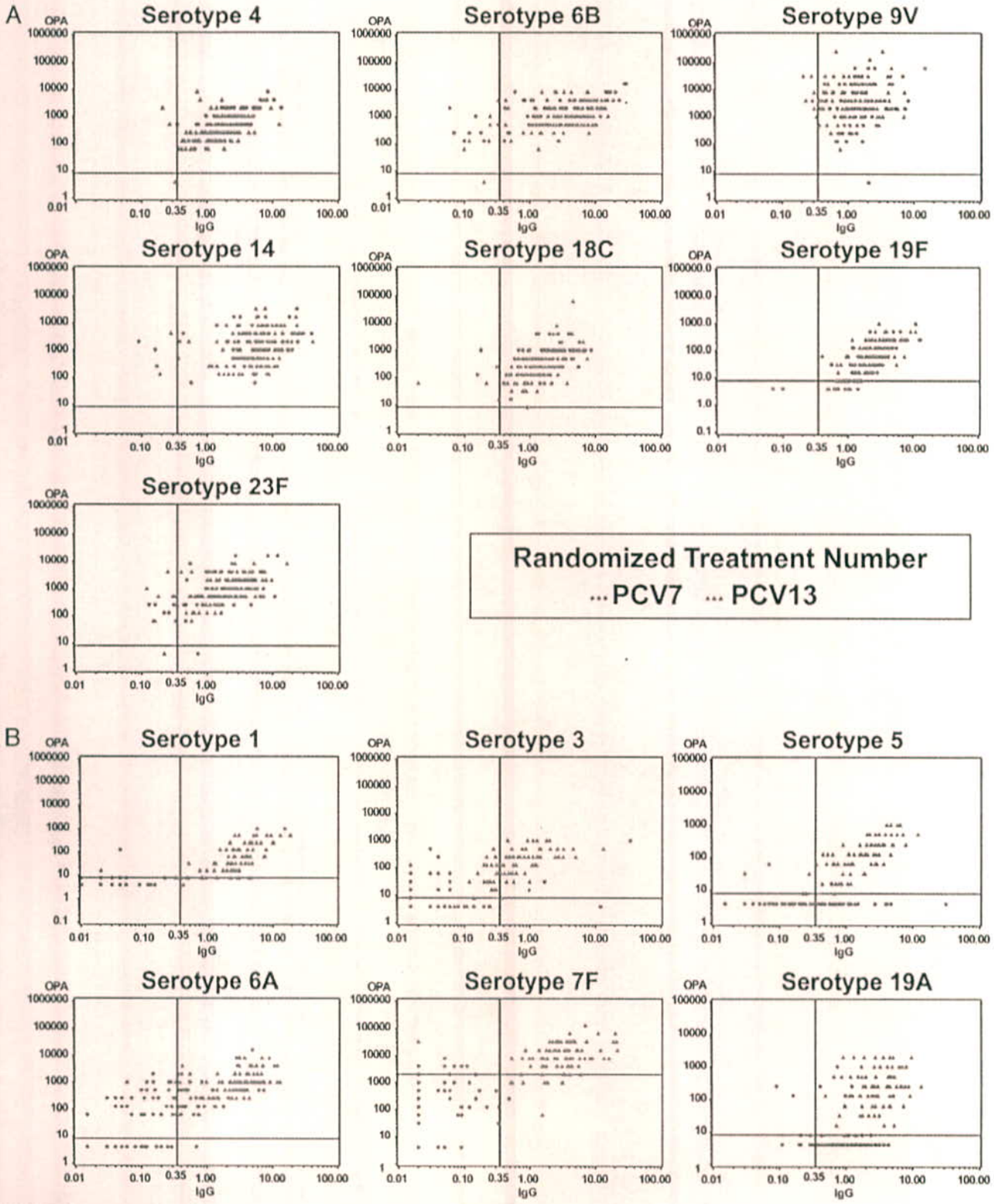

FIGURE 2

Concordance analysis between antipolysaccharide-binding IgG and functional OPA responses. A, Common serotypes; B, additional serotypes. Shown are postinfant series data from samples with IgG concentrations (x-axis) and OPA titers (y-axis). PCV7 or PCV13 recipient data are represented. Vertical lines denote IgG reference concentrations; horizontal lines denote OPA responder titer.

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TABLE 3 Immune Responses to the 6 Additional Serotypes After the Infant Vaccination Series

Immune Measurement	1	3	5	6A	7F	19A
% responders according to antipolysaccharide IgG (95% CI)[a]						
PCV13	95.6 (92.3–97.8)[b]	63.5 (57.1–69.4)	89.7 (85.2–93.1)	96.0 (92.8–98.1)	98.4 (96.0–99.6)	98.4 (96.0–99.6)
PCV7	1.6 (0.4–4.1)	4.6 (2.3–8.0)	31.0 (24.6–37.9)	42.5 (36.2–49.0)	2.8 (1.1–5.7)	86.6 (81.6–90.6)
Antipolysaccharide IgG GMC (95% CI), μg/mL[c]						
PCV13	2.03 (1.78–2.32)[d]	0.49 (0.43–0.55)	1.33 (1.18–1.50)	2.19 (1.93–2.48)	2.57 (2.28–2.89)	2.07 (1.87–2.30)
PCV7	0.02 (0.02–0.03)	0.04 (0.03–0.04)	0.20 (0.16–0.24)	0.25 (0.21–0.29)	0.04 (0.03–0.04)	0.89 (0.79–0.99)
OPA titer, % ≥1:8 (95% CI)[e]						
PCV13	98.9 (94.1–100)[f]	96.8 (91.0–99.3)	92.3 (84.8–96.9)	100 (96.2–100)	100[g] (96.2–100)	91.4 (83.8–96.2)
PCV7	9.8 (4.6–17.8)	21.3 (13.5–30.9)	2.2 (0.3–7.6)	77.7 (67.9–85.6)	76.4[g] (66.2–84.8)	16.3 (9.4–25.5)
OPA GMT (95% CI)[e]						
PCV13	52 (39–69)[h]	121 (92–158)	91 (67–123)	980 (783–1226)	9494 (7339–12 281)	152 (105–220)
PCV7	4 (4–5)	7 (5–9)	4 (4–4)	100 (66–152)	128 (80–206)	7 (5–8)

[a] Proportion of subjects with antipolysaccharide IgG concentrations of ≥0.35 μg/mL (see text).
[b] Exact 2-sided CI is based on the observed proportion of subjects.
[c] GMCs were calculated by using all subjects with available data for the specified blood draw.
[d] CIs are back-transformations of a CI based on the Student's *t* test distribution for the mean logarithm of the concentrations.
[e] Number of subjects with a determinate antibody titer for the specified serotype ranged from 89 to 94.
[f] Exact 2-sided CI is based on the observed proportion of subjects.
[g] 7F responder rate at ≥1:2048 is 90.4% (95% CI: 82.6–95.5) in PCV13 recipients and 13.5% (95% CI: 7.2–22.4) in PCV7 recipients.
[h] CIs are back-transformations of a CI based on the Student's *t* distribution for the mean logarithm of the titers.

TABLE 4 Pneumococcal Antipolysaccharide IgG GMCs (μg/mL) Before and After the Toddler Dose

	Before Toddler Dose		After Toddler Dose		Ratio[c] (95% CI[d])
	PCV13, GMC[a] (95% CI[b])	PCV7, GMC[a] (95% CI[b])	PCV13, GMC[a] (95% CI[b])	PCV7, GMC[a] (95% CI[b])	
Common serotypes					
4	0.35 (0.31–0.39)	0.51 (0.45–0.57)	3.73 (3.28–4.24)	5.49 (4.91–6.13)	0.68 (0.57–0.80)
6B	0.78 (0.69–0.89)	1.01 (0.88–1.15)	11.53 (9.99–13.30)	15.63 (13.80–17.69)	0.74 (0.61–0.89)
9V	0.39 (0.35–0.43)	0.53 (0.48–0.59)	2.62 (2.34–2.94)	3.63 (3.25–4.05)	0.72 (0.62–0.85)
14	1.89 (1.64–2.17)	2.49 (2.17–2.85)	9.11 (7.95–10.45)	12.72 (11.22–14.41)	0.72 (0.60–0.86)
18C	0.34 (0.30–0.37)	0.45 (0.41–0.50)	3.20 (2.82–3.64)	4.70 (4.18–5.28)	0.68 (0.57–0.81)
19F	0.73 (0.65–0.82)	0.65 (0.57–0.74)	6.60 (5.85–7.44)	5.60 (4.87–6.43)	1.18 (0.98–1.41)
23F	0.38 (0.33–0.44)	0.48 (0.42–0.55)	5.07 (4.41–5.83)	7.84 (6.91–8.90)	0.65 (0.54–0.78)
Additional serotypes					
1	0.64 (0.57–0.72)	0.03 (0.02–0.03)	5.06 (4.43–5.80)	0.03 (0.03–0.03)	86.48 (72.95–102.52)
3	0.15 (0.13–0.17)	0.05 (0.04–0.06)	0.94 (0.83–1.05)	0.07 (0.05–0.08)	13.88 (11.42–16.87)
5	0.77 (0.69–0.86)	0.44 (0.37–0.51)	3.72 (3.31–4.18)	0.55 (0.47–0.64)	6.76 (5.44–8.41)
6A	0.83 (0.75–0.92)	0.30 (0.26–0.35)	8.20 (7.30–9.20)	1.87 (1.60–2.19)	8.78 (7.15–10.78)
7F	0.83 (0.75–0.93)	0.04 (0.04–0.05)	5.67 (5.01–6.42)	0.05 (0.04–0.05)	69.30 (58.40–82.24)
19A	0.92 (0.81–1.05)	0.70 (0.61–0.80)	8.55 (7.64–9.56)	3.54 (3.15–3.98)	2.34 (2.01–2.72)

[a] GMCs were calculated by using all subjects with available data for the specified blood draw.
[b] CIs are back-transformations of a CI based on the Student's *t* distribution for the mean logarithm of the concentrations.
[c] Ratio of GMCs; PCV13/PCV7.
[d] CIs for the ratio are back-transformations of a CI based on the Student's *t* distribution for the mean difference of the logarithms of the measures (PCV13–PCV7 reference). Noninferiority was met if the lower limit of the 2-sided CI was >0.5.

reactions within 7 days after any infant-series dose. In most instances, the highest percentages of subjects experiencing local reactions occurred on day 1 or 2 (data not shown).

In general, there were no statistical differences in incidence of "any" systemic event after any dose between groups. For specific reactions, the only statistical difference was in the incidence of moderate fever after dose 1 (2.8% vs 0.0% for PCV13 and PCV7, respectively; $P = .026$). Most cases of fever were mild in severity in each group. Severe fever was reported in only 2 subjects, both in the PCV7 group. There were no differences between groups in the use of antipyretic medications to prevent or treat symptoms after any dose. There were a total of 12 reported cases of hives within 7 days of the infant series (10 for PCV13 and 2 for PCV7). Only 1 case after dose 3 (in the PCV7 group) was confirmed. The other cases were not confirmed. After the toddler dose, 3 subjects in each group reported hives within 7 days

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TABLE 5 Opsonophagocytic Activity Responses to the 7 Common Serotypes After the Toddler Dose

Immune Measurement	OPA Titer % ≥1:8[a]		OPA GMT[a]	
	PCV13, OPA (95% CI)	PCV7, OPA (95% CI)	PCV13, OPA (95% CI)	PCV7, OPA (95% CI)
Common serotypes				
4	98.9 (93.8–100)[b]	98.9 (94.1–100)	1180 (847–1643)[c]	1492 (1114–1999)
6B	98.9 (94.1–100)	100 (96.2–100)	3100 (2337–4111)	4066 (3243–5098)
9V	98.9 (94.0–100)	100 (96.2–100)	11 856 (8810–15 955)	18 032 (14 125–23 021)
14	100 (96.1–100)	100 (96.2–100)	2002 (1453–2760)	2366 (1871–2992)
18C	98.9 (94.0–100)	100 (96.2–100)	993 (754–1308)	1722 (1327–2236)
19F	96.7 (90.8–99.3)	94.8 (88.3–98.3)	200 (144–276)	167 (121–230)
23F	98.9 (94.0–100)	100 (96.1–100)	2723 (1961–3782)	4982 (3886–6387)
Additional serotypes				
1	98.9 (93.9–100)	12.0 (6.1–20.4)	164 (114–237)	5 (4–6)
3	97.8 (92.3–99.7)	43.8 (33.6–54.3)	380 (300–482)	12 (9–16)
5	98.9 (94.0–100)	5.2 (1.7–11.7)	300 (229–393)	5 (4–6)
6A	98.9 (94.1–100)	94.8 (88.3–98.3)	2242 (1707–2945)	539 (375–774)
7F	100[d] (96.0–100)	80.4[d] (70.9–88.0)	11 629 (9054–14 938)	268 (164–436)
19A	97.8 (92.3–99.7)	53.2 (42.6–63.6)	1024 (774–1355)	268 (164–436)

Opsonophagocytic activity responses before the toddler dose were not measured.

[a] Number of subjects with a determinate antibody titer for the specified serotype ranged from 88 to 96.

[b] Exact 2-sided CI is based on the observed proportion of subjects.

[c] CIs are back-transformations of a CI based on the Student's *t* distribution for the mean logarithm of the titers.

[d] 7F responder rate at ≥1:2048 is 93.4% (95% CI: 86.2–97.5) in PCV13 recipients and 23.9% (95% CI: 15.6–33.9) in PCV7 recipients.

of vaccination; none were clinically confirmed.

AEs reported reflected anticipated childhood illnesses (data not shown). One serious AE in each group was judged by the investigator to be related to study vaccine after the infant series: pyrexia with febrile convulsion (PCV13 group) and nephroblastoma (PCV7 group) diagnosed 3 months after dose 3 (thought to be related by the site investigator but not by the study medical monitor). No related serious AEs were reported after the toddler dose.

DISCUSSION

For the 7 common serotypes, antipolysaccharide IgG responses of ≥0.35 μg/mL were found to be noninferior between the 2 vaccines for 5 of the serotypes. Serotypes 6B and 9V did not meet this noninferiority criterion because they were slightly outside the −10% CI. When comparing GMCs, PCV13 met noninferiority for all the common serotypes including 6B and 9V, although the actual titers were lower than those after PCV7 (Table 2). These GMC responses can be seen graphically in the reverse cumulative distributions curves in Fig 3; the curves for PCV13 and PCV7 were similar in shape for each of the common serotypes, with the PCV13 curves being slightly shifted to the left. In addition, PCV13 elicited functional OPA responses similar to PCV7 for each common serotype. Collectively, these data support that PCV13 is immunogenic against the 7 common serotypes, including 6B and 9V.

For the additional 6 serotypes, PCV13 elicited higher concentrations of antipolysaccharide IgGs and notably higher titers of OPA antibodies (Table 3) compared with PCV7. For serotypes 5 and 19A, PCV7 seemed to elicit some IgG antibodies, but these antibodies have no antibacterial functional activity. This lack of OPA activity is likely the reason for the known lack of PCV7 effectiveness against serotype 19A disease.[14,15,31,32] By contrast, PCV13 elicited substantial antitype 19A OPA activity, which suggests that it would provide protection against type 19A disease.

PCV7 is known to provide some cross-protection against serotype 6A-mediated IPD. This was reflected by the antitype 6A binding and functional antibodies elicited by PCV7. Nonetheless, PCV13 elicited a substantially higher level of functional antibody relative to the antipolysaccharide-binding IgG response, which suggests that PCV13 would be more effective than PCV7 in providing direct protection against type 6A-mediated pneumococcal disease.

Although serotype 3 exhibited the lowest IgG responses of the 6 additional serotypes, the functional antibody response for serotype 3 in the PCV13 group exceeded the response in the PCV7 group by 18-fold after the infant series and 32-fold after the toddler dose; accordingly, PCV13 will likely provide added protection against serotype 3 disease.

For the 7 serotypes in Prevnar, an OPA titer of 1:8 after the infant series was shown to correlate with protective levels of anticapsular binding antibody.[28] In the current study, a cutoff of 1:8 was also used to analyze the OPA activity for the 6 additional serotypes. For serotype 6A, there was a high percentage of responders in both the PCV13 (100%)

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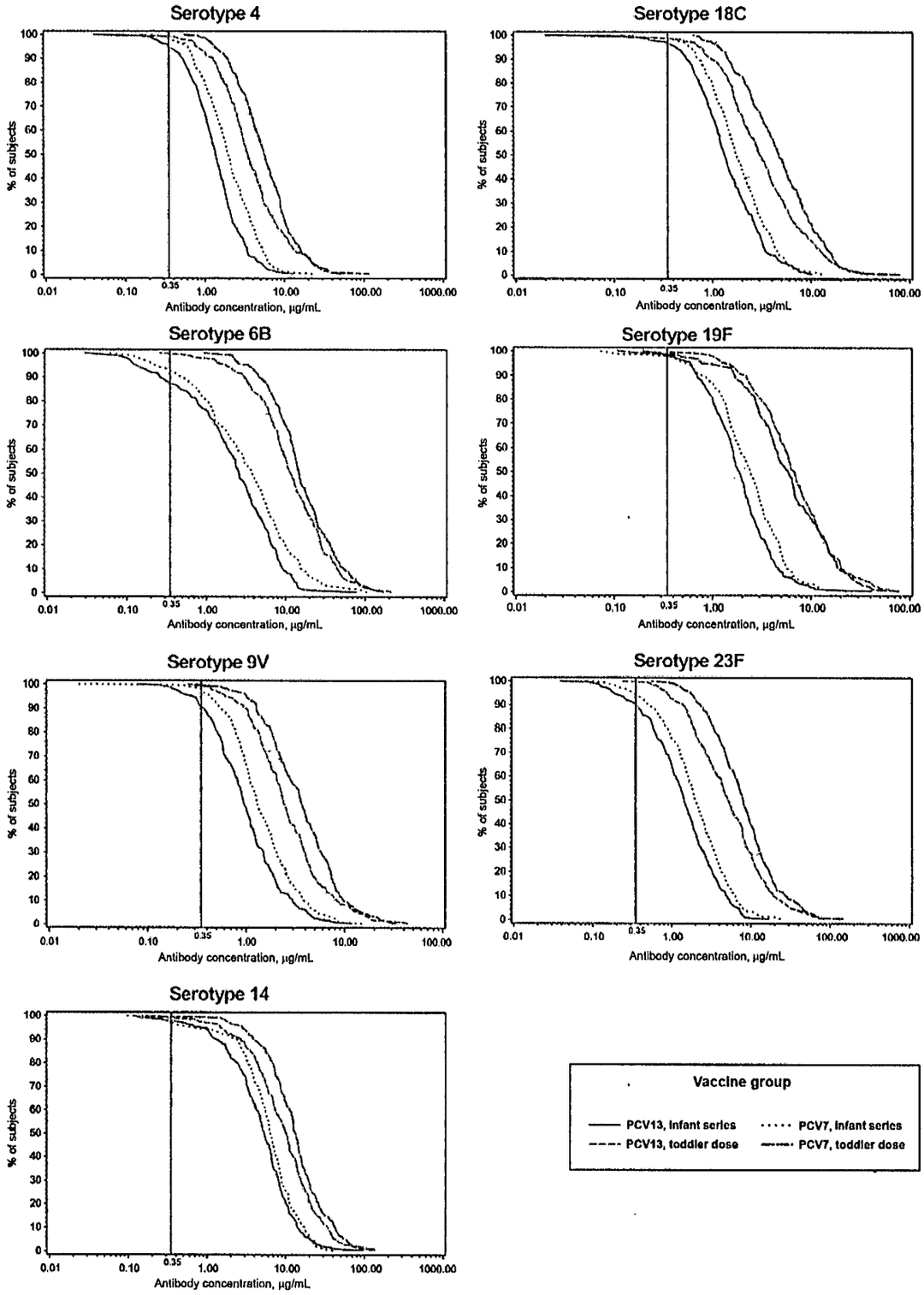

FIGURE 3

Reverse cumulative distribution curves of the antipolysaccharide IgG responses 1 month after the infant and toddler vaccinations for each of the 7 common serotypes. The vertical lines denote the IgG reference concentration of 0.35 μg/mL (see text).

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TABLE 6 Subjects Reporting Local Reactions Within 7 Days

Local Reaction	Dose 1		Dose 2		Dose 3		Toddler Dose	
	PCV13, % (n[a]/N[b])	PCV7, % (n/N)	PCV13, % (n/N)	PCV7, % (n/N)	PCV13, % (n/N)	PCV7, % (n/N)	PCV13, % (n/N)	PCV7, % (n/N)
Tenderness								
Any	72.7 (192/264)	72.2 (195/270)	77.0 (154/200)	75.9 (164/216)	78.7 (140/178)	80.9 (140/173)	81.2 (121/149)	84.4 (124/147)
Significant[c]	13.7 (25/182)	9.2 (18/195)	10.6 (13/123)	11.5 (15/131)	8.8 (8/91)	9.3 (8/86)	15.4 (10/65)	12.2 (6/49)
Swelling								
Any	27.4 (55/201)	23.6 (51/216)	31.2 (44/141)	29.5 (43/146)	37.9 (44/116)	36.9 (38/103)	44.0 (40/91)	50.7 (37/73)
Mild[d]	23.1 (46/199)	21.2 (45/212)	29.8 (42/141)	26.1 (37/142)	35.4 (40/113)	36.9 (38/103)	43.3 (39/90)	46.5 (33/71)
Moderate[d]	6.8 (12/176)	5.2 (10/193)	5.1 (6/118)	7.1 (9/126)	6.6 (6/91)	6.1 (5/82)	14.7 (10/68)	14.3 (7/49)
Severe[d]	0.0 (0/173)	0.0 (0/187)	0.0 (0/116)	0.0 (0/120)	0.0 (0/87)	0.0 (0/79)	0.0 (0/59)	0.0 (0/44)
Redness								
Any	35.6 (72/202)	32.3 (72/223)	45.2 (70/155)	37.8 (62/164)	48.9 (64/131)	50.0 (59/118)	54.4 (56/103)	65.5 (57/87)
Mild[d]	34.5 (69/200)	31.4 (69/220)	44.5 (69/155)	37.0 (60/162)	47.7 (61/128)	48.7 (57/117)	53.9 (55/102)	63.5 (54/85)
Moderate[d]	4.5 (8/177)	2.6 (5/191)	1.7 (2/117)	3.2 (4/124)	5.5 (5/91)	5.0 (4/80)	8.1 (5/62)	14.0 (7/50)
Severe[d]	0.0 (0/173)	0.0 (0/186)	0.0 (0/116)	0.0 (0/120)	0.0 (0/87)	0.0 (0/79)	0.0 (0/59)	0.0 (0/44)

No differences were statistically significant; $P < .05$ (Fisher's exact test, 2-sided).

[a] Number of subjects who reported the specific characteristic.

[b] Number of subjects who reported "yes" for at least 1 day or "no" for all days.

[c] Present and interfered with limb movement.

[d] Mild, 0.5–2.0 cm; moderate, 2.5–7.0 cm; and severe, >7.0 cm.

TABLE 7 Subjects Reporting Systemic Events and Antipyretic Medication Use Within 7 Days

Systemic Reaction	Dose 1		Dose 2		Dose 3		Toddler Dose	
	PCV13, % (n[a]/N[b])	PCV7, % (n/N)	PCV13, % (n/N)	PCV7, % (n/N)	PCV13, % (n/N)	PCV7, % (n/N)	PCV13, % (n/N)	PCV7, % (n/N)
Fever								
≥38°C but ≤39°C	24.0 (47/196)	21.2 (43/203)	43.2 (63/146)	40.4 (61/151)	39.8 (49/123)	37.7 (40/106)	53.5 (53/99)	51.3 (39/76)
>39°C but ≤40°C	2.8 (5/177)	0.0 (0/187)[c]	2.5 (3/118)	4.9 (6/123)	8.5 (8/94)	2.5 (2/81)	6.6 (4/61)	12.5 (6/48)
>40°C	0.0 (0/174)	0.0 (0/187)	0.0 (0/116)	0.8 (1/121)	0.0 (0/87)	1.3 (1/80)	1.7 (1/60)	0.0 (0/45)
Decreased appetite	54.8 (125/228)	45.4 (108/238)	59.9 (106/177)	52.3 (91/174)	59.2 (87/147)	59.6 (81/136)	65.5 (76/116)	73.6 (81/110)
Irritability	89.6 (259/289)	85.9 (249/290)	89.8 (212/236)	91.5 (216/236)	88.4 (191/216)	92.2 (201/218)	92.0 (183/199)	93.1 (163/175)
Increased sleep	79.5 (213/268)	78.5 (212/270)	79.3 (161/203)	73.5 (147/200)	71.3 (117/164)	69.8 (104/149)	70.4 (81/115)	74.3 (81/109)
Decreased sleep	45.7 (101/221)	47.9 (113/236)	49.1 (83/169)	55.8 (96/172)	60.4 (93/154)	63.4 (85/134)	58.4 (66/113)	64.2 (61/95)
Hives	1.7 (3/178)	1.1 (2/188)	2.5 (3/118)	0.0 (0/120)	4.5 (4[d]/89)	0.0 (0[d]/79)	4.8 (3/62)	6.4 (3/47)
Use of antipyretic medication to prevent symptoms	75.0 (204/272)	74.8 (205/274)	86.7 (202/233)	83.5 (193/231)	81.2 (164/202)	84.1 (159/189)	88.4 (145/164)	90.7 (147/162)
Use of antipyretic medication to treat symptoms	78.6 (202/257)	71.8 (191/266)	82.4 (182/221)	81.9 (181/221)	82.3 (167/203)	85.6 (160/187)	84.0 (136/162)	87.7 (121/138)

[a] Number of subjects who reported the specific characteristic.

[b] Number of subjects who reported "yes" for at least 1 day or "no" for all days.

[c] Statistically significant difference; $P < .05$ (Fisher's exact test, 2-sided).

[d] One report of hives was recorded in error on the e-diary of a subject in the PCV13 group; the case was actually in a subject in the PCV7 group.

and PCV7 (77%) groups, which was expected on the basis of cross-reactivity of antigens within serogroup 6 and correlates with the protection that Prevnar has shown against 6A disease. The higher GMT to 6A in PCV13 recipients suggests there would be an added benefit from the inclusion of 6A in the vaccine. For serotypes 1, 3, 5, and 19A, serotypes that are not in Prevnar and where there is no cross-protection from the inclusion of serotype 19A, a cutoff of 1:8 discriminates well between PCV7 recipients who are not protected against these serotypes and PCV13 recipients in whom protection against disease caused by these serotypes is anticipated. However, for serotype 7F, a high percentage of PCV13 (100%) and PCV7 (76%) recipients had titers of ≥1:8, despite the fact that Prevnar does not provide protection against 7F strains. The 2 groups can easily be differentiated by the OPA GMT, which was ~100-fold greater in PCV13 recipients, and by using a cutoff of ≥1:2048 based on the 95th percentile of observed responses in PCV7 recipients. Postlicensure effectiveness studies will provide the data needed to assess the appropriateness of this cutoff.

It was suggested in a recent study that the use of an antipyretic medication led to a reduction in immunogenicity with a 10-valent PCV.[33] This and other PCV13 trials were not designed to assess effect of antipyretic use on immu-

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nogenicity; posthoc analysis did not show any decreased immune response to PCV7 or PCV13 (Supplemental Text 1).

Since the licensure of Prevnar, nasopharyngeal carriage with serotype 6C has been reported in the United States and United Kingdom,[34–36] and rates of IPD attributable to 6C have increased in people age 5 or older in the United States.[37,38] Although antibody to 6B provides some cross-protection to 6A, it does not seem to protect against 6C.[38] The impact of serotype 6A in PCV13 on disease caused by serotype 6C will be determined on the basis of surveillance for IPD in the context of widespread use of PCV13.

Finally, the safety analysis presented no notable concerns for subjects who received PCV13. Overall, observations indicate that the safety profile of PCV13 is similar to that of PCV7.

CONCLUSIONS

Data from this study support that PCV13 will be as effective as PCV7 in preventing disease caused by serotypes common to both vaccines. PCV13 has a safety profile comparable to PCV7. In addition, PCV13 should mediate protection against the 6 additional serotypes, all of which are important worldwide causes of severe pneumococcal disease.

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Immunogenicity and Safety of 13-Valent Pneumococcal Conjugate Vaccine in Infants and Toddlers

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Immunogenicity and Safety of the Eleven Valent Pneumococcal Polysaccharide-Protein D Conjugate Vaccine in Infants

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Background: Development is ongoing to increase the serotype coverage of pneumococcal conjugate vaccines. We report here the immunogenicity and safety of a new 11-valent pneumococcal conjugate vaccine (Pn-PD) in infants.

Methods: In a randomized, single blind study, 154 Finnish infants received 1 of 3 regimens: 4 doses of Pn-PD at 2, 4, 6 and 12–15 months; 3 doses of the Pn-PD at 2, 4 and 6 months and 1 dose of 23-valent polysaccharide vaccine (PncPS) at 12–15 months; or 3 doses of the hepatitis B vaccine at 2, 4 and 6 months and Pn-PD at 12–15 months. Serum IgG antibodies to vaccine serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F were measured with an enzyme immunoassay at the ages of 2, 7 and 12–15 months and at 4 or 28 days after the last vaccination. Local and systemic reactions were recorded by parents during 8 days after each dose. Serious adverse reactions were recorded during the entire study period.

Results: There was a significant increase in the IgG concentrations to vaccine serotypes after 3 doses of Pn-PD. Antibody concentrations after the primary series varied between 1.26 and 4.92 μg/ml depending on the serotype and study group. PncPS vaccine induced a better booster response than the Pn-PD, measured at 28 days after the fourth dose. IgG concentrations after the Pn-PD booster ranged between 1.60 and 9.63 μg/ml and after the PncPS booster between 4.24 and 40.54 μg/ml, depending on the serotype. The antibody concentrations after the first dose of Pn-PD administered at 12–15 months increased significantly but were lower than after the fourth dose at the same age. No significant antibody increase was measured 4 days after the vaccinations at 12–15 months. The safety profile of the vaccine was acceptable.

Conclusions: The Pn-PD we tested was immunogenic and safe in infants.

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During the past 10 years, an array of polysaccharide-protein conjugate vaccines against *Streptococcus pneumoniae* with differing carrier proteins and polysaccharide (PS) compositions have been studied.[1] One of these vaccines, a 7-valent vaccine with a CRM_{197} carrier protein (PncCRM), has been granted marketing authorization in the United States, in the European Union, in Australia and in several other countries.

The only available pneumococcal conjugate vaccine, PncCRM (Prevenar/Prevnar), has been effective in preventing invasive pneumococcal infections in the United States.[2] In high risk populations, American Indians and human immunodeficiency virus-infected children, the efficacy of 7- and 9-valent formulas (respectively) has been good against invasive pneumococcal infections.[3,4] In Finland, PncCRM was moderately efficacious against vaccine type acute otitis media.[5] The 7-valent PncCRM contains serotypes 4, 6B, 9V, 14, 18C, 19F and 23F. It is missing serotypes 1 and 5, which are common in developing countries.[6,7] In Asia, an increase from 7- to 11-valent vaccine by adding serotypes 1, 3, 5 and 7F widened the serogroup coverage of the vaccine against invasive pneumococcal infections from ~45% to 76% in young children.[8]

The carrier proteins used in the experimental and licensed pneumococcal conjugate vaccines have been the same as those used in the *Haemophilus influenzae* type b (Hib) conjugate vaccines: CRM_{197}, diphtheria toxoid, tetanus toxoid and *Neisseria meningitidis* outer membrane protein complex.[9] However, the increase in the load of a carrier might influence the immunogenicity of other concomitantly injected conjugate vaccines that use the same protein carrier[10] and the pneumococcal conjugate vaccine itself.[11] A recombinant non-

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lipidated form of the protein D (PD) from *H. influenzae* has been used in experimental Hib conjugate vaccines,[12] and this protein has been conjugated to pneumococcal polysaccharides.[13] PD is a highly conserved 42-kDa cell surface lipoprotein of *H. influenzae*. In rats, PD induces systemic and local antibodies to PD,[14] and immunization with PD has induced protection against *H. influenzae* otitis media in rat and chinchilla models.[15,16] This vaccine could probably induce immunity against pneumococcus and *H. influenzae*.

It is recommended that the immunogenicity of new pneumococcal conjugate vaccines is evaluated by measuring antibody concentrations after the third dose.[17] The end point would be the immune response after the primary series (usually 3 doses) of the vaccines by calculating the proportions of children with antibody concentration ≥0.35 μg/ml when using enzyme immunoassay (EIA) without adsorption with 22F polysaccharide. This threshold has been proposed in the recent WHO meeting of experts[18] as the primary endpoint for demonstration of immunologic noninferiority in head-to-head comparison trials that use a registered pneumococcal conjugate vaccine as a comparator. Other measures of immune response should be taken into consideration as secondary end points. These are the geometric mean concentrations at various ages, persistence of antibodies and the induction of immunologic B cell memory.[17] The induction of memory can be evaluated by the antibody response after a booster dose of pneumococcal polysaccharide vaccine (PncPS) in age-matched primed and unprimed subjects, but in several pneumococcal conjugate vaccine studies immune memory has been assessed after primary series by the PncPS and or the pneumococcal conjugate response, and the immune memory response has been evaluated at days 7–10 after vaccination.[19,20] There are to date no data showing that primed children respond more quickly after boosting with polysaccharide or conjugate vaccine than unprimed children.

We report here immunogenicity and safety of a new 11-valent pneumococcal conjugate vaccine (Pn-PD) using *H. influenzae* protein D as a carrier. The Pn-PD was given at 2, 4, 6 and 12–15 months of age. Immunogenicity was assessed by measuring IgG antibody concentrations after the third vaccine dose and by demonstration of the development of immunologic memory. To this end, the immune response to the fourth dose, either Pn-PD or PncPS vaccine, given at 12–15 months was measured at days 4 and 28 after vaccination and compared with that after the first dose of Pn-PD at the same age.

SUBJECTS AND METHODS

This was a randomized, single blind, controlled phase II study to evaluate the immunogenicity and safety of the 11-valent pneumococcal conjugate vaccine in infants. We enrolled 154 healthy Finnish infants at 4 child health centers (in Hämeenlinna, Joensuu, Kerava and Salo) after the parents or guardians had signed an informed consent. The study was approved by the Institutional Review Board of Finnish National Public Health Institute (KTL).

Vaccines and Vaccinations. The study vaccine, Pn-PD [GlaxoSmithKline Biologicals (GSK Bio), Rixensart, Belgium], contained 11 pneumococcal capsular polysaccharides of serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F (1 μg of each) conjugated to a recombinant nonlipidated form of protein D, a cell surface lipoprotein of *H. influenzae*. The 23-valent pneumococcal polysaccharide vaccine (PncPS; Pneumovax 23; Aventis Pasteur, Lyon, France) contained 25 μg of PS of each of the 23 serotypes. The control vaccine was hepatitis B vaccine (Engerix-B; GSK Bio). Children were randomized into 3 study groups (Table 1). Group 1 received Pn-PD at 2, 4, 6 and 12–15 months. Group 2 received Pn-PD at 2, 4 and 6 months and PncPS at 12–15 months. Group 3 received the control vaccine at 2, 4 and 6 months and Pn-PD at 12–15 months. Diphtheria-tetanus-acellular pertussis-inactivated polio-Hib vaccine (Infanrix-Polio + Hib; Glaxo SmithKline) was given at 2, 4 and 6 months concomitantly with the pneumococcal vaccines or the control vaccine, but at a separate injection site (opposite limb). Subjects did not receive any other vaccinations during the study period.

Serologic Methods. For assessing the immune response after the primary series of 3 doses of Pn-PD, venous blood samples were taken at the ages of 2 and 7 months. For measuring the antibody response to the booster vaccination, blood samples were taken before and 4 or 28 days after the vaccinations given at 12–15 months. Serum was separated by centrifugation and samples were stored at −20°C until analyzed.

Antipneumococcal IgG concentrations to serotypes included in the vaccine were measured at the Vaccine Immunology Laboratory at KTL by EIA without adsorption with 22F polysaccharide.[21,22] For coating with type 3 PS, methylated human serum albumin (5 μg/ml) was added to the coating solution to promote binding to microtiter plates.[23] The results for serum antipneumococcal PS IgG are given in micrograms/ml). The detection cutoffs were 0.06 μg/ml for type 4, 0.07 μg/ml for type 18C, 0.08 μg/ml for types 9V and 23F, 0.09 μg/ml for types 3 and 6B, 0.1 μg/ml for types 1 and 7F, 0.15 μg/ml for type 5, 0.16 μg/ml for type 19F and 0.19 μg/ml for type 14.

Safety Evaluation. The parents or guardians recorded local and systemic reactions on a diary card during 8 days after each vaccine dose. The followed reactions were pain, redness

TABLE 1. The Vaccine Groups

Group	2 mo	4 mo	6 mo	12–15 mo
1	Pn-PD	Pn-PD	Pn-PD	Pn-PD
2	Pn-PD	Pn-PD	Pn-PD	PncPS
3	Control	Control	Control	Pn-PD

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and swelling; drowsiness; fever; irritability/fussiness; and loss of appetite. Serious adverse events were recorded during the entire study period. A serious adverse event was defined as any untoward medical occurrence resulting in death, a life-threatening condition, persistent or significant disability, hospitalization or prolongation of existing hospitalization.

Statistical Methods. Sample size was calculated to provide at least 89% power to detect a difference between the combined groups 1 and 2, and group 3 in terms of geometric mean concentrations of antipneumococcal polysaccharide antibodies for 11 serotypes 1 month after the third dose. Serum antibody data were log-transformed for statistical analyses. A paired t test was used to compare responses within the groups. Differences in antibody concentrations among the vaccination groups were determined by analysis of variance and independent-sample t test. $p < 0.05$ was considered significant. The results are given as geometric mean concentrations (GMC) with 95% confidence intervals. When concentration was below the detection limit, a value one-half of the cutoff was used to compute GMC. We also calculated the percentages of children with antipneumococcal PS IgG concentration ≥ 0.35 μg/ml. A descriptive analysis was applied to all safety variables.

RESULTS

Antibody Concentrations to the 11 Vaccine Serotypes Before and After the Primary Series. At the age of 2 months, antipneumococcal PS IgG concentrations were similar in the 3 groups, and the GMCs varied between 0.09 (4, group 1) and 0.40 μg/ml (19F, group 2), depending on the serotype and group (data not shown).

The children in groups 1 and 2 had significantly higher antipneumococcal PS IgG concentrations for all vaccine serotypes after 3 doses of Pn-PD at 7 months than before any dose at 2 months of age ($P < 0.001$ for all serotypes). In contrast, the children who had not received a pneumococcal vaccine had lower ($P < 0.001$ for all serotypes) antipneumococcal PS IgG concentrations at 7 months than at 2 months (data not shown). The 7-month concentrations were also lower than those measured at the same age in groups 1 and 2 ($P < 0.001$; Table 2). The threshold of ≥ 0.35 μg/ml was reached by 86–100% of the infants in groups 1 and 2 and by 0–12% in group 3 (Table 3). Among the Pn-PD recipients, the highest concentrations were induced for types 14 and 19F.

By 12 months of age, the antipneumococcal PS IgG concentrations in groups 1 and 2 decreased compared with those at 7 months. In group 3, the antibody concentrations remained at essentially the same level from 7 to 12 months of age (Table 2). The proportions of children with antibody concentrations of ≥ 0.35 μg/ml at 12 months ranged from 59 to 98% in groups vaccinated with 3 doses of Pn-PD. In

TABLE 2. GMC of Antibody Concentrations During a Course of Pneumococcal or Control (Hepatitis B) Vaccination

Age (mo)	Group* (N)	GMC by Serotype (μg/ml)										
		1	3	4	5	6B	7F	9V	14	18C	19F	23F
7	1 (51)	2.42 (1.95–2.99)†	2.06 (1.67–2.54)	2.33 (1.88–2.90)	1.42 (1.15–1.75)	1.26 (0.93–1.72)	1.75 (1.39–2.21)	1.86 (1.50–2.31)	4.66 (3.57–6.08)	1.41 (1.14–1.76)	3.71 (2.94–4.69)	1.37 (1.04–1.80)
	2 (51)	2.74 (2.13–3.53)	2.46 (1.91–3.16)	2.94 (2.26–3.84)	1.86 (1.51–2.30)	1.55 (1.06–2.27)	2.22 (1.73–2.84)	2.25 (1.65–3.06)	4.92 (3.54–6.83)	1.87 (1.44–2.42)	4.42 (3.42–5.70)	1.60 (1.13–2.27)
	3 (52)	0.07 (0.06–0.09)	0.09 (0.07–0.11)	0.04 (0.04–0.05)	0.12 (0.10–0.15)	0.06 (0.05–0.07)	0.07 (0.06–0.08)	0.06 (0.05–0.08)	0.13 (0.11–0.17)	0.05 (0.04–0.05)	0.14 (0.11–0.17)	0.06 (0.05–0.07)
12–15‡	1 (51)	0.58 (0.46–0.73)	0.56 (0.45–0.69)	0.80 (0.64–1.00)	0.88 (0.70–1.09)	0.83 (0.63–1.09)	0.73 (0.57–0.94)	0.87 (0.69–1.09)	2.01 (1.46–2.77)	0.43 (0.35–0.52)	1.62 (1.22–2.16)	0.61 (0.46–0.81)
	2 (51)	0.78 (0.60–1.01)	0.72 (0.53–0.98)	1.01 (0.78–1.31)	1.08 (0.86–1.36)	1.21 (0.87–1.66)	1.00 (0.77–1.31)	1.23 (0.93–1.62)	2.14 (1.55–2.94)	0.62 (0.47–0.83)	2.06 (1.58–2.69)	0.72 (0.50–1.05)
	3 (51)	0.09 (0.07–0.11)	0.10 (0.08–0.13)	0.07 (0.05–0.08)	0.26 (0.21–0.33)	0.08 (0.07–0.10)	0.09 (0.07–0.12)	0.09 (0.07–0.11)	0.12 (0.11–0.14)	0.06 (0.04–0.07)	0.19 (0.15–0.24)	0.07 (0.06–0.09)
13–16§	1 (24)	3.30 (2.61–4.17)	1.60 (1.26–2.03)	3.93 (2.93–5.26)	2.18 (1.66–2.85)	3.50 (2.29–5.36)	3.16 (2.40–4.15)	3.59 (2.50–5.15)	6.21 (3.89–9.90)	1.93 (1.57–2.37)	9.63 (7.00–13.23)	2.20 (1.60–3.01)
	2 (22)	34.01 (22.22–52.06)	7.24 (5.48–9.56)	16.06 (10.43–24.74)	7.98 (5.28–11.97)	7.98 (4.11–15.49)	8.36 (6.26–11.15)	21.01 (11.26–39.23)	22.83 (13.54–38.52)	6.43 (4.09–10.09)	40.54 (25.28–65.02)	4.24 (2.34–7.71)
	3 (25)	0.85 (0.55–1.31)	4.64 (3.30–6.53)	0.98 (0.64–1.51)	0.73 (0.52–1.03)	0.15 (0.09–0.25)	0.93 (0.61–1.43)	0.55 (0.35–0.86)	0.76 (0.49–1.18)	0.84 (0.53–1.32)	0.65 (0.41–1.04)	0.22 (0.14–0.32)

*For groups 1, 2 and 3, see Table 1.
†Numbers in parentheses, 95% confidence intervals.
‡Blood sample taken just before administration of the fourth vaccine dose.
§Blood sample taken 28 days after the fourth vaccination.

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group 3, the respective proportion varied between 2 and 41% (Table 3).

Serum samples were taken either 4 or 28 days after pneumococcal vaccination at 12-15 months (ie, either the 4th dose of Pn-PD, a dose of PncPS or the first dose of Pn-PD). In the samples taken 4 days after the fourth vaccination, the antibody concentrations were similar to those detected in samples obtained just before vaccination, and the individual increases were minimal and few (data not shown). Twenty-eight days after the vaccination, antipneumococcal IgG concentrations were higher than the pre-fourth vaccination concentrations for all serotypes in every vaccine group (Table 2). The GMCs were significantly lower in group 3 than in groups 1 and 2, except for serotype 3. In general, the highest GMCs were found in group 2 subjects who received PncPS vaccine at 12-15 months (Table 2). Proportions with antibody concentrations of ≥0.35 μg/ml varied depending on the serotype between 96 and 100%, 91 and 100% and 20 and 100%, in groups 1, 2 and 3, respectively (Table 3).

Safety. Mild local reactions were common and usually more frequently recorded after the first 3 doses of Pn-PD than after the corresponding dose of hepatitis B vaccine. Mild systemic reactions were common in all study groups. Severe reactions were few. During the study, there were 28 serious adverse events, and none was considered to be caused by the study vaccine.

Local Reactions. Within 8 days of each of the first 3 doses, local reactions were more common in children who received Pn-PD than in those who received hepatitis B vaccine (Table 4). Local pain preventing normal daily activities was rare in all groups and reported in fewer than 6% of the children after each of the first 3 doses. PncPS vaccine, as the fourth given after 3 doses of Pn-PD, seemed to cause more local redness and swelling than the fourth dose of Pn-PD (Table 4).

TABLE 3. Percentages of Samples with Antibody Concentration ≥0.35 μg/ml

Age (mo)	Group* (N)	Serotypes										
		1	3	4	5	6B	7F	9V	14	18C	19F	23F
7	1 (51)	100	100	100	96	90	98	98	100	98	100	94
	2 (51)	100	100	98	100	86	98	96	98	98	100	88
	3 (52)	4	8	0	12	0	4	8	10	0	12	2
12–15†	1 (51)	78	73	86	92	82	86	84	96	59	98	76
	2 (51)	80	78	90	94	86	94	92	96	71	98	73
	3 (51)	4	10	2	41	6	10	6	8	4	31	6
13–16‡	1 (24)	100	100	100	100	96	100	100	96	100	100	100
	2 (22)	100	100	100	100	91	100	100	100	100	100	91
	3 (25)	84	100	92	88	20	92	72	68	88	80	36

*For groups 1, 2 and 3, see Table 1.
†Blood sample taken just before administration of the fourth vaccine dose.
‡Blood sample taken 28 days after the fourth vaccination.

TABLE 4. Local Reactions Within 8 Days of Each Vaccination by Subject

	Vaccination	Group 1*		Group 2		Group 3	
		Any (%)	Grade 3† (%)	Any (%)	Grade 3 (%)	Any (%)	Grade 3 (%)
Pain†	1	31.4	2.0	43.1	2.0	9.6	0
	2	29.4	0	27.5	3.9	3.8	0
	3	27.5	2.0	21.6	0	3.9	0
	4	37.3	5.9	49.0	0	49.0	3.9
		Any (%)	> 30 mm (%)	Any (%)	>30 mm (%)	Any (%)	> 30 mm (%)
Redness	1	25.5	0	47.1	2.0	30.8	0
	2	37.3	2.0	43.1	0	25.0	0
	3	49.0	0	47.1	5.9	27.7	5.9
	4	39.2	2.0	52.9	13.7	29.4	7.8
Swelling	1	15.7	2.0	25.5	0	5.8	0
	2	17.6	2.0	23.5	2.0	7.7	0
	3	15.7	2.0	27.5	2.0	9.8	0
	4	17.6	0	27.5	7.8	15.7	3.9

*For groups 1, 2 and 3, see Table 1.
†Pain was considered to be of grade 3 intensity if it prevented normal activity.

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Systemic Reactions. Mild drowsiness, irritability and loss of appetite were common in all 3 study groups. Irritability preventing normal daily life was reported similarly in all groups, in fewer than 6% of the children. Drowsiness preventing normal activity or complete loss of appetite was not recorded after any dose during the 8-day routine follow-up (Table 5).

Fever reactions (>38°C) were more frequently recorded during the primary series of Pn-PD (27.5–49.0%) than during the primary series of hepatitis B vaccine (9.6–27.5%). Fever reactions seemed to increase by age and were recorded in all vaccine groups more often after the fourth than after the first vaccination (Table 5).

DISCUSSION

We found that the 11-valent Pn-PD conjugate vaccine was immunogenic and safe in infants. Three doses of Pn-PD induced a clear antibody response when compared with a control vaccine. After the fourth dose of the Pn-PD, there was a significant booster response as shown by the higher Pn-PD and PncPS response observed in the primed groups 1 and 2, than the Pn-PD response observed in the unprimed study group 3.

Three doses of Pn-PD induced similar immune responses in the current study as in a previous study in the Philippines, using a 6-10-14-week immunization schedule.[13] The immune response was comparable with other experimental pneumococcal conjugate vaccines studied in infants in Finland. There are, however, notable serotype specific differences in antiboily profiles among various vaccines[5,24–27]; eg after 4 doses of pneumococcal conjugate vaccine, geometric mean IgG concentration to serotype 18C varied between 1.41 and 7.8 μg/ml depending on the vaccine.

Children in group 3 received their first dose of Pn-PD at 12–15 months of age. The antibody concentrations measured in these children indicate 2 results: (1) even 1 dose of Pn-PD can induce an antibody increase in toddlers for most of the 11 serotypes but not for types 6B and 23F; (2) antibody concentrations were significantly lower than after 4 doses of the Pn-PD.

Children in group 2 received PncPS vaccine after 3 doses of Pn-PD. PncPS boosting is believed to give information about the development of B cell memory and to mimic a natural contact with pneumococcus. The antibody concentrations were high for all serotypes, even higher than after the Pn-PD booster. Hence we can assume that Pn-PD evoked the development of memory B cells that were then triggered by PS for antibody production. The significantly higher antipneumococcal antibody concentrations induced by PncPS booster than by Pn-PD booster might be caused by the higher PS content in the PncPS than in the Pn-PD vaccine. The same has been reported earlier with other pneumococcal conjugate vaccines.[19,26,28] In any case, it is apparent that the 3 doses of Pn-PD primed the children efficiently, except for serotype 3.

Previous studies have shown that there is a brisk antibody response on days 7–10 to a booster vaccination with either PncPS or a conjugate vaccine.[19,20] We wanted to see whether the antibody increase can be seen even earlier. To

TABLE 5. Systemic Reactions During 8-Day Follow-up Period After Each Vaccination (an Adverse Event That Prevents Normal Everyday Activities) by Subject.

Adverse Event*	Vaccination	Group 1†		Group 2		Group 3	
		Any (%)	Grade 3† (%)	Any (%)	Grade 3 (%)	Any (%)	Grade 3 (%)
Drowsiness	1	76.5	0	58.8	0	50.0	0
	2	39.2	0	25.5	0	32.7	0
	3	43.1	0	23.5	0	23.5	0
	4	47	0	49.0	0	56.9	0
Irritability	1	78.4	5.9	74.5	5.9	59.6	3.8
	2	64.7	0	58.8	3.9	50.0	5.8
	3	62.7	3.9	54.9	0	41.2	0
	4	74.5	0	66.7	2	78.4	3.9
Loss of appetite	1	39.2	0	35.3	0	21.2	0
	2	37.3	0	27.5	0	13.5	0
	3	37.3	0	25.5	0	33.3	0
	4	35.3	0	39.2	0	37.3	0
		≥38°C (%)	>39°C (%)	≥38°C (%)	>39°C (%)	≥38°C (%)	>39°C (%)
Fever	1	33.3	2.0	35.3	3.9	13.5	0
	2	27.5	2.0	37.3	11.8	9.6	0
	3	49.0	5.9	37.3	0	27.5	3.9
	4	41.2	3.9	45.1	9.8	43.1	5.9

*Drowsiness and irritability were considered to be of grade 3 intensity if they prevented normal activity. Loss of appetite was considered to be of grade 3 if the child was not eating at all.

†For groups 1, 2 and 3, see Table 1.

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this end, we took a blood sample on day 4 after vaccination at 12–15 months, but the antibody concentrations were still essentially the same as before the 4th vaccination. Thus no antibody response could be seen as early as 4 days after vaccination although a clear cut booster response was demonstrated at 28 days. This indicates that serum samples taken at 4 days are not useful for evaluating the immune responses after booster vaccination.

The local and systemic reaction rates after vaccination with Pn-PD were similar to those seen in Finnish infants vaccinated with other pneumococcal conjugate vaccines.[5,26] As with other pneumococcal conjugate vaccines, administration of Pn-PD was followed by local reactions and fever more often than was administration of hepatitis B vaccine. PncPS given after 3 doses of Pn-PD was relatively reactogenic: redness and swelling with a diameter of >30 mm were seen in 13.7 and 7.8%, respectively; and fever higher than 39°C occurred in almost 10%. This is noteworthy because in a previous study,[26] PncPS, when given after 3 doses of a 7-valent pneumococcal polysaccharide-meningococcal outer membrane protein complex conjugate vaccine, caused redness and swelling exceeding 25 mm in fewer than 1% and fever higher than 39°C in fewer than 1% of the vaccinees.

Well-characterized and predictive laboratory correlates are needed because of the expense, difficulty and ethical issues involved in conducting further efficacy trials. The measures for predicting the efficacy of a vaccine are difficult to estimate. At the moment, a threshold antibody concentration of ≥0.35 μg/ml (determined without adsorption with 22F polysaccharide) after the primary series has been proposed as a primary endpoint when comparing a new pneumococcal conjugate vaccine with a licensed vaccine.[18] In this study, almost 100% of the children achieved the 0.35 μg/ml threshold concentration of antibodies to 9 serotypes after the first 3 doses measured with the non-22F EIA. As for serotypes 6B and 23F, ~90% of infants achieved this concentration. The functional activity (opsonophagocytic activity and avidity) and the ability to induce immunologic memory are important secondary endpoints.[17,29] The opsonophagocytic activity of the Pn-PD antibodies in our subjects will be reported later.

We have shown that a novel 11-valent vaccine is well-tolerated and immunogenic. Presently there is only 1 licensed pneumococcal conjugate vaccine available for general use. The licensed vaccine contains 7 pneumococcal serotypes and an increase in the coverage would be beneficial in many countries. The efficacy of this vaccine against acute otitis media caused by vaccine serotypes and by nontypable *H. influenzae* is now tested in the Czech Republic and in the Slovakian Republic. In the Czech Republic, an 11-valent vaccine would cover 75% of the pneumococcal acute otitis media instead of a 51% coverage of a 7-valent vaccine.[30] The shortage in the supply of the licensed vaccine[31,32] supports further the need for licensure of new pneumococcal conjugate vaccines in the market.

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A RANDOMIZED, PLACEBO-CONTROLLED STUDY TO EVALUATE THE IMMUNOGENICITY OF AN 11-VALENT PNEUMOCOCCAL PROTEIN D CONJUGATE VACCINE ADMINISTERED AS PRIMARY VACCINATION TO INFANTS AT 6, 10 AND 14 WEEKS OF AGE

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Objective: To assess immunogenicity of an eleven-valent pneumococcal (11-Pn) *H influenzae* Protein D (PD) conjugate vaccine in infants.

Method: 300 subjects were randomly allocated either to receive the 11-Pn-PD vaccine (SBBiologicals or to serve as controls. Both groups also received concomitantly a DTPw-HBV/Hib vaccir (SBBiologicals). 11-Pn-PD vaccine contains 1 µg of pneumococcal serotypes 1, 3, 4, 5, 6B, 7F, 9V, 1· 18C, 19F, 23F, conjugated to PD. Infants were injected at 6, 10 and 14 weeks of age. Sera wei collected immediately before dose I and one month after dose III. Anticapsular antibodies ar opsonophagocytic titers were measured and reactogenicity was reported.

Results: One month post-dose III, 92 % to 100 % of subjects had titers ≥ 0.5 µg/mL, excep for anti-6B (82 %) and anti-23F (86 %). Moreover, 11 to 29-fold higher GMCs were observed i vaccinees compared to controls. 57 % to 98 % of subjects who received 11-Pn-PD wer seropositive for opsonophagocytic antibodies, compared to 0 % to 11 % for controls. Excep for serotype 3, opsonophagocytic anti-Pn GMTs were 4 to 50-fold higher in subjects wh received 11-Pn-PD than in controls. The immune responses induced by the concomitantl administered DTPw-HBV/Hib vaccine were similar in both groups. Safety profiles were simila between both groups, and between 11-Pn-PD and DTPw-HBV/Hib.

Conclusion: 11-Pn-PD vaccine is immunogenic, well tolerated, and does not impa immunogenicity of co-administered DTPw-HBV/Hib in infants.

Vaccine Profile

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EXPERT REVIEWS

10-valent pneumococcal nontypeable *Haemophilus influenzae* PD conjugate vaccine: Synflorix™

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The global burden of disease due to *Streptococcus pneumoniae* remains high. The licensed 7-valent pneumococcal conjugate vaccine (7vCRM, Prevenar™/Prevnar™) has successfully reduced invasive disease in the USA, but serotype coverage is incomplete and some evidence suggests that serotype replacement has occurred. Recently, a new 10-valent pneumococcal nontypeable *Haemophilus influenzae* (NTHi) protein D (PD) conjugate vaccine (PHiD-CV, Synflorix™) has been licensed in more than 40 countries, including Europe, for the prevention of invasive disease and acute otitis media (AOM) due to pneumococcus in infants and children. PHiD-CV is immunogenic in infants when administered as a three-dose primary vaccination in a range of schedules and has a safety profile comparable to that of 7vCRM. Additional serotypes in PHiD-CV (1, 5 and 7F) increase overall serotype coverage and improve coverage in specific age groups and against specific disease syndromes. The use of the PD carrier, which provided protection against AOM caused by NTHi in a large efficacy trial testing a prototype of the final vaccine formulation, suggests that PHiD-CV will also provide some protection against AOM due to NTHi.

Keywords: booster immunization • *Haemophilus influenzae* • immunization • otitis • pneumococcal conjugate vaccine • *Streptococcus pneumoniae*

Streptococcus pneumoniae is a major human pathogen, causing infections of the respiratory tract and spreading to peripheral tissues via the bloodstream (invasive pneumococcal disease [IPD]). *S. pneumoniae* is one of the major causes of bacterial meningitis in children, is a leading cause of bacterial community-acquired pneumonia and, along with nontypeable *Haemophilus influenzae* (NTHi), is the most common cause of bacterial acute otitis media (AOM) in children. According to the WHO, pneumococcal disease is responsible for 700,000–1 million deaths annually in children under 5 years of age [1]. Most of these deaths occur in children living in developing countries [2,3].

Invasive pneumococcal disease

Invasive pneumococcal disease manifests as meningitis, frank sepsis, bacteremic pneumonia, joint or other local infections, and occult bacteremia. In countries with widespread vaccination against *H. influenzae* type b (Hib), *S. pneumoniae* and *Neisseria meningitidis* are usually the major causes of bacterial meningitis in children aged 1 month or older. The highest incidence of IPD is found at the extremes of age. In the USA, the incidence of IPD in children under 1 year of age in 1998, prior to introduction of routine vaccination against pneumococcal disease, was 165 out of 100,000 [4]. The incidence in children 12–23 months of age in 1998 was 203 out of 100,000. Reported IPD incidence rates vary substantially between countries, most likely owing to differences in outpatient blood culture collection practices between countries [5–7]. It is likely that IPD rates are within the same range across industrialized countries, and are at least as high, or higher in other countries [8,9]. Much higher IPD incidence rates have been reported among indigenous populations in industrialized countries, such as Aboriginal Australians and Native Alaskans [10,11].

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Otitis media

Acute otitis media is a common childhood disease with the peak age-specific attack rate occurring between 6 and 18 months of age. It is estimated that at least 70% of AOM is bacterial in origin, with *S. pneumoniae* and NTHi being the leading causes [12]. Despite the widespread use of antibiotics, complications of AOM still occur and may include secretory OM, mastoiditis and meningitis [13].

In European countries, the reported AOM rates range between 0.125 and 1.24 episodes per child-year [14–16], with the latter figure approaching rates reported in the USA [17]. Otitis rates in indigenous populations are distinctly higher than in other populations [18,19].

Pneumonia

Pneumonia is the largest single infectious disease cause of infant mortality globally [20]. The incidence (episodes per child-year) of pneumonia before the age of 5 years is estimated to be 0.05 in industrialized countries, and almost six-times higher than this (0.29) in developing countries [21,22]. Notwithstanding difficulties in identifying the responsible etiological agent, pneumococcus, along with Hib, are generally believed to be the major bacterial pathogens. In studies of vaccine effectiveness, vaccination with 7-, 9- or 11-valent pneumococcal conjugate vaccines (PCVs) reduced radiologically diagnosed pneumonia by 20–37% [23–26].

Given its role as a major cause of AOM in children, an etiological role for NTHi in other infections of the respiratory tract seems likely. NTHi has accounted for 11% of pneumonia cases in children, second after *S. pneumoniae* (33%) [27], although the available evidence suggests that there is regional variation in its importance as a lower respiratory pathogen [27–29].

Licensed pneumococcal conjugate vaccines

To date, two vaccines have been licensed for use: a seven-valent PCV containing polysaccharides from serotypes 4, 6B, 9V, 14, 18C, 19F and 23F, all conjugated to the mutated nontoxic diphtheria toxin (7vCRM; Prevenar™ or Prevnar™) from Wyeth Lederle S.A. (Pearl River, NY, USA) and the 10-valent PCV (PHiD-CV, Synflorix™) from the GlaxoSmithKline (GSK) group of companies, Belgium.

After the introduction of 7vCRM into the US vaccination calendar in 2000, IPD was reduced by 77% in children under 5 years of age compared with prevaccination levels [30–33], and hospital admissions due to all-cause pneumonia declined by 39% in children under 2 years of age, with an estimated 65% decline in hospitalized pneumonia coded as pneumococcal infection in this age group [34].

Why develop a new 10-valent pneumococcal vaccine?

There are at least 91 immunologically distinct pneumococcal serotypes, of which 10–15 are implicated in the vast majority of invasive disease worldwide [35]. Although the serotypes included in 7vCRM are estimated to already cover 80–90% of serotypes causing IPD in young children in North America and Australia, this decreases to 70–75% in western Europe and Africa, and to only 50–65% in Latin America and Asia [35–38,201].

Serotypes not found in 7vCRM appear to play an important role in certain disease syndromes and in some age groups. Serotypes 1, 5 and 7F in particular have been associated with severe clinical syndromes [39–41], and the risk of severe disease and death due to pneumococcal infection has been found to be among the highest following serotype 7F infection [42]. Serotype 1 is implicated in complicated pneumonias and pleural empyema in children [40,43–46], is commonly isolated in children over 2 years of age, and is an important cause of IPD in Asia and Africa [35,47–49]. Serotype 5 is the third most important serotype causing IPD in young children in Latin America [35,38].

The recently licensed 10-valent PCV PHiD-CV includes three additional serotypes (1, 5 and 7F) and therefore increases the serotype coverage against IPD to 80% or more in most regions [35,37,201], including the serotypes responsible for disease in older children [50,51].

Availability of a second PCV will also contribute to increasing global supply and improving coverage in developing countries for which the WHO recommends routine vaccination of children with PCV [1]. However, as of 2008, only 26 out of 193 WHO member states included PCVs in their vaccination programs [30], and in 2009 only one of the 72 Global Alliance for Vaccines and Immunisation-eligible countries provides PCV [201,202].

Why select a new carrier protein?

Coadministration of vaccines conjugated to a protein carrier similar to an antigen included in the coadministered vaccine has been linked with immune suppression due to carrier-mediated epitopic suppression or bystander interference [52,53]. The use of the novel protein D (PD) carrier protein instead of carrier proteins closely related to coadministered antigens therefore minimizes the risk of interference related to the carrier protein and, importantly, by virtue of its antigenic properties, may prevent AOM due to NTHi. A direct protective effect of PD on AOM caused by NTHi has been demonstrated in children vaccinated with an 11-valent PCV that was the predecessor to PHiD-CV [14].

The initial 11-valent PCV (11Pn-PD) contained serotype 3 in addition to the ten serotypes in PHiD-CV. 1 µg of polysaccharide for each of the 11 serotypes contained in 11Pn-PD was conjugated to PD. A large, randomized, double-blind, placebo-controlled Pneumococcal Otitis Efficacy Trial (POET) was conducted in Europe between 2000 and 2004 [14]. Infants received 11Pn-PD or the hepatitis A control vaccine at 3, 4, 5 and 12–15 months of age. Follow-up for according-to-protocol vaccine efficacy began 2 weeks after dose three and continued until 24–27 months of age. A total of 4968 infants were enrolled and vaccinated, of which 2489 received 11Pn-PD.

POET was the first study to demonstrate a statistically significant and clinically relevant protective effect of vaccination on the overall AOM disease burden (33.6% reduction; 95% CI: 20.8–44.3). AOM episodes caused by *S. pneumoniae* vaccine serotypes were reduced by 57.6% (95% CI: 41.4–69.3) and AOM episodes caused by NTHi were reduced by 35.3% (95% CI: 1.8–57.4).

These results confirm the potential of the PD carrier protein to exert a protective immune response against disease due to NTHi, and indicate that PD functions well as a carrier protein for pneumococcal polysaccharides, because consistent functional and boostable immune responses are induced after immunization for all conjugated serotype polysaccharides contained in PHiD-CV [14,54].

Introduction to PHiD-CV

Composition & indications

PHiD-CV contains 1 µg of each capsular polysaccharide of the pneumococcal serotypes 1, 5, 6B, 7F, 9V, 14 and 23F, and 3 µg of serotype 4, conjugated individually to PD, a recombinant nonlipidated form of a highly conserved 42-kDa cell-surface lipoprotein of NTHi; 3 µg of serotype 18C capsular polysaccharide conjugated to tetanus toxoid; and 3 µg of serotype 19F capsular polysaccharide conjugated to diphtheria toxoid. The total polysaccharide content of the vaccine is the same as that of 7vCRM, despite the additional serotypes present. The pneumococcal conjugates are adsorbed onto an aluminium phosphate adjuvant.

PHiD-CV has been licensed in Europe via the centralized procedure (under the name Synflorix) and in several other countries around the globe, and regulatory review is ongoing elsewhere. Depending on local regulatory approvals, PHiD-CV can be indicated for active immunization against diseases caused by *S. pneumoniae* (including sepsis, meningitis, pneumonia, bacteremia and AOM) and against AOM caused by NTHi. Given that PHiD-CV is mainly licensed based on immunological data, some regulatory authorities granted licensure with more restricted indications, pending availability of additional clinical data. In infants, the primary vaccination schedule consists of three 0.5 ml doses with an interval of at least 1 month between doses. A booster dose is recommended 6 months after the last priming dose, preferably between 12 and 15 months of age. In infants aged 7–11 months the vaccination schedule consists of two doses of 0.5 ml with an interval of at least 1 month between doses, and a third dose recommended in the second year of life with an interval of at least 2 months after the previous dose. In children 12–23 months of age, two doses should be given with an interval of at least 2 months between doses. The need for a booster dose after this immunization schedule has not been evaluated.

Changes to the first formulation

Three key changes were made to 11Pn-PD tested in previous clinical trials [14,55]. In the final PHiD-CV formulation, the carrier protein for serotype 18C polysaccharide was changed to tetanus toxoid and the carrier protein for serotype 19F polysaccharide was changed to diphtheria toxoid. In addition, it was decided that serotype 3 should be removed from the vaccine, since protective efficacy against pneumococcal serotype 3 AOM episodes could not be demonstrated in the POET study. Another reason for removing this serotype was due to the observation of an atypical immune response against serotype 3, for which postbooster antibody concentrations remained below those measured postprimary and even significantly below serotype 3 antibody concentrations measured after a single dose of conjugate vaccine in the second year of life [55,56]. In addition, functional opsonophagocytic activity against serotype 3, although measurable following primary immunization and inducing a good booster response, did not correlate with clinical protection [57]. The particular characteristics of *S. pneumoniae* serotype 3 that appear to reduce its susceptibility to host defense mechanisms have recently been reviewed [57].

Overview of studies conducted with PHiD-CV

Published immunogenicity, reactogenicity and safety data of PHiD-CV summarized in this review have been generated in five primary vaccination studies, including large-scale randomized, controlled clinical trials, involving in total 3806 healthy infants receiving PHiD-CV of which 2949 were evaluated for immunogenicity (Table 1). PHiD-CV booster data are reviewed from three studies involving 2801 healthy toddlers receiving a PHiD-CV booster dose, of which 1577 were included in immunogenicity evaluations (Table 2). Studies were conducted in industrialized and developing countries in Europe and Asia. Commonly used three-dose primary vaccination schedules were utilized, including the WHO Expanded Program on Immunization recommended schedule of dosing at 6, 10 and 14 weeks of age. The two-dose primary vaccination schedule followed by a booster dose (2 + 1 schedule) used in some Northern European countries and Italy, was also assessed.

Studies with vaccine control groups were double-blind unless there were differences in the appearances between PHiD-CV and the control vaccine, in which case the study was single-blind. All enrolled infants and children were in good health and had no previous pneumococcal disease or exposure to pneumococcal vaccines. In line with routine clinical practice, commonly recommended pediatric vaccines were coadministered with PHiD-CV in all studies.

Immune responses following PHiD-CV primary & booster vaccination

Methods used to measure pneumococcal immune responses

Vaccine-induced protection against *S. pneumoniae* can be conferred by antibodies directed against the polysaccharide capsule that facilitate phagocytosis via opsonization of the pathogen. It is generally accepted that protection against IPD is achieved when circulating antibodies reach a minimal level [58–60]. Immunological thresholds have been put forward as licensure criteria for demonstration of noninferiority of the immune response induced by a new candidate vaccine compared with a licensed vaccine with proven efficacy [61]. These licensure criteria reflect the minimum criteria required to guarantee sufficient protective efficacy. Different vaccines have been licensed in the past based on such criteria (e.g., Hib conjugate and meningococcal conjugate vaccines), despite differences in the immunological profile.

A consensus on licensure criteria for vaccine-induced protection against IPD was reached at a WHO Expert Committee meeting in 2003 [61] and was recently confirmed [62]. The primary criterion

Table 1. Summary of primary vaccination studies evaluating the immunogenicity and safety of PHiD-CV.

Study number	Country	Schedule (months)	Pneumococcal vaccine	Coadministered vaccines	Number enrolled	Number evaluated for immunogenicity*	Mean age (SD) at first dose (weeks)	Ref.
Primary vaccination studies in Europe								
001	Finland, France	2, 3, 4	PHiD-CV	DTPa–HBV–IPV/Hib[†]	1235	1108	9.0 (2.03)	[70]
NCT00307554	Poland		7vCRM	DTPa–HBV–IPV/Hib[†]	415	376	8.8 (2.00)	
011	Germany	2, 4, 6	PHiD-CV	DTPa–HBV–IPV/Hib + MenC-CRM[‡]	385	171	8.0 (2.23)	[77]
NCT00334334	Poland		PHiD-CV	DTPa–HBV–IPV/Hib + MenC-TT[‡]	387	178	8.1 (2.13)	
	Spain		PHiD-CV	DTPa–HBV–IPV + HibMenC-TT	386	175	8.1 (2.19)	
			7vCRM	DTPa–HBV–IPV + HibMenC-TT	390	174	8.1 (2.37)	
012	Poland	2, 4, 6	PHiD-CV	DTPw–HBV/Hib + IPV	303	285	7.4 (1.50)	[72]
NCT00344318			7vCRM	DTPw–HBV/Hib + IPV	103	96	7.6 (1.57)	
010[§] NCT00370318	Czech Republic	3, 4, 5	PHiD-CV (NPP)	DTPa–HBV–IPV/Hib (+HRV at 3 and 4 months)	233	227	12.3 (2.21)	[75]
			PHiD-CV (PP)	DTPa–HBV–IPV/Hib (+HRV at 3 and 4 months)	226	208	12.2 (2.06)	
002	Denmark, Norway	3, 5 or	PHiD-CV	DTPa–HBV–IPV/Hib[¶]	175	158	12.0 (1.91)	[73]
NCT307034	Slovakia, Sweden	3, 4, 5			176	154	12.2 (1.90)	
Primary vaccination study in Asia								
012	The Philippines	1.5, 2.5, 3.5	PHiD-CV	DTPw–HBV/Hib + OPV	300	285	7.5 (1.65)	[72]
NCT00344318			7vCRM	DTPw–HBV/Hib + OPV	100	95	7.4 (1.52)	

*Pneumococcal responses.
†In France, the second pneumococcal vaccine dose was coadministered with DTPa–IPV/Hib.
‡MenC-CRM and MenC-TT were only administered at 2 and 4 months of age.
§Subjects in study 010 were randomized to receive prophylactic paracetamol with and soon after vaccination (PP group) or to receive no paracetamol prophylaxis (NPP group); see footnote of Table 2 for details of coadministered vaccines.
¶DTPa–IPV/Hib was administered in Denmark and Norway.
7vCRM: 7-valent pneumococcal conjugate vaccine; DTPa–HBV–IPV/Hib: Diphtheria–tetanus–acellular pertussis–hepatitis B virus–inactivated polio virus/*Haemophilus influenzae* type b vaccine; DTPw–HBV/Hib: Diphtheria–tetanus–whole-cell pertussis–hepatitis B virus/*H. influenzae* type b vaccine; HibMenC-TT: *H. influenza* type b–*Neisseria meningitidis* serogroup C–tetanus toxoid vaccine; NPP: No paracetemol prophylaxis; OPV: Oral polio vaccine; PHiD-CV: 10-valent pneumococcal nontypeable *H. influenzae* (NTHi) protein D (PD) conjugate vaccine; PP: Prophylactic paracetamol; SD: Standard deviation.

Table 2. Summary of booster vaccination studies evaluating the immunogenicity and safety of PHiD-CV.

Study number	Country	Schedule (months)	Primary vaccination		Booster vaccination		Number enrolled	Number evaluated for immunogenicity	Mean age (SD) at booster (months)	Ref.
			Pneumococcal vaccine	Coadministered vaccines	Pneumococcal vaccine	Coadministered vaccines				
007 (Booster 001)	Finland	12–18	PHiD-CV	DTPa–HBV–IPV/Hib	PHiD-CV	DTPa–HBV–IPV/Hib	737	343	15.3 (2.08)	[70]
NCT00370396	Poland		7vCRM	DTPa–HBV–IPV/Hib	7vCRM	DTPa–HBV–IPV/Hib	92	91	14.2 (2.27)	
	France		7vCRM	DTPa–HBV–IPV/Hib	PHiD-CV	DTPa–HBV–IPV/Hib	283	134	14.2 (2.24)	
017 (Booster 011) NCT00463437	Germany, Poland, Spain	11–18	PHiD-CV	DTPa–HBV–IPV/Hib + MenC-CRM	PHiD-CV	DTPa–HBV–IPV/Hib[†] + MenC-CRM	359	158	14.2 (1.68)	[77]
			PHiD-CV	DTPa–HBV–IPV/Hib + MenC-TT	PHiD-CV	DTPa–HBV–IPV/Hib[†] + MenC-TT	363	160	14.2 (1.68)	
			PHiD-CV	DTPa–HBV–IPV + HibMenC-TT	PHiD-CV	DTPa–HBV–IPV[‡] + HibMenC-TT	358	161	14.2 (1.68)	
			7vCRM	DTPa–HBV–IPV + HibMenC-TT	7vCRM	DTPa–HBV–IPV[‡] + HibMenC-TT	357	156	14.2 (1.72)	
014[§] (Booster 010) NCT00496015	Czech Republic	12–15	PHiD-CV (NPP)	DTPa–HBV–IPV/Hib + HRV	PHiD-CV (NPP)	DTPa–HBV–IPV/Hib	172	168	12.7 (0.77)	[75]
			PHiD-CV (PP)	DTPa–HBV–IPV/Hib + HRV	PHiD-CV (PP)	DTPa–HBV–IPV/Hib	178	141	12.6 (0.77)	
002 (Booster phase) NCT307034	Denmark, Norway, Slovakia, Sweden	11–12	PHiD-CV (three doses)	DTPa–HBV–IPV/Hib[¶]	PHiD-CV	DTPa–HBV–IPV/Hib[¶]	175	158	11.1 (0.61)	[73]
		11–12	PHiD-CV (two doses)	DTPa–HBV–IPV/Hib[¶]	PHiD-CV	DTPa–HBV–IPV/Hib[¶]	176	154	11.2 (0.63)	

*Pneumococcal responses.
[†]Subjects in Spain received a booster dose of DTPa–IPV/Hib or DTPa–IPV.
[§]Subjects in study 014 were randomized before the protocol amendment (subjects after protocol amendment not shown) to receive prophylactic paracetamol with and soon after vaccination (PP group) or to receive no paracetamol prophylaxis (NPP group).
[¶]DTPa–IPV/Hib was administered in Denmark and Norway.
Coadministered vaccines were manufactured by GSK except where indicated: DTPa–HBV–IPV/Hib = Infanrix hexa™; DTPa–HBV–IPV = Infanrix™ penta/Pediarix™; DTPa–IPV/Hib = Infanrix™ IPV Hib; DTPa–IPV = Infanrix™ IPV; DTPw–HBV/Hib = Tritanrix™HepB/Hib; IPV = Poliorix™; OPV = Polio Sabin™; HibMenC-TT = Menitorix™; HRV = Rotarix™; MenC-TT = NeisVac-C™, Baxter Healthcare SA; MenC-CRM = Meningitec™, Wyeth Lederle Vaccines.
7vCRM: 7-valent pneumococcal conjugate vaccine; DTPa–HBV–IPV/Hib: Diphtheria–tetanus–acellular pertussis–hepatitis B virus–inactivated polio virus/*Haemophilus influenzae* type b vaccine; DTPw: Diphtheria–tetanus–whole-cell pertussus vaccine; HibMenC-TT: *H. influenza* type b–*Neisseria meningitidis* serogroup C–tetanus toxoid vaccine; NPP: No paracetamol prophylaxis; OPV: Oral polio vaccine; PHiD-CV: 10-valent pneumococcal nontypeable *H. influenzae* protein D conjugate vaccine; PP: Prophylactic paracetamol; SD: Standard deviation.

was based on the percentage of vaccinated subjects who reached a reference serotype-specific IgG antibody concentration of at least 0.35 µg/ml, as measured by ELISA applied during 7vCRM efficacy trials in the USA and South Africa. Although this threshold is sometimes referred to as the 'putative protective antibody concentration', variability between studies, serotypes and individuals has hampered the definition of a real individual correlate of protection. Therefore, the predefined threshold should not be used as an absolute measure of protection, but only as a comparative threshold for immune responses between different vaccines as an indication of relative protection at the population level compared with a vaccine for which efficacy has been documented.

Antipolysaccharide IgG antibody measurements by ELISA are influenced by a number of factors, such as the purity of the polysaccharides used to coat the ELISA plates and the implementation of specific process steps to increase the specificity of the antibody measurements. Preadsorption of sera with the common cell wall polysaccharide as well as with heterologous 22F polysaccharide is now recommended to increase the specificity of ELISA [63,64]. This preadsorption method (22F ELISA) is therefore employed by most laboratories, including the WHO reference laboratory and GSK Biologicals' laboratories in Belgium. An antibody concentration of 0.35 µg/ml, as measured with the non-22F ELISA at the WHO reference lab, was shown to correspond to a concentration of 0.20 µg/ml using GSK Biologicals' 22F-inhibition ELISA [65].

Secondary criteria for licensure include demonstration of the functional capacity of antibodies through measurement of opsonophagocytic activity (OPA) [61]. The biological function of vaccine-induced antibodies to bind to the pneumococcal surface and to induce complement activation and phagocytosis of the pneumococcus by polymorphonuclear leukocytes can be measured *in vitro* in an OPA assay. Data from clinical trials show better correlations between 7vCRM vaccine effectivenesss against IPD [32] and measurable OPA (percentage of subjects with an OPA titer ≥8) compared with the percentage of subjects reaching the 0.2 µg/ml 22F-ELISA threshold (Table 3). OPA is therefore considered to be the most reliable correlate of protection [58,60,66]. GSK Biologicals developed and validated an OPA killing assay using a HL 60 cell line [67,68] and the results are expressed as the dilution of serum (opsonic titer) needed to sustain 50% killing of live pneumococci under assay conditions.

Secondary licensure criteria also require demonstration of the induction of immunological memory following primary vaccination with PCVs, since induction of memory is considered a key feature that differentiates conjugate from simple polysaccharide vaccines [69].

Immune responses following three-dose primary vaccination studies

Data are available from five studies evaluating immune responses following three-dose primary vaccination with PHiD-CV in healthy infants, including three studies with a 7vCRM control group (Table 1).

Geometric mean concentrations (GMCs) for antibodies against pneumococcal serotypes contained in both vaccines tended to be higher in the 7vCRM groups compared with the PHiD-CV groups, with the exception of antibodies against serotypes 18C (within the same range for both vaccines) and 19F (higher in the PHiD-CV groups in several studies). The percentage of subjects with antipneumococcal antibody concentrations 0.2 µg/ml or higher was, however, within the same range in PHiD-CV and 7vCRM groups for each of the vaccine serotypes, except for serotypes 6B and 23F, which tended to be lower in the PHiD-CV groups (Table 4).

Table 3. Correlation between IgG antibody responses and opsonophagocytic activity titers following three-dose primary vaccination with 7vCRM, and 7vCRM vaccine effectiveness against invasive pneumococcal disease.

Serotype	ELISA ≥0.2 µg/ml			Vaccine effectiveness[a]		OPA ≥1:8		
	n[b]	%	95% CI	%	95% CI	n[b]	%	95% CI
4	734	100	100–100	93	65–99	334	100	99–100
6B	732	85	83–88	94	77–98	332	95	92–97
9V	734	100	99–100	100	88–100	331	100	98–100
14	734	100	99–100	94	81–98	338	98	95–99
18C	734	99	98–100	97	85–99	331	99	97–100
19F	736	99	98–100	87	65–95	331	91	88–94
23F	734	95	93–96	98	80–100	327	99	97–100
6A	450	52	47–57	76	39–90	275	78	72–83
19A	451	32	28–37	26	-45–62	279	2	1–4

[a]CDC case control [32].
[b]Pooled data of 7vCRM vaccinees from studies 001 [70], 011 [77] and 012 [72].
7vCRM: 7-valent pneumococcal conjugate vaccine; CI: Confidence interval; N: Number; OPA: Opsonophagocytic activity.

For common serotypes, OPA geometric mean titers (GMTs) were higher in 7vCRM groups than PHiD-CV groups, with some exceptions, most notably serotype 19F for which OPA GMTs were consistently higher after PHiD-CV priming. The percentage of subjects who achieved an OPA titer of 8 or more was, however, within the same range for all common serotypes. OPA seropositivity was usually lower for serotype 1 compared with the other serotypes (Table 5).

Noninferiority of PHiD-CV compared with 7vCRM

In line with the WHO licensure criteria, the primary objective of study 001 [70] was to demonstrate noninferiority of immune responses following PHiD-CV (n = 1108) compared with 7vCRM (n = 376). Noninferiority in terms of the percentage of subjects achieving the 0.2 µg/ml antibody concentration was demonstrated for eight out of ten vaccine serotypes. Despite a greater than 10% difference in the percentage of subjects achieving the 0.2 µg/ml antibody concentration against serotypes 6B and 23F, high functional OPA responses were measured for all common serotypes, with limited differences between groups in the percentage of subjects with OPA titers of 8 or more, including serotypes 6B and 23F. This is of particular relevance since ELISA IgG antibody measurements may underestimate the serotype-specific effectiveness for serotypes 6B and 23F [67]. The biological activity of antibodies against serotypes 6B and 23F was previously documented in the POET study with a clinical efficacy of 87.6% (95% CI: 58.4–96.3) and 72.3% (95% CI: 24.8–89.8) against serotype 6B and 23F AOM, respectively, using the predecessor 11Pn-PD vaccine containing the same 6B–PD and 23F–PD conjugates as PHiD-CV [14]. In addition, since the antibody levels needed for protection against OM are likely to be higher than those needed for protection against IPD [71], it is unlikely that the lower antibody responses against 6B and 23F observed following vaccination with PHiD-CV indicate reduced efficacy against these serotypes.

Immune responses with the 6-, 10- and 14-week immunization schedule in the Philippines

The accelerated immunization schedule advocated by the WHO was specifically assessed in the Philippines [72]. In this study, PHiD-CV or 7vCRM were coadministered at 6, 10 and 14 weeks of age with a whole-cell pertussis-based combination vaccine (diphtheria–tetanus–whole-cell pertussis–hepatitis B virus [DTPw–HBV/Hib]). Antipneumococcal antibody and OPA responses in Philippino infants (Tables 5 & 6) were substantially higher for all serotypes compared with the response measured in European infants, including a group of infants vaccinated in the same study at 2, 4 and 6 months of age with PHiD-CV coadministered with DTPw–HBV/Hib in Poland [72]. For the common serotypes, antibody GMCs and OPA GMTs against serotypes 18C and 19F were substantially higher in the PHiD-CV group compared with the 7vCRM group, whereas the OPA GMT against serotype 23F was significantly higher in the 7vCRM group.

Booster vaccination studies

Immunogenicity data following a fourth consecutive (booster) dose of PHiD-CV are available from four studies (Table 2). Robust increases were observed from pre- to postbooster in terms of antibody GMCs (between a 4- and 16.7-fold increase) as well as OPA GMTs (between a 3.9- and 103.6-fold increase), surpassing postprimary levels for each serotype and therefore indicating effective priming during the infant immunization course.

Opsonophagocytic activity titers of 8 or more were observed in more than 90% of subjects against all serotypes, with the exception of a somewhat lower response in one study against serotype 1 (77.8%) (Table 7). Postbooster OPA titers tended to be lower in PHiD-CV groups compared with the 7vCRM primed and boosted subjects, with the exception of serotypes 18C and 19F, for which OPA GMTs were comparable (18C) or higher (19F) in PHiD-CV groups compared with 7vCRM groups.

Interchangeability of the booster dose

In study 007 [70], a random subset of children primed with three doses of 7vCRM received a PHiD-CV booster dose in their second year of life. In this group, the percentage of subjects with antibody concentrations of 0.2 µg/ml or more and the percentage of subjects with OPA titers of 8 or more for the common serotypes were within the same range as that observed in 7vCRM primed and boosted subjects. Boosting was also evident from increases in GMC/GMT postbooster, although antibody GMCs and OPA GMTs tended to be higher following the 7vCRM booster dose for the common serotypes.

For serotypes 1, 5 and 7F, ELISA antibody and OPA responses were substantially higher compared with the group that received primary and booster vaccination with 7vCRM. Antibody GMCs and OPA GMTs did not, however, reach the same levels as those observed after a fourth consecutive PHiD-CV dose. It is not clear whether a single booster dose of PHiD-CV in the second year of life will be sufficient to confer protection against diseases due to serotypes 1, 5 and 7F.

These data demonstrate that a booster dose of PHiD-CV in 7vCRM-primed children induces an adequate immune response to the seven 7vCRM serotypes, and a modest response to the three new serotypes as an additional benefit.

Two-dose primary plus booster vaccination

Immunogenicity following a two-dose PHiD-CV primary vaccination at approximately 3 and 5 months of age followed by a booster dose at 11–12 months (2 + 1 schedule) was compared with a conventional three-dose priming (at 3, 4 and 5 months of age) plus booster vaccination (3 + 1 schedule) in study 002 [73]. The percentage of two-dose primed subjects with antibody concentrations of 0.2 µg/ml or more was within the same range compared with the percentages observed following three-dose primary schedules studied in Europe for all serotypes, except 6B and 23F for which the percentage of subjects with antibody concentrations of 0.2 µg/ml or more was 55.7 and 69.3%, respectively (Table 4). These percentages are comparable to the serotype 6B results measured 2 months after the second dose of PHiD-CV administered at 2 and 4 months of

Table 4. IgG antibody responses against pneumococcal serotypes following primary vaccination (primary ATP cohort for immunogenicity, except [§]).

Schedule	2, 3, 4 months		2, 4, 6 months					
Study (Ref.)	007 [10]		011 [5,6]				012 [7]	
Group and number of subjects (n)[†]	PHiD-CV (n = 1107)	7vCRM (n = 375)	PHiD-CV + MenC-CRM (n = 169)	PHiD-CV + MenC-TT (n = 175)	PHiD-CV + HibMenC-TT (n = 173)	7vCRM + HibMenC-TT (n = 170)	PHiD-CV (n = 285)	7vCRM (n = 96)
% ≥0.2 µg/ml								
1	97.3	4.0	96.4	97.7	93.1	0.6	98.2	3.1
4	97.1	100.0	100.0	99.4	98.3	100.0	98.9	100.0
5	99.0	1.9	100.0	100.0	98.8	2.4	98.9	2.1
6B	65.9	79.0	94.1	88.6	87.3	92.9	85.6	94.8
7F	99.5	4.5	100.0	99.4	98.8	3.0	100.0	5.2
9V	98.1	99.5	98.8	97.7	98.3	98.8	100.0	100.0
14	99.5	99.5	100.0	100.0	100.0	99.4	100.0	100.0
18C	96.0	98.9	98.8	98.9	99.4	98.8	98.6	99.0
19F	95.4	99.2	98.2	99.4	98.8	100.0	98.9	99.0
23F	81.4	94.1	95.9	96.0	92.5	94.1	94.4	99.0
GMC (µg/ml)								
1	1.05	0.03	1.15	1.09	1.00	0.03	1.04	0.03
4	1.45	2.78	1.88	1.96	1.70	2.78	1.64	2.14
5	1.70	0.03	1.96	1.87	1.69	0.03	1.62	0.03
6B	0.33	0.59	0.96	0.85	0.71	1.32	0.73	1.23
7F	1.72	0.04	2.82	2.57	2.25	0.04	2.25	0.04
9V	1.32	2.68	1.77	1.72	1.58	3.17	1.51	2.70
14	2.90	4.49	3.75	3.79	3.36	5.97	3.31	5.23
18C	1.66	2.46	2.43	3.92	2.34	3.01	3.74	2.64
19F	1.84	3.42	4.93	4.71	3.81	2.56	5.30	2.38
23F	0.53	1.34	1.30	1.20	0.96	2.46	1.11	2.20

[†]n: Number of subjects in ATP cohort for immunogenicity with available results for at least one serotype (the actual number of subjects tested can vary slightly for the different serotypes, depending on the number of sera available).
[‡]Subjects in study 010 were randomized to receive prophylactic paracetamol with and soon after vaccination (PP group) or to receive no paracetamol prophylaxis (NPP group).
[§]Post-dose 2 subset based on booster ATP cohort for immunogenicity.
7vCRM: 7-valent pneumococcal conjugate vaccine; GMC: Geometric mean concentration; HibMenC-TT: *Haemophilus influenzae* type b–*Neisseria meningitidis* serogroup C–tetanus toxoid vaccine; PHiD-CV: 10-valent pneumococcal nontypeable *H. influenzae* protein D conjugate vaccine.

age in study 011 (64.1%), and higher than the 30.8% 6B responses following two doses of 7vCRM in that same study (Table 4).

Study 002 was the first to report functional OPA responses for a 2 + 1 schedule. The percentage of subjects reaching an OPA titer of 8 or more following two primary PHiD-CV doses was within the same range as the OPA response rate following a three-dose priming for serotypes 1, 4, 9V and 14, but was lower for the other serotypes (Table 5). As with the antibody responses, lower OPA responses were also measured 2 months after the second 7vCRM dose in study 011, especially for serotype 6B (34.9% with OPA titer ≥8) compared with 62.8% 2 months after the second PHiD-CV dose (Table 5).

Booster responses at 11–12 months of age were observed in the 2 + 1 group against all vaccine serotypes, although the percentage of subjects with postbooster antibody concentrations of 0.2 µg/ml or more remained lower for serotype 6B following a 2 + 1 schedule as compared with a 3 + 1 schedule (Table 6). Postbooster OPA titers rose substantially for all serotypes in both groups in study 002, but remained lower in the 2 + 1 group compared with those measured in the 3 + 1 group, particularly for serotypes 1, 5 and 6B (Table 7).

The lower immunogenicity of the two-dose primary schedule compared with the three-dose primary schedule is in line with previous observations with 7vCRM [74] and highlights the

Table 4. IgG antibody responses against pneumococcal serotypes following primary vaccination (primary ATP cohort for immunogenicity, except [§]) (cont.).

3, 4, 5 months			6, 10, 14 weeks		3, 5 months	2, 4 months	
010 [¶]		002 [¶]	012 [¶]		002 [¶]	011 [¶]	
PHiD-CV NPP (n = 227)	PHiD-CV AP (n = 208)	PHiD-CV (n = 154)	PHiD-CV (n = 285)	7vCRM (n = 95)	PHiD-CV (n = 158)	PHiD-CV pooled (n = 1561)	7vCRM + HibMenC-TT (n = 1461)
% ≥ 0.2 µg/ml (cont.)							
99.1	97.6	98.7	100	3.2	97.4	95.8	4.2
99.6	99.5	99.3	99.3	100	98.0	98.7	99.3
99.6	99.5	100.0	100	3.2	96.1	96.5	2.1
75.6	62.1	63.1	91.2	86.3	55.7	64.1	30.8
99.6	99.0	99.3	99.6	9.5	96.7	98.6	6.4
98.7	98.0	99.3	99.6	100	93.4	96.1	96.6
99.6	99.5	100.0	100	100	96.1	99.4	97.9
99.6	95.7	99.3	99.6	100	96.1	87.8	97.3
100.0	97.6	96.1	100	98.9	92.8	96.2	99.3
87.1	80.4	77.6	97.2	94.7	69.3	75.0	74.7
GMC (µg/ml) (cont.)							
1.45	0.92	1.23	3.23	0.03	1.03	0.76	0.03
2.13	1.33	1.71	4.96	5.68	1.37	1.24	1.97
2.04	1.42	1.85	4.87	0.03	1.32	1.18	0.03
0.46	0.26	0.31	1.19	1.06	0.19	0.28	0.12
2.16	1.57	2.14	4.84	0.05	1.28	1.27	0.05
1.48	1.03	1.47	4.04	5.07	0.92	0.94	2.20
3.57	2.30	2.57	6.45	5.88	1.72	2.13	2.54
2.65	1.19	3.42	11.56	3.71	1.26	1.21	1.78
5.59	3.46	4.43	10.46	4.68	2.43	2.08	2.15
0.76	0.49	0.52	2.23	2.28	0.38	0.42	0.44

*n: Number of subjects in ATP cohort for immunogenicity with available results for at least one serotype (the actual number of subjects tested can vary slightly for the different serotypes, depending on the number of sera available).
[¶]Subjects in study 010 were randomized to receive prophylactic paracetamol with and soon after vaccination (PP group) or to receive no paracetamol prophylaxis (NPP group).
[§]Post-dose 2 subset based on booster ATP cohort for immunogenicity.
7vCRM: 7-valent pneumococcal conjugate vaccine; GMC: Geometric mean concentration; HibMenC-TT: *Haemophilus influenzae* type b–*Neisseria meningitidis* serogroup C–tetanus toxoid vaccine; PHiD-CV: 10-valent pneumococcal nontypeable *H. influenzae* protein D conjugate vaccine.

importance of the early booster dose when the 2 + 1 schedule is employed.

Coadministration with other recommended pediatric vaccines

The immunogenicity of a wide range of pediatric vaccines was evaluated when coadministered with PHiD-CV and compared with immune responses following their coadministration with 7vCRM as the standard of care at the time that the studies were conducted [75,76]. The following coadministered vaccines were evaluated: MenC-CRM (Meningitec™), MenC-TT (NeisVac-C™), HibMenC–TT (Menitorix™), DTPa–IPV/Hib (Infanrix™ IPV Hib), DTPa–IPV (Infanrix™ IPV), DTPa–HBV–IPV (Infanrix penta™ or Pediarix™), DTPa–HBV–IPV/Hib (Infanrix hexa™), DTPw–HBV/Hib (Tritanrix™ HepB/Hib), OPV (Polio Sabin™), IPV (Poliorix™) and HRV (Rotarix™).

Immune responses to the coadministered vaccines were within the same range when given with PHiD-CV or 7vCRM, with the following exceptions: anti-tetanus and antipolyribosylribitol phosphate antibody GMCs were both higher after PHiD-CV than 7vCRM coadministration, reflecting an immune-enhancing effect of tetanus toxoid used as carrier protein for serotype 18C

Table 5. Opsonophagocytic activity against pneumococcal serotypes following primary vaccination (primary ATP cohort for immunogenicity, except [§]).

Schedule	2, 3, 4 months		2, 4, 6 months					
Study (Ref.)	001 [70]		010 [71]				012 [72]	
Group and number of subjects (n)	PHiD-CV (n = 268)	7vCRM (n = 89)	PHiD-CV + MenC-CRM (n = 162)	PHiD-CV + MenC-TT (n = 168)	PHiD-CV + HibMenC-TT (n = 161)	7vCRM + HibMenC-TT (n = 156)	PHiD-CV (n = 145)	7vCRM (n = 49)
% ≥8								
1	65.7	4.5	54.3	51.2	50.3	1.3	43.1	0.0
4	99.6	100	100	100	97.5	100	98.6	100
5	90.9	3.4	92.8	86.5	88.7	2.0	88.2	0.0
6B	92.4	95.5	87.8	84.7	81.8	97.4	84.1	89.8
7F	99.6	18.2	100	98.8	96.8	15.2	97.9	10.2
9V	100	100	99.3	98.7	100	99.3	100	100
14	99.6	98.9	98.1	97.0	96.3	98.1	97.9	98.0
18C	93.6	95.5	96.8	98.2	91.7	100	95.1	98.3
19F	87.7	92.1	98.1	93.9	94.3	90.5	99.3	91.8
23F	93.9	97.7	95.2	94.9	90.4	99.3	92.3	100
GMT								
1	25.3	4.7	23.9	18.8	19.7	4.2	14.8	4.0
4	734.9	1010.4	697.4	755.6	669.8	926.2	602.9	513.0
5	59.9	4.5	91.7	71.4	77.4	4.2	67.2	4.0
6B	457.4	999.4	459.1	404.6	354.2	1575.3	361.9	805.0
7F	2253.9	10.2	2513.3	2821.3	2290.5	8.7	2002.2	6.9
9V	1399.7	1233.3	1005.6	1108.8	1122.6	1305.0	1171.7	1166.0
14	1061.0	1890.6	797.8	879.0	779.9	1539.4	640.0	947.6
18C	130.1	212.3	174.9	282.8	142.7	212.8	174.9	127.0
19F	148.6	52.0	387.5	298.4	261.0	52.0	337.8	35.9
23F	1010.0	4412.9	1066.0	1219.6	880.7	5469.2	920.6	3895.4

[*]n: Number of subjects in ATP cohort for immunogenicity with available results for at least one serotype (the actual number of subjects tested can vary slightly for the different serotypes, depending on the number of sera available).
[‡]Subjects in study 010 were randomized to receive prophylactic paracetamol with and soon after vaccination (PP group) or to receive no paracetamol prophylaxis (NPP group).
[§]Post-dose 2 subset based on booster ATP cohort for immunogenicity.
7vCRM: 7-valent pneumococcal conjugate vaccine; GMT: Geometric mean titer; MenC-TT: *Neisseria meningitidis* serogroup C–tetanus toxoid vaccine; PHiD-CV: 10-valent pneumococcal nontypeable *Haemophilus influenzae* protein D conjugate vaccine.

in PHiD-CV. Although not consistently observed, antidiphtheria antibody GMCs tended to be higher after 7vCRM coadministration, reflecting the mutant diphtheria toxoid CRM197 used as the carrier protein in 7vCRM [76].

Cross-reactive serotypes 6A & 19A

PHiD-CV induced cross-reactive antibodies and functional OPA responses against cross-reacting serotypes 6A and 19A after primary and booster vaccination [70,72,77]. The cross-reacting immune response to serotype 6A appears within the same range following PHiD-CV and 7vCRM vaccination (postprimary antibody concentrations ≥0.2 µg/ml: 22.2–63.2% for PHiD-CV, 31.2–58.3% for 7vCRM; OPA titers ≥8: 58.0–85.6 and 68.5–87.4%, respectively) [70,72,77]. Antibody and OPA responses against cross-reacting serotype 19A were higher after PHiD-CV compared with 7vCRM, with postprimary antibody concentrations 0.2 µg/ml or more in 45.0–68.4% of PHiD-CV and 27.7–45.3% of 7vCRM vaccinees; and OPA titers of 8 or more in 25.5–32.4 and 0.0–3.4%, respectively. This could potentially provide improved protection against serotype 19A following PHiD-CV compared with 7vCRM vaccination [70,72,77].

Safety & reactogenicity of PHiD-CV

Methods to assess safety

In all clinical trials, diary cards were provided to the parents or guardians of all enrolled subjects on which specific local

Table 5. Opsonophagocytic activity against pneumococcal serotypes following primary vaccination (primary ATP cohort for immunogenicity, except [§]) (cont.).

3, 4, 5 months			6, 10, 14 weeks		3, 5 months	2, 4 months	
010[¶]		002[‡§]	012[‡§]		002[‡§]	011[‡§]	
PHiD-CV NPP (n = 176)	PHiD-CV AP (n = 164)	PHiD-CV (n = 154)	PHiD-CV (n = 142)	7vCRM (n = 46)	PHiD-CV (n = 158)	PHiD-CV pooled (n = 140-1)	7vCRM ⇔ HibMenC-TT (n = 137-1)
% ≥8 (cont.)							
55.1	34.8	62.9	82.4	2.2	60.8	48.8	0.0
100	100	99.2	99.3	100	100	97.8	95.6
93.0	79.9	90.8	99.3	0.0	82.6	74.6	0.0
93.2	82.2	88.9	93.0	93.0	74.4	63.0	35.2
99.4	100	98.5	100	22.7	90.6	96.8	0.0
99.4	100	100	100	100	100	98.5	99.3
99.4	98.8	100	97.2	93.5	98.5	97.1	93.3
98.2	91.7	96.2	99.3	100	82.8	59.8	77.0
94.5	91.6	93.8	98.6	91.3	87.0	84.3	67.9
97.6	93.8	97.7	100	97.7	86.3	97.1	87.8
GMT (cont.)							
23.7	10.3	26.5	93.7	4.2	21.9	14.4	4.0
744.6	788.3	758.9	1008.7	1229.9	462.6	244.1	240.6
72.5	32.2	68.4	209.3	4.0	48.3	24.1	4.0
684.8	386.7	379.6	963.5	1762.2	157.8	92.9	23.9
2345.3	2458.2	2176.5	5196.4	14.2	844.8	1192.9	4.0
1230.3	1658.1	1343.4	1631.9	1713.3	875.1	712.7	686.7
1161.6	897.6	1125.3	1669.1	2117.4	692.6	396.1	412.6
202.2	135.0	218.6	673.3	283.7	56.2	21.6	24.5
369.5	244.6	356.7	1121.7	81.6	101.0	65.8	19.5
1497.2	1163.6	1233.7	2186.6	4126.6	489.7	917.1	561.9

'n: Number of subjects in ATP cohort for immunogenicity with available results for at least one serotype (the actual number of subjects tested can vary slightly for the different serotypes, depending on the number of sera available).
[¶]Subjects in study 010 were randomized to receive prophylactic paracetamol with and soon after vaccination (PP group) or to receive no paracetamol prophylaxis (NPP group).
[§]Post-dose 2 subset based on booster ATP cohort for immunogenicity.
7vCRM: 7-valent pneumococcal conjugate vaccine; GMT: Geometric mean titer; MenC-TT: *Neisseria meningitidis* serogroup C–tetanus toxoid vaccine; PHiD-CV: 10-valent pneumococcal nontypeable *Haemophilus influenzae* protein D conjugate vaccine.

and general symptoms were recorded for 4 days after each vaccine dose. All other unsolicited symptoms were recorded when occurring within 31 days after vaccination and any serious adverse events were reported throughout the entire duration of each study.

Reactogenicity & safety profile

Safety and reactogenicity in comparative clinical trials have been reviewed recently [78]. Primary and booster vaccination with PHiD-CV was well-tolerated, with local and systemic symptoms similar to those commonly observed following other routinely used pediatric vaccines. In all comparative primary and booster vaccination studies, the percentages of doses followed by pain, redness or swelling at the injection site, or with systemic symptoms, including fever, were within the range observed in the 7vCRM groups (Tables 8 & 9) [78,79].

Higher incidences of solicited local and general symptoms were reported when PHiD-CV was coadministered with a DTPw-based combination vaccine (Table 8).

Large swelling reactions (i.e., swelling with a diameter >50 mm, noticeable diffuse swelling or noticeable increase of limb circumference) were reported following 0.6% of the PHiD-CV booster doses, compared with 1.3% of the booster doses of DTPa-based combination vaccines and 1.6% of the 7vCRM booster doses [78]. Those occurring after PHiD-CV administration did not involve the adjacent joint.

Table 6. IgG antibody responses against pneumococcal serotypes after booster vaccination (ATP cohort for immunogenicity).

Study [Ref]	007 [44]			017 [45]				014* [46]		012 [51]	
Group and number of subjects (n†)	4th dose PHiD-CV (n = 347)	4th dose 7vCRM (n = 89)	7vCRM priming ⇨ PHiD-CV booster (n = 134)	4th dose PHiD-CV ⇨ MenC-CRM (n = 168)	4th dose PHiD-CV ⇨ MenC-TT (n = 153)	4th dose PHiD-CV ⇨ HibMenC-TT (n = 160)	4th dose 7vCRM ⇨ HibMenC-TT (n = 153)	4th dose PHiD-CV NPP (n = 168)	4th dose PHiD-CV PP (n = 149)	PHiD-CV (3 ⇨ 1) (n = 154)	PHiD-CV (2 ⇨ 1) (n = 158)
% ≥0.2 µg/ml											
1	99.4	4.9	85.0	100	100	100	2.6	100	100	100	99.4
4	99.7	100	100	100	100	100	100	100	100	100	100
5	99.4	6.1	85.7	100	100	98.8	7.3	100	100	100	100
6B	96.5	97.7	98.5	98.1	96.7	97.5	99.3	99.4	95.7	96.6	88.5
7F	100	7.1	95.5	100	100	100	1.3	100	100	100	100
9V	100	100	100	99.4	99.3	100	100	100	100	100	99.4
14	99.1	100	100	99.4	100	99.4	100	99.4	100	98.6	99.4
18C	100	100	99.3	100	100	100	100	100	100	99.3	100
19F	99.4	100	97.8	99.4	99.3	100	98.7	98.8	97.9	98.0	96.2
23F	97.4	98.9	97.0	98.7	99.3	98.8	99.3	97.6	96.4	95.9	96.1
GMC (µg/ml)											
1	1.53	0.04	0.67	2.26	2.14	2.12	0.03	2.62	1.67	1.88	1.85
4	3.35	4.40	4.47	3.94	4.15	3.53	5.26	4.21	3.01	3.47	3.06
5	2.20	0.05	0.74	2.94	2.66	2.54	0.05	3.68	2.30	3.21	2.65
6B	1.94	3.53	1.74	2.48	2.16	2.04	4.96	2.45	1.35	1.85	1.12
7F	3.50	0.04	1.83	4.94	4.87	4.21	0.03	4.13	2.90	3.88	2.81
9V	3.25	6.09	1.94	4.73	4.41	4.03	8.23	4.39	2.86	3.97	2.95
14	5.56	9.29	4.76	5.91	5.87	5.62	11.28	5.95	4.58	5.47	4.19
18C	5.01	5.21	4.98	7.19	9.99	7.20	7.58	7.00	4.96	7.20	6.24
19F	6.05	3.35	5.06	7.77	7.38	6.78	3.72	7.55	6.00	6.95	5.58
23F	2.38	6.67	2.42	3.49	3.27	2.55	9.42	2.92	1.99	2.78	2.41

*Subjects in study 014 were randomized before the protocol amendment (subjects after protocol amendment not shown) to receive prophylactic paracetamol with and soon after vaccination (PP group) or to receive no paracetemol prophylaxis (NPP group).
†n: Number of subjects in ATP cohort for immunogenicity with available results for at least one serotype (the actual number of subjects tested can vary slightly for the different serotypes depending on the number of sera available).
7vCRM: 7-valent pneumococcal conjugate vaccine; GMC: Geometric mean concentration; MenC-TT: *Neisseria meningitidis* serogroup C–tetanus toxoid vaccine; NPP: No paracetemol prophylaxis; PHiD-CV: 10-valent pneumococcal nontypeable *Haemophilus influenzae* protein D conjugate vaccine; PP: Prophylactic paracetamol.

Table 7. Opsonophagocytic activity against pneumococcal serotypes following booster vaccination (ATP cohort for immunogenicity).

Study [Ref.]	007 [71]			017 [72]				014* [73]		002 [51]	
Group and number of subjects (n[a])	4th dose PHiD-CV (n = 327)	4th dose 7vCRM (n = 84)	7vCRM priming + PHiD-CV booster (n = 126)	4th dose PHiD-CV + MenC-CRM (n = 145)	4th dose PHiD-CV + MenC-TT (n = 143)	4th dose PHiD-CV + HibMenC-TT (n = 145)	4th dose 7vCRM + HibMenC-TT (n = 143)	4th dose PHiD-CV NPP (n = 158)	4th dose PHiD-CV PP (n = 130)	PHiD-CV (3+1) (n = 154)	PHiD-CV (2+1) (n = 138)
% ≥8											
1	91.0	3.6	31.4	94.3	95.7	90.7	8.8	98.1	90.8	77.8	80.9
4	100	100	100	100	100	100	100	100	100	99.0	96.8
5	96.3	1.2	36.9	97.6	97.7	96.3	3.1	100	97.7	97.5	87.2
6B	96.6	98.7	94.9	94.4	94.1	95.1	98.6	98.7	90.0	90.3	81.1
7F	99.7	31.1	98.3	100	100	100	47.3	100	100	100	100
9V	100	100	100	100	100	100	100	100	100	100	100
14	100	100	100	100	100	100	100	100	100	100	100
18C	99.7	100	98.3	100	98.4	100	96.5	100	100	98.5	97.8
19F	94.9	92.5	98.3	97.1	96.4	100	98.5	98.7	96.2	96.1	96.2
23F	99.7	98.8	98.4	100	100	100	100	99.4	100	98.3	92.5
GMT											
1	192.2	4.3	8.3	226.8	205.8	153.9	5.0	417.0	144.6	100.6	109.9
4	1856.3	2812.6	1528.9	1919.6	2068.1	1539.9	3281.0	2297.0	1547.9	1204.0	634.6
5	144.1	4.1	9.5	192.9	170.0	148.8	4.3	289.3	134.3	157.2	102.1
6B	981.2	3459.6	640.2	693.5	702.8	627.5	4670.0	985.7	496.7	468.5	220.3
7F	4330.3	25.2	2397.2	4539.3	5462.6	4950.2	61.4	4674.7	4025.8	3290.6	1843.4
9V	2343.5	5357.4	886.8	2317.7	26 66.1	2334.1	4800.6	2403.7	2234.8	1706.9	1068.1
14	2085.9	2134.2	977.8	1751.4	1808.3	1537.7	3020.8	1865.2	1581.7	1280.7	835.5
18C	810.3	968.7	610.7	813.7	1030.5	717.1	754.2	737.8	652.9	490.8	330.0
19F	624.3	287.8	530.1	875.9	763.6	551.3	321.3	1062.2	629.4	734.7	251.3
23F	2830.1	13900.7	2828.8	3032.2	3378.9	2836.7	25321.1	3154.0	2335.7	1528.9	1047.3

*Subjects in study 014 were randomized before the protocol amendment (subjects after protocol amendment not shown) to receive prophylactic paracetamol with and soon after vaccination (PP group) or to receive no paracetamol prophylaxis (NPP group).
[a]n: Number of subjects in ATP cohort for immunogenicity with available results for at least one serotype (the actual number of subjects tested can vary slightly for the different serotypes, depending on the number of sera available).
7vCRM: 7-valent pneumococcal conjugate vaccine; GMT: Geometric mean titer; HibMenC-TT: *Haemophilus influenza* type b–*Neisseria meningitidis* serogroup C–tetanus toxoid vaccine; PHiD-CV: 10-valent pneumococcal nontypeable *H. influenzae* protein D conjugate vaccine.

Table 8. Overall per dose incidence (%) of local and general symptoms within 4 days following PHiD-CV or 7vCRM primary vaccination (total vaccinated cohort).

		Studies with DTPa coadministration								Study with DTPw coadministration			
Schedule		2, 3, 4 months		2, 4, 6 months				3, 4, 5 months		6, 10, 14 weeks		2, 4, 6 months	
Study		001		011				010		012		012	
Group and number of subjects (n†)		PHiD-CV (n = 3683)	7vCRM (n = 1240)	PHiD-CV + MenC-CRM (n = 1130)	PHiD-CV + MenC-TT (n = 1129)	PHiD-CV + HibMenC-TT (n = 1130)	7vCRM + HibMenC-TT (n = 1146)	PHiD-CV NPP (n = 697)	PHiD-CV PP (n = 675)	PHiD-CV (n = 891)	7vCRM (n = 297)	PHiD-CV (n = 895)	7vCRM (n = 300)
Symptom	Intensity‡												
Pain	Any	33.2	31.0	34.3	34.2	35.1	29.8	28.6	17.9	67.2	58.9	63.1	60.7
	Grade 3§	2.4	1.9	3.1	2.7	3.0	2.0	1.7	0.3	9.4	9.4	12.6	12.3
Redness	Any	45.4	48.0	46.8	44.3	45.8	42.8	42.0	38.5	47.0	41.8	68.3	64.7
	>30 mm	1.7	1.6	2.5	1.5	1.1	1.4	1.7	0.9	2.4	2.4	5.2	4.7
Swelling	Any	36.1	34.7	37.4	32.4	36.1	31.2	29.0	26.5	36.4	31.3	52.1	53.3
	>30 mm	3.7	3.1	3.1	1.9	1.2	1.7	4.4	4.3	9.3	8.1	9.0	8.0
Drowsiness	Any	46.2	45.1	43.7	45.9	43.5	37.0	40.7	35.4	38.8	32.0	61.1	57.7
	Grade 3	0.8	0.9	1.2	1.5	0.9	1.1	0.7	0.0	1.0	1.3	2.7	1.0
Fever (rectal)	≥38.0°C	32.5	33.7	37.6	38.5	32.7	28.4	39.0	19.9	60.9	63.0	60.0	51.3
	>39.0°C	2.0	2.1	3.6	3.9	2.6	2.3	2.9	0.6	6.1	4.0	5.5	5.0
	>40.0°C	0.0	0.0	0.0	0.2	0.1	0.2	0.0	0.1	0.0	0.0	0.0	0.3
Irritability	Any	59.6	60.6	53.7	52.1	52.9	45.8	47.2	38.4	66.2	57.2	81.8	73.3
	Grade 3	3.8	3.5	4.8	4.0	3.6	3.0	1.3	0.3	2.9	2.4	13.3	9.3
Loss of appetite	Any	23.5	24.6	30.0	28.6	29.6	27.4	14.6	10.5	25.9	21.9	44.2	33.0
	Grade 3	0.2	0.2	0.7	0.7	0.2	0.4	0.0	0.0	0.2	0.3	0.2	0.3

*Subjects in study 010–014 were randomized to receive prophylactic paracetamol with and soon after vaccination (PP group) or to receive no paracetamol prophylaxis (NPP group).
†n: Number of documented doses.
‡Grade 3 defined as: pain – the child cried when the limb was moved/was spontaneously painful; irritability/fussiness – the child cried and could not be comforted/prevented normal activity; drowsiness – prevented normal activity; loss of appetite – the child did not eat at all.
7vCRM: 7-valent pneumococcal conjugate vaccine; DTPa: Diphtheria–tetanus–acellular pertussis vaccine; DTPw: Diphtheria–tetanus–whole-cell pertussis vaccine; MenC-TT: *Neisseria meningitidis* serogroup C–tetanus toxoid vaccine; NPP: No paracetamol prophylaxis; PHiD-CV: 10-valent pneumococcal nontypeable *Haemophilus influenzae* protein D conjugate vaccine; PP: Prophylactic paracetamol.

Table 9. Overall incidence per dose (%) of local and general symptoms within 4 days following PHiD-CV or 7vCRM booster vaccination (total vaccinated cohort).

Study		007 (Booster 001)			017 (Booster 011)				014 (Booster 010)	
Group		4x PHiD-CV (n = 735)[a]	4x 7vCRM (n = 91)	7vCRM + PHiD-CV (n = 282)	PHiD-CV + MenC-CRM (n = 358)	PHiD-CV + MenC-TT (n = 357)	PHiD-CV + HibMenC-TT (n = 355)	7vCRM + HibMenC-TT (n = 353)	PHiD-CV NPP (n = 172)	PHiD-CV PP (n = 178)
Symptom	Intensity[b]									
Pain	Any	61.5	52.7	53.2	55.1	54.6	54.4	46.5	45.9	30.3
	Grade 3	6.4	3.3	6.4	6.2	7.8	7.0	4.5	5.8	1.1
Redness	Any	61.4	64.8	54.3	54.5	51.3	52.4	48.7	43.0	50.0
	>30 mm	13.1	7.7	6.7	11.0	9.0	10.4	9.9	8.1	4.5
Swelling	Any	46.0	46.2	39.7	45.5	41.5	42.3	37.7	29.1	29.2
	>30 mm	9.1	7.7	7.1	7.3	7.6	6.2	7.9	5.2	1.1
Drowsiness	Any	41.2	52.7	46.1	34.8	39.8	39.2	29.6	48.8	51.1
	Grade 3	0.7	0.0	1.8	0.8	0.3	1.1	0.6	0.6	0.0
Fever (rectal)	≥38.0°C	33.3	36.3	39.7	30.6	34.2	29.6	30.4	58.1	36.0
	>39.0°C	3.3	7.7	3.5	4.8	4.5	3.1	2.3	8.1	2.2
	>40.0°C	0.1	2.2	1.1	0.3	0.6	0.3	0.0	0.6	0.6
Irritability	Any	59.6	60.4	62.4	47.8	53.2	53.8	44.2	61.0	48.3
	Grade 3	2.0	2.2	4.3	2.2	2.5	2.5	2.3	1.2	0.6
Loss of Appetite	Any	31.3	34.1	32.6	28.4	27.7	31.5	26.2	26.7	26.4
	Grade 3	0.5	0.0	1.1	1.1	0.6	0.8	0.3	2.3	0.0

[a]Subjects in study 014 were randomized before the protocol amendment (subjects after protocol amendment not shown) to receive prophylactic paracetamol with and soon after vaccination (PP group) or to receive no paracetamol prophylaxis (NPP group).
[b]n: Number of documented doses.
[c]Grade 3 defined as: pain – the child cried when the limb was moved/was spontaneously painful; irritability/fussiness – the child cried and could not be comforted/prevented normal activity; drowsiness – prevented normal activity; loss of appetite – the child did not eat at all.
7vCRM: 7-valent pneumococcal conjugate vaccine; HibMenC-TT: *Haemophilus influenza* type b–*Neisseria meningitidis* serogroup C–tetanus toxoid vaccine; NPP: No paracetamol prophylaxis; PHiD-CV: 10-valent pneumococcal nontypeable *Haemophilus influenzae* protein D conjugate vaccine; PP: Prophylactic paracetamol.
Data taken from [75,78].

The incidence of fever after vaccination with PHiD-CV warrants special note. All comparative studies of PHiD-CV and 7vCRM evaluated the incidence of fever above 39°C as a key study objective. There was no evidence that PHiD-CV induced more febrile reactions over 39°C than 7vCRM after primary or booster vaccination [78]. Febrile convulsions within 10 days after vaccination were uncommon, reported after 1 in 5400 primary immunizations [78]. All were reported in the first 48 h after vaccination. No convulsions were reported within 10 days of PHiD-CV booster vaccination. No safety concerns were identified in a review of 2996 subjects vaccinated with PHiD-CV [78].

In studies 010 (primary) and 014 (booster), the effects of prophylactic paracetamol administration were studied in a prospective manner. Fever, pain and irritability were reduced in the group receiving prophylactic paracetamol compared with controls. However, lower antibody responses to some of the DTPa–HBV–IPV/Hib and PHiD-CV antigens were also observed in the group receiving prophylactic paracetamol. *Post-hoc* analysis on past GSK-sponsored pediatric trials identified similar effects on vaccine immunogenicity when paracetamol was administered on the day of vaccination, including for 7vCRM. These findings suggest a potentially general effect of antipyretic prophylaxis on vaccine immunogenicity of some antigens in diverse inactivated vaccines. However, since the seroprotection and seropositivity rates were mostly not impacted and because priming and boostability were adequate, these findings may be of limited clinical impact. They do, however, indicate that antipyretic prophylaxis should no longer be routinely recommended, but should be used judiciously [75].

Expert commentary

IPD prevention

Immunogenicity following primary and booster vaccination with PHiD-CV tended to be lower for most common serotypes compared with 7vCRM in most clinical trials. However, the difference in immunogenicity between conjugate vaccines does not necessarily result in differences in clinical efficacy and in the case of PHiD-CV, there are a number of elements that support the conclusion that the observed differences in immunogenicity are likely to have no clinical impact on IPD prevention.

- Immune responses were induced for all ten pneumococcal vaccine serotypes, with similar immunological characteristics observed for PHiD-CV and 7vCRM (the same ranking of serotypes, with the lowest responses for serotype 6B and the highest for serotype 14);
- High percentages of subjects reached the reference antibody threshold put forward for the immunological evaluation of new PCVs compared with a licensed vaccine with proven efficacy, with comparable proportions of vaccinees reaching this threshold for most serotypes;
- Functional OPA responses were measured for all serotypes, including those serotypes for which a lower percentage of subjects reached the reference antibody concentration, with minimal differences observed between PHiD-CV and 7vCRM in terms of the percentage of subjects with a measurable OPA titer;
- There was a high degree of boostability of the ELISA and OPA responses, indicative of effective priming against all serotypes in infancy, including serotypes with more limited postprimary responses compared with either other serotypes contained in the vaccine, or with 7vCRM;
- Efficacy against overall pneumococcal AOM in general with statistically significant efficacy against AOM due to serotypes 6B, 14, 19F and 23F with the predecessor 11Pn-PD vaccine [14] further testifies for the functionality of the antibodies induced by PD-conjugate vaccination.

AOM prevention

Protection against overall clinical AOM, pneumococcal AOM and AOM caused by NTHi was demonstrated with the predecessor 11Pn-PD vaccine [14]. Efficacy of PHiD-CV against pneumococcal AOM is expected to be at least similar to the efficacy observed with the 11Pn-PD vaccine, based on comparable antibody and functional OPA [56] responses for those serotypes shown to be the most important cause of AOM.

With the use of the NTHi-derived PD as a carrier protein, PHiD-CV also provides the potential to prevent AOM due to NTHi, resulting in an overall impact on clinical AOM that can be expected to be substantially higher than the overall impact of any CRM conjugate vaccine not containing the PD carrier protein [80].

The functionality of the PHiD-CV-induced anti-PD antibodies against NTHi OM has been further validated in a widely accepted *in vivo* juvenile chinchilla OM model [80–85]. Antibodies against PD were shown to prevent NTHi OM in chinchillas following active and passive immunization [83,86]. In a comparison of the passive protective capacity of human infant sera from the POET study and sera obtained after infant immunization with PHiD-CV, a comparable degree of protection against OM due to NTHi was observed [87]. These results strongly suggest, in line with the observations with 11Pn-PD in POET, that PHiD-CV can be expected to also provide protection against NTHi disease.

However, the magnitude of the public health impact of the PD component of PHiD-CV remains to be documented. Since the implementation of widespread use of 7vCRM in the USA, NTHi has become the most common cause of recurrent and refractory OM [88]. This is consistent with prelicensure studies of 7vCRM and another PCV candidate that showed an increase in *H. influenzae* AOM episodes following vaccination [16,89]. If PHiD-CV successfully reduces AOM disease caused by *H. influenzae* and NTHi, then the clinical and public health impact is potentially substantial [90,91].

Pneumonia prevention

Experience with PCVs thus far seems to confirm that pneumococcal pneumonia is vaccine-preventable. Protective efficacy was demonstrated against pneumonia for several PCVs (7vCRM [25,92], 9-valent pneumococcal conjugate vaccine [9vCRM] [23,24] and an 11-valent diphtheria and tetanus toxoid conjugate vaccine [26]).

Despite likely variability in vaccine efficacy and immunogenicity, and in the proportion of pneumonia due to pneumococcal vaccine serotypes, it is interesting to note that when using the WHO definition of consolidated pneumonia, the vaccine efficacy estimates all fall within a 20–40% range. These findings have been confirmed by postmarketing surveillance data following 7vCRM introduction in the USA [34].

Taking these previous experiences into account, it is reasonable to conclude that PHiD-CV will provide protection against pneumonia, given the induction of antibody and functional OPA responses that:

- Provide protection against IPD that is at least comparable to that obtained with 7vCRM (WHO licensure criteria);
- Provide protection against clinical AOM (POET) that exceeds that seen with 7vCRM;
- Reduce nasopharyngeal carriage of vaccine serotype *S. pneumoniae* (42.8% reduction; 95% CI: -16.7–71.9) and *H. influenzae* (42.6% reduction;95% CI: 1.3–66.6) 3 months after booster vaccination with the related 11Pn-PD vaccine in the POET study [14].

However, given the difficulties in establishing an etiological diagnosis of pneumonia, more clinical data are needed to further quantify the magnitude of the clinical impact of the dual pathogen PHiD-CV vaccine on clinical pneumonia.

Immunization schedule

PHiD-CV has been studied in a variety of immunization schedules. Immune responses were consistent throughout European studies conducted using standard 3 + 1 schedules. Somewhat reduced postprimary antibody responses were observed for serotypes 6B and 23F in the accelerated schedules of 2, 3, 4 months versus 2, 4, 6 months. However, these effects were not apparent when assessing the functional OPA responses.

There is now ample evidence that early infant immunization with protein-conjugated polysaccharide vaccines requires booster vaccination in order to guarantee long-lasting protection [93,94]. For PCVs, evidence is accumulating that also highlights the importance of booster vaccination. 7vCRM was initially developed as a four-dose vaccine (three-dose priming and second year of life boosting) and although a number of countries decided to introduce 7vCRM universal mass vaccination using a 2 + 1 schedule, the question remains unanswered as to whether this schedule provides the same level of protection against mucosal pneumococcal infections (AOM and pneumonia) and the same level of herd immunity compared with the standard 3 + 1 schedule.

The WHO Expanded Program on Immunization currently recommends three-dose priming at 6, 10 and 14 weeks of age, without a subsequent booster dose. Several problems exist with this schedule in the context of pneumococcal vaccination. First, evidence exists that HIV-infected children, among whom very high incidences of pneumococcal infections are registered [95–98], can obtain reasonable protection from PCV primary vaccination [24], but in the absence of a booster dose they suffer from more rapidly waning efficacy due to the loss of T-cell memory [99]. Second, it is intriguing that no protection could be demonstrated against serotype 1 IPD in the Gambian trial with a 9vCRM PCV administered according to the WHO recommended schedule without a booster dose in the second year of life, despite good overall efficacy against vaccine-serotype IPD [100]. The four cases of serotype 1 IPD in the vaccine group all occurred in children 18 months of age or older [100], thereby leaving the question open as to whether they could have been avoided with a booster dose. Unlike for other serotypes, no peak in the incidence of IPD is observed for serotype 1 in the first year of life, for which the incidence remains more constant throughout life, resulting in more than 90% of the cases occurring after 12 months of age [40,101]. The lack of efficacy against serotype 1 disease in clinical trials where 9vCRM was administered without a booster dose seems to fit with the observation of relatively low functional OPA responses prior to the booster dose for serotype 1 compared with other vaccine serotypes, and this is regardless of the PCV used (Tables 5 & 7) [102]. Although an evaluation of the functionality of antibodies is not available from the African trials, the lack of protection against IPD caused by serotype 1 in The Gambia and the age at which serotype 1 disease occurred, suggest that a booster dose might be required in order to obtain a sufficient level of OPA and hence protective efficacy against serotype 1 disease.

Five-year view

In the coming years, results of ongoing clinical trials evaluating the efficacy of PHiD-CV in preventing IPD, AOM and community-acquired pneumonia will become available [103,203,204]. These data will provide additional information on the potential clinical and public health benefits of PHiD-CV in pneumococcal disease prevention.

Additional PCVs are also likely to become available in the coming years. The most advanced PCV is a 13-valent CRM197 PCV that contains the additional serotypes 3, 6A and 19A. Some of the diseases caused by these serotypes are probably already covered by the cross-protection provided by 7vCRM and PHiD-CV [32]. It will be important to document protection against serotype 3 disease, given the specific immunological characteristics that make the use of immunological licensure criteria for serotype 3 questionable [57]. Acceptable immune responses to coadministered vaccine antigens will finally need to be documented given the possibility of carrier or bystander interferences known to be associated with CRM197 conjugate vaccines [104].

Serotype replacement after widespread use of PCVs remains a potential concern, requiring continued IPD surveillance to identify the emergence of new clinically important strains. New or adapted vaccine formulations including, for example, conserved pneumococcal protein antigens, might be needed in the future in order to meet this challenge.

The most important evolution in the near future is that the availability of two PCVs with extended serotype coverage, together with the new mechanisms for funding extensive vaccination programs in countries with limited resources [105,205], will make pneumococcal disease prevention worldwide a reality, even for the most impoverished populations.

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Key issues

- *Streptococcus pneumoniae* is a major cause of bacterial meningitis, community-acquired pneumonia and, along with nontypeable *Haemophilus influenzae* (NTHi), acute bacterial otitis media (AOM) in children.
- The first licensed pneumococcal conjugate vaccine (PCV); (7-valent pneumococcal conjugate vaccine [7vCRM]), containing seven pneumococcal serotypes, has been proven highly effective against vaccine-serotype invasive pneumococcal disease (IPD). However, the included serotypes are estimated to account for only 70–75% of IPD in young children in western Europe and Africa, and 50–65% or less in Latin America and some parts of Asia. For these countries, a vaccine with wider serotype coverage is needed.
- Serotypes 1, 5 and 7F, not included in 7vCRM, are associated with severe clinical syndromes, and are important causes of IPD in Asia and Africa (serotype 1) and Latin America (serotype 5).
- 10-valent pneumococcal NTHi protein D conjugate vaccine (PHiD-CV) contains ten vaccine serotypes: the seven serotypes also found in 7vCRM (4, 6B, 9V, 14, 18C, 19F and 23F), as well as serotypes 1, 5 and 7F. Eight out of ten serotypes are conjugated to a novel carrier protein: PD, a nonlipidated and highly conserved form of a 42-kDa cell-surface lipoprotein of NTHi.
- In clinical trials conducted in infants and toddlers, a high percentage of PHiD-CV vaccinees achieved ELISA antibodies 0.2 µg/ml or more and functional opsonophagocytic activity (OPA) titers of 8 or more. Robust antibody and OPA booster responses attest to effective priming in infancy.
- PHiD-CV may be coadministered with other killed and live-attenuated pediatric vaccines.
- Primary and booster vaccination with PHiD-CV was well-tolerated, with a safety profile similar to that of 7vCRM.
- Efficacy against AOM due to pneumococcal vaccine serotypes and NTHi was demonstrated after vaccination with the PD-containing 11-valent predecessor vaccine (11Pn-PD). PHiD-CV induced antibodies against PD have similar biological activity compared with those induced by the predecessor 11Pn-PD vaccine, for which protection against NTHi AOM was demonstrated.
- Availability of a second PCV, which uses a novel PD carrier and with expanded serotype coverage, is a significant step forward in the prevention of pneumococcal infections and AOM due to *H. influenzae* in infants and children worldwide.

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PRODUCT MONOGRAPH

Prevnar® 13

Pneumococcal 13-valent Conjugate Vaccine (Diphtheria CRM_{197} Protein)

Suspension For Intramuscular Injection

Active Immunizing Agent

©Wyeth Canada
1025 Boulevard Marcel Laurin
St. Laurent (Montreal)
Quebec, Canada
H4R 1J6

Date of Preparation:
November 27, 2009

Submission Control No: 122881

Date of Approval: December 21, 2009

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Prevnar® 13

Pneumococcal 13-valent Conjugate Vaccine (Diphtheria CRM197 Protein)

PART I: HEALTH PROFESSIONAL INFORMATION

SUMMARY PRODUCT INFORMATION

Route of Administration	**Dosage Form / Strength**	**Clinically Relevant Nonmedicinal Ingredients**
Intramuscular injection,	Suspension for injection 1 dose (0.5 mL pre-filled syringe) contains: Pneumococcal polysaccharide serotype 1 2.2 µg[a] Pneumococcal polysaccharide Serotype 3 2.2 µg[a] Pneumococcal polysaccharide Serotype 4 2.2 µg[a] Pneumococcal polysaccharide Serotype 5 2.2 µg[a] Pneumococcal polysaccharide Serotype 6A 2.2 µg[a] Pneumococcal polysaccharide Serotype 6B 4.4 µg[a] Pneumococcal polysaccharide Serotype 7F 2.2 µg[a] Pneumococcal polysaccharide Serotype 9V 2.2 µg[a] Pneumococcal polysaccharide Serotype 14 2.2 µg[a] Pneumococcal polysaccharide Serotype 18C 2.2 µg[a] Pneumococcal polysaccharide Serotype 19A 2.2 µg[a] Pneumococcal polysaccharide Serotype 19F 2.2 µg[a] Pneumococcal polysaccharide Serotype 23F 2.2 µg[a]	Sodium Chloride, Succinic Acid, Polysorbate 80, Water for Injection

a. conjugated to CRM197

INDICATIONS AND CLINICAL USE

Prevnar® 13, Pneumococcal 13-valent Conjugate Vaccine (Diphtheria CRM_{197} Protein), is indicated for the active immunization against Streptococcus pneumoniae serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F, causing invasive pneumococcal disease (including sepsis, meningitis, bacteraemic pneumonia, pleural empyema and bacteraemia), in infants and children from 6 weeks through 5 years of age (See PART II, CLINICAL TRIALS).

CONTRAINDICATIONS

Hypersensitivity to any component of the vaccine, including diphtheria toxoid.

(For a complete listing, see DOSAGE FORMS, COMPOSITION AND PACKAGING section)

WARNINGS AND PRECAUTIONS

Serious Warnings and Precautions

Safety and immunogenicity data on Prevnar 13 are not available for children in specific groups at higher risk for invasive pneumococcal disease (e.g., children with congenital or acquired splenic dysfunction, HIV infection, malignancy, nephrotic syndrome). Children in these groups may have reduced antibody response to active immunization due to impaired immune responsiveness. Vaccination in high-risk groups should be considered on an individual basis.[57]

General

- As with all injectable vaccines, appropriate medical treatment and supervision must always be readily available in case of a rare anaphylactic event following the administration of the vaccine. [22]

- Minor illnesses, such as mild respiratory infection, with or without low-grade fever, are not generally contraindications to vaccination. The decision to administer or delay vaccination because of a current or recent febrile illness depends largely on the severity of the symptoms and their etiology. The administration of Prevnar 13 should be postponed in subjects suffering from acute severe febrile illness.

- As with any intramuscular injection, Prevnar 13 should be given with caution to infants or children with thrombocytopenia or any coagulation disorder, or to those receiving anticoagulant therapy. [23]

- Prevnar 13 will not protect against Streptococcus pneumoniae serotypes not included in the vaccine. Prevnar 13 will not protect against other microorganisms that cause invasive disease, pneumonia, or otitis media. This vaccine is not intended to be used for treatment of active infection.

- As with any vaccine, Prevnar 13 may not protect all individuals receiving the vaccine from pneumococcal disease.

- The use of pneumococcal conjugate vaccine does not replace the use of 23-valent pneumococcal polysaccharide vaccine (PPV23) in children ≥ 24 months of age with sickle cell disease, asplenia, HIV infection, chronic illness, or who are otherwise immunocompromised. Data on sequential vaccination with Prevnar 13 followed by 23-valent pneumococcal polysaccharide vaccine are not available; data on sequential vaccination with Prevnar (7-valent) vaccine followed by PPV23 are limited. [70]

- As with all injectable pediatric vaccines, the potential risk of apnea should be considered when administering the primary immunization series to premature infants. The need for monitoring for at least 48 hours after vaccination should be considered for very premature infants (born ≤ 30 weeks of gestation) who remain hospitalized at the time of the recommended administration. As the benefit of vaccination is high in this group of infants, vaccination should not be withheld or delayed.

- Immunization with Prevnar 13 does not substitute for routine diphtheria immunization.

Special Populations

Pregnant Women:
Safety during pregnancy has not been established.

Nursing Women:
Safety during lactation has not been established.
It is not known whether vaccine antigens or antibodies are excreted in human milk.

Pediatrics:
The safety and immunogenicity of Prevnar 13 in children below the age of 6 weeks or on or after the 6th birthday have not been established

Geriatrics:
The safety and immunogenicity of Prevnar 13 in geriatric populations have not been established

ADVERSE REACTIONS

Adverse Drug Reaction Overview

Clinical Trial Adverse Drug Reactions

The safety of the vaccine was assessed in 13 controlled clinical trials where approximately 15,000 doses were given to 4,729 healthy infants in ages ranging in age from 6 weeks to 16 months of age. In all trials, Prevnar 13 was co-administered with routine pediatric vaccines. In a catch-up study, 354 children (7 months to 5 years of age) receiving at least one dose of Prevnar 13 were also assessed for safety.

Expected frequency of adverse reactions is presented in CIOMS frequency categories:

Very common	≥ 10%
Common:	≥ 1 % and < 10 %
Uncommon:	≥ 0.1% and < 1%
Rare:	≥ 0.01% and < 0.1%
Very rare:	< 0.01%

System Organ Class	Adverse Reaction
Metabolism and nutrition disorders	
Very common	**Decreased appetite**
Psychiatric disorders	
Very common	**Irritability**
Uncommon	**Crying**
Nervous system disorders	
Very common	**Drowsiness/increased sleep; restless sleep/decreased sleep**
Uncommon	**Seizures (including febrile seizures)**
Gastrointestinal disorders	
Common	**Diarrhea; vomiting**
Immune system disorders	
Rare	**Hypersensitivity reaction including face edema, dyspnea, bronchospasm**
Skin and subcutaneous tissue disorders	
Common	**Rash**
Uncommon	**Urticaria or urticaria-like rash**

System Organ Class	Adverse Reaction
General disorders and administration site conditions	
Very common	**Fever; any injection-site erythema, induration/swelling or pain/tenderness;** **Injection-site erythema or induration/swelling 2.5 cm – 7.0 cm (after toddler dose and in older children [age 2 to 5 years]).**
Common	**Fever greater than 39°C; injection-site erythema or induration/swelling 2.5 cm - 7.0 cm (after infant series); injection-site pain/tenderness interfering with movement**
Uncommon	**Injection-site induration/swelling or erythema greater than 7.0 cm**

Additional Adverse Reactions from Prevnar (7-valent)

Although the following adverse drug reactions were not observed in the clinical trials for Prevnar 13, they are considered adverse drug reactions for Prevnar (7-valent) and are considered adverse drug reactions for Prevnar 13 as well. These reactions are listed as follows with the frequency seen with Prevnar (7-valent).

Adverse Reactions from Prevnar (7-valent) Clinical Trials

System Organ Class	*Adverse Reaction*
Nervous system disorders	
Rare	**Hypotonic-hyporesponsive episode**

Adverse Reactions from Prevnar (7-valent) Postmarketing Experience

These frequencies are based on spontaneous reporting rates for Prevnar (7-valent) and have been calculated using number of reports and number of doses distributed.

System Organ Class	*Adverse Reaction*
Blood and lymphatic system disorders	
Very rare	**Lymphadenopathy localized to the region of the injection-site**

System Organ Class	*Adverse Reaction*
Immune system disorders	
Very rare	**Anaphylactic/anaphylactoid reaction including shock**
Skin and subcutaneous tissue disorders	
Very rare	**Angioneurotic edema; erythema multiforme**
General disorders and administration site conditions	
Very rare	**Injection-site dermatitis; injection-site urticaria; injection-site pruritus**

Serious Adverse Events (SAEs)

During the 13 controlled clinical trials, SAEs that the investigator considered related to study vaccine were reported for 11 out of 7,489 total subjects (0.1%); 6 out of 4,729 subjects (0.1%) in the Prevnar 13 group and 5 out of 2,760 (0.2%) in Prevnar (7-valent) group. Of these 11 cases, there were 3 cases of febrile convulsions and 4 cases of fever. Out of the 4 cases with fever, 1 subject had diffuse body rash, 1 subject had respiratory distress, and 1 subject had tense fontanelle. The remaining related SAE cases were crying (1), bronchitis (1), infantile spasms (1) and nephroblastoma (1).

Deaths

During the 13 controlled clinical trials, death occurred in 4 (out of 7,489) infants. All 4 cases were attributable to Sudden Infant Death Syndrome (SIDS). Three (out of 4,729 cases receiving 15,739 doses) cases were among Prevnar 13 recipients, and one (out of 2,760 cases receiving 9,030 doses) was in the Prevnar (7-valent) group. None of these cases were assessed by the investigator as causally related to vaccination.

DRUG INTERACTIONS

Overview

Prevnar 13 can be given, at a different injection site, with any of the following vaccine antigens, either as monovalent or combination vaccines: diphtheria, tetanus, acellular pertussis, Haemophilus influenzae type b, inactivated poliomyelitis, hepatitis B, meningococcal serogroup C, measles, mumps, rubella and varicella. Clinical studies demonstrated that the immune responses and the safety profiles of the administered vaccines were unaffected.

In the clinical trials, some infants and children were given rotavirus vaccine (oral administration) or hepatitis A vaccine (at a different site) with Prevnar 13. The safety profiles were comparable for these children but immunogenicity data were not available.

Different injectable vaccines should always be given at different injection-sites.

DOSAGE AND ADMINISTRATION

For intramuscular use only.

The dose is 0.5 mL given intramuscularly, with care to avoid injection into or near nerves and blood vessels. The preferred sites are the anterolateral aspect of the thigh in infants or the deltoid muscle of the upper arm in older children. The vaccine should not be injected in the gluteal area. **Do not administer Prevnar 13 intravascularly.**

The vaccine should not be injected intradermally, subcutaneously or intravenously, since the safety and immunogenicity of these routes have not been evaluated.

Parenteral products should be inspected visually for particulate matter or discoloration prior to use.

Data on the interchangeability of Prevnar 13 with other pneumococcal conjugate vaccines containing a protein carrier different from CRM_{197} are not available.

It is recommended that infants who receive a first dose of Prevnar 13 complete the vaccination course with Prevnar 13.

The vaccine is not to be mixed with other vaccines/products in the same syringe.

Vaccination Schedule

Primary Immunization
For infants, the recommended immunization series of Prevnar 13 consists of three doses of 0.5 mL each, at approximately 2-month intervals, followed by a fourth dose (booster) of 0.5 mL at 12-15 months of age (3+1 schedule). The customary age for the first dose is 2 months of age, but it can be given as young as 6 weeks of age. The recommended dosing interval is 4 to 8 weeks. The fourth dose should be administered at approximately 12-15 months of age, and at least 2 months after the third dose.

Table 1: Prevnar 13 Vaccine Schedule for Infants and Toddlers

Dose	*Dose 1* [a]	*Dose 2* [b]	*Dose 3* [b]	*Dose 4* [c]
Age at Dose	*2 months*	*4 months*	*6 months*	*12-15 months*

a. Dose 1 may be given as early as 6 weeks of age.
b. The recommended dosing interval is 4 to 8 weeks.
c. The fourth dose should be administered at approximately 12-15 months of age, and at least 2 months after the third dose.

Previously unvaccinated older infants and children:

For children who are beyond the age of routine infant schedule, the following Prevnar 13 schedule applies:

Table 2: Prevnar 13 Schedule for Previously Unvaccinated Children ≥7 Months through 5 Years of Age

Age at First Dose	Total Number of 0.5 mL Doses
7-11 months of age	3[a]
12-23 months of age	2[b]
≥24 months through 5 years of age (prior to the 6th birthday)	1

a. 2 doses at least 4 weeks apart; third dose after the one-year birthday, separated from the second dose by at least 2 months.
b. 2 doses at least 2 months apart.

If Prevnar 13 is given as part of a routine infant immunization program, a three dose (2+1) schedule may be considered. The first dose may be given from the age of 2 months, with a second dose 2 months later, and a third (booster) dose is recommended between 11 - 12 months of age. Lower immunogenicity responses for serotypes 6B and 23F were observed when Prevnar 13 is given as a 2-dose (e.g., at 2 and 4 months of age, or at 3 and 5 months of age) schedule in infants up to 6 months of age. After the booster dose, all vaccine serotypes had immune responses consistent with adequate priming with a two-dose primary series (See PART II, CLINICAL TRIALS section).

Infants and Children Previously Vaccinated with Prevnar (7-valent) (Streptococcus pneumoniae serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F):
Prevnar 13 contains the same 7 serotypes contained in Prevnar (7-valent) and is manufactured based on the same conjugate technology using the CRM_{197} carrier protein. A booster dose of

Prevnar 13 in children (n = 100) previously vaccinated with 3 doses of Prevnar (7-valent) elicited anti-capsular antibody levels (GMCs) to each of the seven common serotypes that were comparable to those elicited by 4 doses of Prevnar (7-valent) (see Table 7 of PART II, CLINICAL TRIALS section).

Children who have completed the infant series with Prevnar (7-valent) can receive a single dose of Prevnar 13 in the second year of life. A single dose of Prevnar 13 in children >1 year of age elicits immune responses to the six additional serotypes that are comparable to those elicited by a 3-dose infant series of Prevnar 13, but are generally lower than those elicited by a 3 infant doses + 1 toddler dose schedule of Prevnar 13.

OVERDOSAGE

Overdose with Prevnar 13 is unlikely due to its presentation as a pre-filled syringe. However, there have been reports of overdose with Prevnar 13 defined as subsequent doses administered closer than recommended to the previous dose. In general, adverse events reported with overdose are consistent with those that have been reported with doses given in the recommended schedules of Prevnar 13.

ACTION AND CLINICAL PHARMACOLOGY

Mechanism of Action

Prevnar 13 contains the 7 pneumococcal capsular polysaccharides that are in Prevnar (7-valent) (4, 6B, 9V, 14, 18C, 19F, 23F) plus 6 additional polysaccharides (1, 3, 5, 6A, 7F, 19A) all conjugated to CRM_{197} carrier protein. B-cells produce antibodies in response to antigenic stimulation via T-dependent and T-independent mechanisms. The immune response to most antigens is T-dependent and involves the collaboration of CD4+ T-cells and B-cells, recognizing the antigen in a linked fashion. CD4+ T-cells (T-helper cells) provide signals to B-cells directly through cell surface protein interactions, and indirectly through the release of cytokines. These signals result in proliferation and differentiation of the B-cells, and production of high-affinity antibodies. CD4+ T-cell signaling is a requisite for the generation of long-lived B-cells called plasma cells, which continuously produce antibodies of several isotypes (with an IgG component) and memory B-cells that rapidly mobilize and secrete antibodies upon re-exposure to the same antigen. [66]

Bacterial capsular polysaccharides (PSs), while varied in chemical structure, share the common immunological property of being largely T-independent antigens. In the absence of T-cell help, PS stimulated B-cells predominantly produce IgM antibodies; there is generally no affinity maturation of the antibodies, and no memory B-cells are generated. As vaccines, PSs are associated with poor or absent immunogenicity in infants less than 24 months of age and failure to induce immunological memory at any age. Conjugation of PSs to a protein carrier overcomes the T-cell–independent nature of PS antigens. Protein carrier-specific T-cells provide the signals

needed for maturation of the B-cell response and generation of B-cell memory. Conversion of Streptococcus pneumoniae PSs to a T cell dependent antigen by covalent coupling to the immunogenic protein carrier CRM_{197} enhances the antibody response, induces immune memory, and elicits booster responses on re-exposure in infants and young children to pneumococcal polysaccharides.

Pharmacodynamics

Disease Burden

S. pneumoniae is an important cause of morbidity and mortality in persons of all ages worldwide. The organism causes invasive infections, such as bacteremia and meningitis, as well as pneumonia and upper respiratory tract infections, including otitis media and sinusitis. In 2002, the World Health Organization (WHO) estimated that pneumococcal disease was the most common vaccine-preventable cause of death among children <5 years of age worldwide, causing an estimated 716,000 deaths in this age group. [15]

Prior to the licensure of 7-valent pneumococcal conjugate vaccine (Prevnar) in Canada in 2001, the observed incidence rates of invasive pneumococcal disease (IPD) among children <2 years of age and <5 years of age were 58.8 – 112.2 per 100,000 and 35.0 to 63.8 per 100,000 per year, respectively. [47] The overall fatality rate was 2.0%.[61] The incidence rate of pneumococcal meningitis among children <2 years of age was 9.0 per 100,000 per year, with a case fatality rate of 5.9%. [7]

Pneumonia may be accompanied by bacteremia or complicated by empyema, a local invasion into a normally sterile space; both of these invasive manifestations of pneumonia are more severe and are associated with higher morbidity and mortality than noninvasive pneumonia. Prior to the introduction of Prevnar (7-valent), the estimated incidence of children < 2 years who were hospitalized with pneumococcal pneumonia in Canada was 2.25/1000 persons per year.[54]

Since the introduction of Prevnar (7-valent) in Canada in 2001, it has been shown that the incidence of IPD caused by Prevnar (7-valent) serotypes decreased by 92% in Vancouver[8], 94% in Calgary[39] and by 72% in Quebec[32].

A ten-year population-based surveillance of all cases of invasive pneumococcal infection occurring in the Calgary Health Region reported a decrease of 92% in 2007 vs. 1998-2001 in Prevnar (7-valent) serotypes among adults 65 years and older suggesting a herd immunity, a phenomenon that occurs via interruption of transmission of disease to otherwise susceptible populations.[39]

Point-prevalence surveys conducted in Calgary have showed that routine Prevnar (7-valent) vaccination led to significant reductions in nasopharyngeal colonization of vaccine-serotype strains of *S. pneumoniae* in both vaccinated and unvaccinated children, indicating a direct and herd effect.[41]

A laboratory-based surveillance study in Quebec[31] showed that the resistance rates to penicillin G decreased from 17.6% in 2004, the year Prevnar (7-valent) was introduced, to 8.1% in 2007. Resistance rates to erythromycin decreased from 43.4% in 2004 to 21.6% in 2007. In Alberta, resistance rates to penicillin G dropped significantly from 14% in 2000 to 4.6% in 2006 ($p<0.0001$).[69]

Data from the International Circumpolar Surveillance System for Invasive Pneumococcal Disease, 1999 – 2005, showed that in northern Canada, incidence of IPD among all indigenous persons decreased from 44.2 cases/100,000 persons in the pre- Prevnar (7-valent) period (1999–2002) to 25.0 cases/100,000 persons in the vaccine implementation period (2003–2005; $p = 0.0005$). Among Canadian indigenous children <2 years of age, incidence decreased from 229.3/100,000 persons in the pre- Prevnar (7-valent) period to 92.6/100,000 persons in the vaccine implementation period (2003–2005; $p = 0.01$). The incidence of serotypes in Prevnar (7-valent) in all age groups decreased from 12.6 cases/100,000 persons in the pre– Prevnar (7-valent) period (1999–2002) to 3.8 cases/100,000 persons in the vaccine implementation period (2003–2005; $p<0.0001$)[9].

While the effect of routine use of the Prevnar (7-valent) in infants and young children has been dramatic, with a near-total elimination of the serotypes contained in this vaccine, an increase in other serotypes causing IPD has been observed (as an increasing percentage of residual disease). Data from Canadian surveillance systems [32] (National Centre for Streptococcus [NCS]; Immunization Monitoring Program, Active [IMPACT]; and Institut National de Santé Publique du Québec [INSPQ]) showed that serotypes 19A, 6A and 3 have emerged as the predominant pneumococcal serotypes causing IPD in Canadian children, accounting for approximately one-third of the residual IPD in 2007 in children <5 years of age. The 6 additional serotypes included in Prevnar 13 caused up to 48% of residual IPD. Compounding the issue of the predominance of emerging serotype 19A is that it is increasingly likely to be nonsusceptible to commonly used first-line antimicrobial agents.[32]

STORAGE AND STABILITY

Store refrigerated at +2 °C to +8 °C, (36 °F to 46 °F).

Do not freeze. Discard if the vaccine has been frozen.

Store in original package.

Prevnar 13 has been shown to be stable at temperatures of up to 40°C for 4 days. These data are not recommendations for shipping or storage, but may guide decisions for use in case of temporary temperature excursions.

Do not use after the expiry date shown on the label.

SPECIAL HANDLING INSTRUCTIONS

Prevnar 13 is a suspension containing an adjuvant. The vaccine should be shaken well to obtain a homogeneous white suspension prior to expelling air from the syringe, and should be inspected visually for any particulate matter and/or variation of physical aspect prior to administration. Do not use if the content appears otherwise.

The vaccine is not to be mixed with other vaccines/products in the same syringe.

DOSAGE FORMS, COMPOSITION AND PACKAGING

The vaccine is a white suspension for injection and provided in a single-dose, pre-filled syringe (0.5 mL). Pack size of 10, without needles.

PART II: SCIENTIFIC INFORMATION

PHARMACEUTICAL INFORMATION

Drug Substance

Proper name: Pneumococcal 13-valent Conjugate Vaccine (Diphtheria CRM_{197} Protein)

Product Characteristics

Pneumococcal 13-valent conjugate vaccine is a sterile solution of saccharides of the capsular antigens of Streptococcus pneumoniae serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F individually conjugated by reductive amination to non-toxic diphtheria CRM_{197} protein. The polysaccharides are chemically activated, and then covalently linked to the protein carrier CRM_{197} to form the glycoconjugate.

Individual conjugates are compounded, and then polysorbate 80 and aluminum phosphate are added to formulate the vaccine. The potency of the vaccine is determined by the quantity of the saccharide antigens and the saccharide-to-protein ratios in the individual glycoconjugates. Each 0.5 mL dose is formulated to contain 2.2 µg of each saccharide for serotypes 1, 3, 4, 5, 6A, 7F, 9V, 14, 18C, 19A, 19F, and 23F and 4.4 µg of saccharide for serotype 6B, conjugated to CRM_{197} carrier protein, and 0.125 mg of aluminum as aluminum phosphate adjuvant.

CLINICAL TRIALS

Overview of Prevnar 13 Clinical Studies

Prevnar 13 Immunogenicity Clinical Studies

Table 3: Overview of 13vPnC Clinical Studies

Study	Study Design	Primary Objectives	Study Vaccine Schedule (Months)	Concomitant Vaccine Schedule (Months)	N Vaccinated per Group (as Randomized)
Pivotal Pneumococcal Studies					
6096A1-004	Multi-Centre, randomized, double-blind, controlled	Demonstrate that the PnC serotype-specific IgG responses (proportion of responders at ≥0.35 µg/mL) induced by 13vPnC are non-inferior to those induced by 7vPnC or 7vPnC reference[a] measured 1 month after the infant series. Demonstrate that the serotype-specific geometric mean IgG concentrations induced by 13vPnC are non-inferior to those induced by 7vPnC or 7vPnC reference[a] measured 1 month after the toddler dose. Assess the non-inferiority of antigen-specific response (Dip, PT, FHA, PRN, Hib) 1 month after dose 3 of PnC and concomitant vaccine in the 13vPnC group relative to the 7vPnC group.	13vPnC or 7vPnC (2, 4, 6, 12-15)	Pediarix (2, 4, 6) ActHIB (2, 4, 6) PedvaxHIB (12-15) ProQuad (12-15) VAQTA (12-15)	13vPnC: 332 7vPnC: 331

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Table 3: Overview of 13vPnC Clinical Studies (Cont'd)

Study	Study Design	Primary Objectives	Study Vaccine Schedule (Months)	Concomitant Vaccine Schedule (Months)	N Vaccinated per Group (as Randomized)
6096A1-006	Multi-centre, randomized, double-blind, controlled	Demonstrate that the PnC serotype-specific IgG responses induced by 13vPnC are non-inferior to those induced by 7vPnC or 7vPnC reference[a] measured 1 month after the infant series. Assess the non-inferiority of antigen-specific response (Dip, HBV, Hib) 1 month after dose 3 of PnC and concomitant vaccine in the 13vPnC group relative to the 7vPnC group.	13vPnC or 7vPnC (2, 3, 4, 11 12)	Infanrix hexa (2, 3, 4, 11-12)	13vPnC: 300 7vPnC: 303
Additional Vaccine Schedules and Concomitant Vaccine Immunogenicity Trials					
6096A1-007	Multi-centre, randomized, double-blind, controlled	Evaluate the immune response after NeisVac-C (MnC using SBA) and 13vPnC relative to NeisVac-C and 7vPnC measured 1 month after the infant series. Evaluate the immune response after Pediacel (antigens assessed: PT, FHA, PRN, FIM, Hib) and 13vPnC relative to Pediacel and 7vPnC measured 1 month after the infant series. Assess the immune response (IgG) to 13vPnC measured 1 month after the infant series and before and 1 month after the toddler dose.	13vPnC or 7vPnC (2, 4, 12)	NeisVac C (2, 4) Pediacel (2, 3, 4) Menitorix (12)	13vPnC: 139 7vPnC: 139
6096A1-008	Multi-centre, randomized, double-blind, controlled	Demonstrate that the immune responses after Pentavac (antigens assessed: PT, FHA, Hib, Dip, Tet,	13vPnC or 7vPnC (2, 3, 4, 12)	Pentavac (2, 3, 4, 12)	13vPnC: 302 7vPnC: 309

Table 3: Overview of 13vPnC Clinical Studies (Cont'd)

Study	Study Design	Primary Objectives	Study Vaccine Schedule (Months)	Concomitant Vaccine Schedule (Months)	N Vaccinated per Group (as Randomized)
		polio types 1, 2, 3) and 13vPnC are non-inferior to the response after Pentavac and 7vPnC measured 1 month after the infant series. Assess the immune responses to 13vPnC measured 1 month after the infant series. Assess immune responses, 1 month after the toddler dose, to the following (infant series/ toddler dose) sequences: 13vPnC/13vPnC and 7vPnC/13vPnC relative to 7vPnC/7vPnC. Post toddler dose pneumococcal responses induced by 13vPnC/13vPnC relative to 7vPnC/13vPnC were also assessed.			
6096A1-011	Multi-centre, randomized, double-blind, controlled	Assess the PnC immune response after 13vPnC relative to 7vPnC measured 1 month after the infant series. Assess the immune response after Easyfive, ie, DTP-Hib-HBV vaccine (antigens assessed: PT, FHA, PRN) and 13vPnC relative to DTP-Hib-HBV and 7vPnC measured 1 month after the infant series.	13vPnC or 7vPnC (6, 10, 14 weeks, 12 months)	Easyfive (6, 10, 14 weeks) Biopolio (6, 10, 14 weeks)	13vPnC: 178 7vPnC:175
6096A1-500	Multi-centre, randomized, double-blind, controlled	Demonstrate that the immune response after Infanrix hexa (antigen assessed: HBV) and 13vPnC is non-inferior to the	13vPnC or 7vPnC (3, 5, 11)	Infanrix hexa (3, 5, 11)	13vPnC: 302 7vPnC: 302

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Table 3: Overview of 13vPnC Clinical Studies (Cont'd)

Study	Study Design	Primary Objectives	Study Vaccine Schedule (Months)	Concomitant Vaccine Schedule (Months)	N Vaccinated per Group (as Randomized)
		response after Infanrix hexa and 7vPnC measured 1 month after the toddler dose. Assess the immune response to 13vPnC measured 1 month after the infant series and just before the toddler dose. Assess the immune responses induced by 13vPnC relative to 7vPnC measured 1 month after the toddler dose.			
6096A1-501	Multi-centre, randomized, double-blind, controlled	Demonstrate that the immune response after Meningitec (antigen assessed: MnC by SBA) and 13vPnC is non-inferior to response after Meningitec and 7vPnC measured 1 month after a 2-dose Meningitec infant series. Assess the non-inferiority of antigen specific response to PT, FHA, PRN, Dip, Tet, and polio types 1, 2, 3 after Infanrix hexa and 13vPnC relative to Infanrix hexa and 7vPnC. Assess the immune responses to 13vPnC measured 1 month after dose 2 and 1 month after dose 3 of the infant series and 1 month after the toddler dose.	13vPnC or 7vPnC (2, 4, 6, 15)	Infanrix hexa (2, 4, 6) Meningitec (2, 4, 15) Infanrix-IPV+Hib (15) MMR II (12)	13vPnC: 314 7vPnC: 302
6096A1-3007	Multi-centre, randomized, double-blind, controlled	Demonstrate the non-inferiority of immune response after NeisVac-C and 13vPnC relative to NeisVac-C (antigen assessed: MnC using SBA) and 7vPnC measured 1 month after	13vPnC or 7vPnC (2, 4, 6, 15)	Infanrix hexa (2, 4, 6) NeisVac-C (2, 4, 15) Priorix (12) Infanrix-IPV+Hib (15)	13vPnC: 218 7vPnC: 226

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Table 3: Overview of 13vPnC Clinical Studies (Cont'd)

Study	Study Design	Primary Objectives	Study Vaccine Schedule (Months)	Concomitant Vaccine Schedule (Months)	N Vaccinated per Group (as Randomized)
		a 2-dose NeisVac-C infant series. Demonstrate the non-inferiority of immune response after Infanrix hexa (antigens assessed: Dip, Tet) and 13vPnC relative to Infanrix hexa and 7vPnC measured 1 month after a 3-dose infant series. Assess the immune responses to 13vPnC measured 1 month after dose 2 and 1 month after dose 3 of the infant series			
Other Clinical Studies					
6096A1-009	Multi-centre, randomized, double-blind, controlled	Demonstrate that the immune response to 13 serotypes after administration of 13vPnC+P80 is non-inferior relative to 13vPnC P80 measured 1 month after the infant series.	13vPnC+P80 or 13vPnC P80 (2, 3, 4, 12)	Pentaxim (2, 3, 4) Engerix-B (2) Priorix (12)	13vPnC+P80: 250 13vPnC-P80: 250
6096A1-3000	Multi-centre, randomized, double-blind, controlled	Assess the PnC response induced by manufacturing scale 13vPnC relative to pilot scale 13vPnC measured 1 month after the infant series. (Immunogenicity was not assessed for the toddler dose, as specified in the protocol.)	13vPnC pilot lot or 13vPnC man lot (2, 3, 4, 12)	Pentaxim (2, 3, 4) Engerix-B (2) Priorix (12)	13vPnC pilot: 134 13vPnC man: 134
6096A1-3005	Multi-centre, randomized, double-blind, controlled	Demonstrate that the immune responses induced by 3 lots of 13vPnC are equivalent at 1 month after the infant series. Demonstrate the non-inferiority of	13vPnC pilot lot 1, 13vPnC pilot lot 2, or 13vPnC man lot or 7vPnC (2, 4, 6, 12)	Pediarix (2, 4, 6) Act-HIB (2, 4, 6) MMR II and Varivax (12) Havrix (12)	13vPnC pilot 1: 486 13vPnC pilot 2: 484 13vPnC man: 485 7vPnC: 244

Table 3: Overview of 13vPnC Clinical Studies (Cont'd)

Study	Study Design	Primary Objectives	Study Vaccine Schedule (Months)	Concomitant Vaccine Schedule (Months)	N Vaccinated per Group (as Randomized)
		immune response induced by Pediarix given with 13vPnC relative to Pediarix given with 7vPnC 1 month after the infant series (antigens assessed: Tet; polio types 1, 2, 3; HBV).			
Trial in Older Infants and Young Children					
6096A1-3002	Multi-centre, Non-randomized, open-label, uncontrolled	Assess the PnC response induced by 13vPnC when measured 1 month after the last scheduled dose in each age group.	13vPnC: Group 1: (3 doses) 7 to <12 months, 1 month later, and 12-16 months Group 2: (2 doses) 12 to <24 months and 56 to 70 days later Group 3: (1 dose) 24 to <72 months	N/A	Group 1: 90 Group 2: 112 Group 3: 152
Phase 1-2 Trials					
6096A1-002	Single-centre, randomized, open-label, controlled	(NOTE: Immunogenicity was a secondary objective in this study.) Assess the post vaccination responses to the 13 pneumococcal serotypes in 13vPnC. Obtain sera to be used as reagents for further development, validation, and standardization of pneumococcal assays.	Single dose of 13vPnC or 23vPS	N/A	23vPS: 15 13vPnC: 15
6096A1-003	Single-centre, randomized, double-blind, controlled	Compare the percentages of subjects achieving a predefined antibody level to each of the 7 common serotypes after 3 doses of 13vPnC relative to 3 doses of 7vPnC.	13vPnC or 7vPnC (2, 4, 6, 12-15)	Pediarix (2, 4, 6) ActHIB (2, 4, 6, 12-15)	13vPnC: 121 7vPnC: 126

Table 3: Overview of 13vPnC Clinical Studies (Cont'd)

Study	Study Design	Primary Objectives	Study Vaccine Schedule (Months)	Concomitant Vaccine Schedule (Months)	N Vaccinated per Group (as Randomized)

a. In studies 004 and 006, values for the additional serotypes in the 13vPnC group are compared with the 7vPnC reference value, defined as the lowest value among the 7 common serotypes in the 7vPnC group.

Abbreviations: 13vPnC+P80 = 13vPnC formulated with polysorbate 80; 13vPnC-P80 = 13vPnC formulated without polysorbate 80; 23vPS = 23-valent pneumococcal polysaccharide vaccine; Dip = diphtheria; FHA = filamentous hemagglutinin; FIM = fimbrial agglutinogens; HBV = hepatitis B virus vaccine; Hib = Haemophilus influenzae type b; man = manufacturing; MnC = meningococcal C; NA = not applicable; PnC = pneumococcal conjugate vaccine; polio types 1, 2, 3 = poliovirus vaccine type 1, type 2, and type 3; PRN = pertactin; PT = pertussis toxoid; SBA = serum bactericidal assay; Tet = tetanus.

Components of vaccines by trade name: ActHIB = Hib; Biopolio = OPV; Easyfive = DTP (whole cell pertussis), Hib, and HBV; Engerix-B = HBV; Havrix = HAV; Infanrix hexa = DTaP, Hib, HBV, and IPV; Infanrix-IPV+Hib = DTaP, IPV, and Hib; Meningitec = meningococcal C vaccine; Menitorix = Hib and meningococcal C vaccine; MMR II = measles, mumps, and rubella vaccine; NeisVac-C = meningococcal C vaccine; Pediacel = DTaP, Hib, and IPV; Pediarix = DTaP, HBV, and IPV; PedvaxHIB = Hib; Pentavac = DTaP, Hib, and IPV; Pentaxim = DTaP, Hib, and IPV; Priorix = MMR; ProQuad = MMR and varicella vaccine; Varivax = varicella vaccine; VAQTA = HAV.

The World Health Organization (WHO) has recommended a serum anti-capsular polysaccharide antibody concentration of 0.35 µg/ml measured one month after the primary infant series as a single antibody reference concentration to estimate the efficacy of new pneumococcal conjugate vaccines against Invasive Pneumococcal Disease (IPD). This recommendation is based upon the observed correlation between immunogenicity and IPD efficacy from three placebo-controlled trials with either Prevnar (7-valent) or the investigational 9-valent CRM_{197} conjugate polysaccharide vaccine. This reference concentration is only applicable on a population basis and cannot be used to predict protection against IPD on an individual basis.

The WHO also requires demonstration of functionality of the elicited antibodies. Opsonophagocytosis (antibody mediated killing of the bacteria) is recognized as the main mechanism of protection against pneumococcal disease, which can be measured by an opsonophagocytosis activity assay (OPA). The percentage of subjects with an OPA titre ≥ 1:8 is used for comparison between vaccines, although the data to support the OPA titre ≥ 1:8 as a marker of protection are currently insufficient.

Finally, the ability to induce immune memory is also required.

In line with WHO recommendations, post-marketing studies will be undertaken to confirm the effectiveness of Prevnar 13.

Immune Responses Following a Three-Dose Primary Infant Series

The immunological comparison between Prevnar 13 and Prevnar (7-valent) was conducted in two pivotal studies (USA study 004 and Germany study 006) and was performed post-hoc in USA study 3005. The percentage of infants achieving pneumococcal anti-capsular polysaccharide IgG antibody concentrations ≥ 0.35 µg/mL one month after a three-dose primary infant series for the 7 common serotypes are presented in Table 4. The immunological non-inferiority was met when the lower bound of the 95% CI for the difference between groups (Prevnar 13 minus Prevnar (7-valent)) in terms of subjects with antibody concentration ≥ 0.35 µg/mL was greater than -10%.

As shown in Table 4, Prevnar 13 non-inferiority was demonstrated by ELISA for all serotypes in study 3005, all except 6B in study 006 and all except for 6B and 9V in study 004, each of which missed non-inferiority by a small margin at the lower bound of the 95% CI for the difference between groups. The clinical relevance of these differences is not known.

Table 4: Percentage of Subjects with Pneumococcal Anti-capsular Polysaccharide IgG Antibody Concentrations ≥ 0.35 µg/ml One Month after the Infant Series for the 7 Common Serotypes

Antibody	*Prevnar 13*		*Prevnar (7-valent)*		*Difference in % of ≥ 0.35 µg/mL (Prevnar 13 minus Prevnar (7-valent))*	
	N	*%*	*N*	*%*	*%*	*95% CI*
USA study 004 (one month after 3-dose primary infant series at 2, 4, and 6 months of age)						
Anti-4	*252*	*94.4*	*251*	*98.0*	*-3.6*	*-7.3, -0.1*
Anti-6B	*252*	*87.3*	*250*	*92.8*	*-5.5*	*-10.9, -0.1*
Anti-9V	*252*	*90.5*	*252*	*98.4*	*-7.9*	*-12.4, -4.0*
Anti-14	*251*	*97.6*	*252*	*97.2*	*0.4*	*-2.7, 3.5*
Anti-18C	*252*	*96.8*	*252*	*98.4*	*-1.6*	*-4.7, 1.2*
Anti-19F	*252*	*98.0*	*251*	*97.6*	*0.4*	*-2.4, 3.4*
Anti-23F	*252*	*90.5*	*252*	*94.0*	*-3.6*	*-8.5, 1.2*
Germany study 006 (one month after 3-dose primary infant series at 2, 3, and 4 months of age)						
Anti-4	*285*	*98.2*	*279*	*98.2*	*0.0*	*-2.5, 2.6*
Anti-6B	*284*	*77.5*	*278*	*87.1*	*-9.6*	*-16.0, -3.3*
Anti-9V	*285*	*98.6*	*279*	*96.4*	*2.2*	*-0.4, 5.2*
Anti-14	*284*	*98.9*	*279*	*97.5*	*1.5*	*-0.9, 4.1*
Anti-18C	*285*	*97.2*	*277*	*98.6*	*-1.4*	*-4.2, 1.2*
Anti-19F	*284*	*95.8*	*277*	*96.0*	*-0.3*	*-3.8, 3.3*
Anti-23F	*284*	*88.7*	*277*	*89.5*	*-0.8*	*-6.0, 4.5*
USA study 3005 (one month after 3-dose primary infant series at 2, 4, and 6 months of age) – post-hoc analysis						
Anti-4	*1216*	*97.2*	*165*	*98.8*	*-1.6*	*(-3.2, 1.4)*
Anti-6B	*1209*	*93.0*	*165*	*97.0*	*-4.0*	*(-6.6, -0.1)*
Anti-9V	*1212*	*95.8*	*158*	*98.7*	*-2.9*	*(-4.7, 0.2)*
Anti-14	*1175*	*98.8*	*163*	*99.4*	*-0.6*	*(-1.7, 2.1)*
Anti-18C	*1214*	*97.2*	*165*	*98.8*	*-1.6*	*(-3.2, 1.4)*
Anti-19F	*1208*	*98.2*	*165*	*97.6*	*0.6*	*(-1.3, 4.0)*
Anti-23F	*1214*	*88.9*	*165*	*95.2*	*-6.3*	*(-9.5, -1.8)*

The percentage of subjects, who received Prevnar 13 and reached the threshold of ≥0.35 µg/mL for serotypes 1, 5, 6A, 7F and 19A, was at 89.7% to 99.5%; and the non-inferiority criterion was met when compared to the lowest response of the 7 common serotypes in Prevnar (7-valent) recipients. For serotype 3, the percentage of subjects, who received Prevnar 13 and reached the threshold, was 63.5% and 73.3% in the USA studies and the non-inferiority criterion was not met when compared to Prevnar (7-valent) response against serotype 6B (92.8% study 004) or serotype 23F (95.2% study 3005) respectively. In the Germany study the 98.2% of subjects responded to serotype 3 and the non-inferiority was met against serotype 6B (87.1% study 006).

The geometric mean concentrations (GMCs) of anti-capsular polysaccharide IgG antibody pneumococcal antibodies for the 7 common serotypes following the primary infant series are presented in Table 5. Geometric mean ratios were calculated (Prevnar 13/ Prevnar (7-valent)) and non-inferiority criteria was met if the lower 95% CI was greater than 0.5, a 2-fold criterion. The non-inferiority criteria were met for all serotypes in the 3 studies; although the post-primary GMCs elicited by Prevnar 13 against the 7 common serotypes were generally lower than those elicited by Prevnar (7-valent).

Table 5: Pneumococcal Anti-capsular Polysaccharide IgG Antibody GMC (µg/mL) One Month after the Infant Series

Antibody	*Prevnar 13*		*Prevnar(7-valent)*		*Geometric Mean Ratio (Prevnar 13/ Prevnar (7-valent))*	
	N	*GMC (95% CI)*	*N*	*GMC (95% CI)*	*Ratio*	*95% CI*
USA study 004 (2, 4, 6 months of age)						
Anti-4	*252*	*1.31 (1.19, 1.45)*	*251*	*1.93 (1.75, 2.13)*	*0.68*	*(0.59, 0.78)*
Anti-6B	*252*	*2.10 (1.77, 2.49)*	*250*	*3.14 (2.64, 3.74)*	*0.67*	*(0.52, 0.85)*
Anti-9V	*252*	*0.98 (0.89, 1.08)*	*252*	*1.40 (1.27, 1.55)*	*0.70*	*(0.61, 0.80)*
Anti-14	*251*	*4.74 (4.18, 5.39)*	*252*	*5.67 (5.02, 6.40)*	*0.84*	*(0.70, 1.00)*
Anti-18C	*252*	*1.37 (1.24, 1.52)*	*252*	*1.79 (1.63, 1.96)*	*0.77*	*(0.67, 0.88)*
Anti-19F	*252*	*1.85 (1.69, 2.04)*	*251*	*2.24 (2.01, 2.50)*	*0.83*	*(0.72, 0.96)*
Anti-23F	*252*	*1.33 (1.17, 1.51)*	*252*	*1.90 (1.68, 2.15)*	*0.70*	*(0.59, 0.84)*
Germany study 006 (2, 3, 4 months of age)						
Anti-4	*285*	*2.18 (1.98, 2.40)*	*279*	*2.99 (2.68, 3.33)*	*0.73*	*(0.63, 0.84)*
Anti-6B	*284*	*0.98 (0.84, 1.14)*	*278*	*1.49 (1.27, 1.75)*	*0.65*	*(0.52, 0.82)*
Anti-9V	*285*	*1.65 (1.51, 1.80)*	*279*	*1.96 (1.77, 2.17)*	*0.84*	*(0.74, 0.96)*
Anti-14	*284*	*4.14 (3.68, 4.66)*	*279*	*4.61 (4.07, 5.23)*	*0.90*	*(0.76, 1.07)*
Anti-18C	*285*	*1.94 (1.76, 2.14)*	*277*	*2.25 (2.04, 2.49)*	*0.86*	*(0.75, 0.99)*
Anti-19F	*284*	*1.73 (1.56, 1.92)*	*277*	*2.86 (2.53, 3.24)*	*0.60*	*(0.51, 0.71)*
Anti-23F	*284*	*1.26 (1.11, 1.43)*	*277*	*1.44 (1.25, 1.65)*	*0.88*	*(0.73, 1.06)*

Table 5: Pneumococcal Anti-capsular Polysaccharide IgG Antibody GMC (µg/mL) One Month after the Infant Series (Cont'd)

Antibody	*Prevnar 13*		*Prevnar (7-valent)*		*Geometric Mean Ratio (Prevnar 13/ Prevnar (7-valent))*	
	N	*GMC (95% CI)*	*N*	*GMC (95% CI)*	*Ratio*	*95% CI*
USA study 3005 (2, 4, 6 months of age) – post-hoc analysis						
Anti-4	*1216*	*1.46 (1.40, 1.52)*	*165*	*1.95 (1.73, 2.19)*	*0.75*	*(0.66, 0.85)*
Anti-6B	*1209*	*2.52 (2.36, 2.69)*	*165*	*2.93 (2.47, 3.48)*	*0.86*	*(0.71, 1.04)*
Anti-9V	*1212*	*1.09 (1.05, 1.14)*	*158*	*1.35 (1.23, 1.49)*	*0.81*	*(0.72, 0.90)*
Anti-14	*1175*	*5.10 (4.85, 5.36)*	*163*	*6.21 (5.40, 7.14)*	*0.82*	*(0.71, 0.95)*
Anti-18C	*1214*	*1.37 (1.32, 1.43)*	*165*	*1.61 (1.44, 1.80)*	*0.85*	*(0.76, 0.95)*
Anti-19F	*1208*	*2.15 (2.06, 2.25)*	*165*	*2.30 (2.02, 2.61)*	*0.94*	*(0.83,1.07)*
Anti-23F	*1214*	*1.18 (1.11, 1.25)*	*165*	*1.71 (1.48, 1.97)*	*0.69*	*(0.59, 0.81)*

Opsonophagocytic activity assay (OPA) was measured in 2 studies (004 and 006) in a randomly selected subset of about 100 subjects per group in each study. Prevnar 13 elicited functional antibodies to all vaccine serotypes. For each of the 7 common serotypes, 90.4% to 100% of Prevnar 13 vaccinees and 92.6% to 100% of Prevnar (7-valent) vaccinees achieved an OPA titre ≥1:8 one month after 3-dose primary infant series. For the 6 additional serotypes, at least 91.4% of Prevnar 13 vaccinees achieved an OPA titre ≥1:8 one month after 3-dose primary infant series.

The OPA GMT was generally lower in the Prevnar 13 vaccinees compared with the Prevnar (7-valent) vaccinees (i.e., the OPA GMT ratios of Prevnar 13 vaccinees versus Prevnar (7-valent) vaccinees was less than 1) for the 7 common serotypes, but most were within 2-fold.

Immune Responses Following a Two-Dose Primary Series

The immunogenicity after two doses in infants has been documented in four studies. The proportion of infants achieving a pneumococcal anti-capsular polysaccharide IgG concentration ≥ 0.35 µg/ml one month after the second dose of Prevnar 13 ranged from 79.6% to 98.5% across 11 of the 13 vaccine serotypes. Smaller proportions of infants achieved this antibody concentration threshold for serotype 6B (27.9% to 57.3%) and 23F (55.8% to 68.1%) for all studies using a 2,4 month regimen, compared to 58.4% for serotype 6B and 68.6% for 23F for a trial (Italy Study 500) using a 3,5 month regimen. After the booster dose, the proportion of infants who achieved this antibody concentration threshold was increased for all vaccine serotypes including 6B and 23F and ranged from 88.2%-100% in Study 007 and 93.9%-100% in study 500. In UK study 007, the post-hoc analysis showed that the functional antibody (OPA) responses were comparable for the 7 common serotypes including 6B and 23F in the Prevnar (7-valent) (n= 45-60) and Prevnar 13 (n= 64-77) arms after the primary series at two and four months of age and after the booster dose at 12 months of age.

Booster Responses Following Two-Dose and Three-Dose Primary Schedules

Antibody responses to booster doses following two-dose or three-dose infant primary series in different studies were comparable for all 13 vaccine serotypes.

Post-booster antibody concentrations were higher for 12 serotypes than those achieved after the infant primary series, which is consistent with adequate priming (the induction of immunologic memory). For serotype 3, antibody concentrations declined after the infant series; after the booster dose antibody concentrations were lower than those after the infant primary series in some clinical trials, and higher in others (See the following table. In all studies where OPA was assessed, including both pivotal non-inferiority studies, the anti-type 3 opsonophagocytic activity of 13vPnC-induced antibody was clearly demonstrated. The proportion of subjects with OPA titers $\geq$1:8 ranged from 96.8% to 100% after the infant series and from 97.8% to 100% after the toddler dose. Functional antibody (OPA) GMTs were higher after the toddler dose than after the infant series in 3 of 4 studies).

The serotype 3 serum IgG GMC induced by only one toddler dose of Prevnar 13 was slightly greater than that induced by 3-infant doses plus one toddler dose of Prevnar 13. However, the point estimate of the proportion of responders in both groups exceeded 93% at $\geq$ 0.35 µg/mL and was slightly higher in the 13vPnC/13vPnC group (94.8%) than the 7vPnC/13vPnC group (93.8%). The proportion of OPA responders at $\geq$ 1:8 was high in both groups (97.8 and 100%) , but OPA GMT was greater in the subjects who received only one toddler dose of Prevnar 13 (428.88; 95% CI: 346.69, 530.56) compared to those who received 3-infant doses plus one toddler dose of Prevnar 13 (345.33; 95% CI: 296.06, 402.80) . The OPA GMT (504.66; 95% CI: 435.71, 584.53) from Study 500 was even greater in the subjects who received 2-infant doses plus one toddler dose of Prevnar 13 (2+1) compared to those who received only one toddler dose of Prevnar 13 (7vPnC/13vPnC group) in study 008 (See Table 6). The clinical significance of these observations is unknown.

Table 6: Pneumococcal Anti-capsular Polysaccharide IgG Antibody Geometric Mean Concentrations (µg/mL), OPA Percentage of Subject ≥1:8 and OPA Geometric Mean Titers for Serotype 3

Study	*Group*	*GMC (95% CI) for the serotype 3*			*N*	*OPA ≥1:8*	*OPA GMT*	*N*	*OPA ≥1:8*	*OPA GMT*
		After infant series	*Before toddler dose*	*After toddler dose*	*After infant series*			*After toddler dose*		
004 [a]	*13vPnC*	*0.49 (0.43, 0.55)*	*0.15 (0.13, 0.17)*	*0.94 (0.83, 1.05)*	*94*	*96.8*	*120.67*	*91*	*97.8*	*380.41*
006 [a]	*13vPnC*	*1.55 (1.41, 1.72)*	*0.25 (0.23, 0.28)*	*1.02 (0.92, 1.13)*	*100*	*99.0*	*250.73*	*98*	*98.0*	*187.54*
009 [a]	*13vPnC*	*1.50 (1.38, 1.63)*	*0.14 (0.13, 0.16)*	*1.09 (0.99, 1.20)*						
	13vPnC	*1.62 (1.49, 1.75)*	*0.18 (0.16, 0.20)*	*1.16 (1.05, 1.29)*						
007 [d]	*13vPnC*	*0.63 (0.56, 0.71)*	*0.14 (0.10, 0.18)*	*0.98(0.78, 1.22)*	*70*	*100.0*	*109.25*	*77*	*100.0*	*306.50*
008 [a]	*13vPnC/13vPnC* [c]	*1.25 (1.13, 1.37)*	*0.2 (0.2, 0.2)*	*1.0 (0.9, 1.1)*				*88*	*100.0*	*345.33*
	7vPnC/13vPnC [b]	-	*0.1 (0.1, 0.1)*	*1.32 (1.1, 1.5)*				*90*	*97.8*	*428.88*
500 [d]	*13vPnC*	*1.15 (1.04, 1.28)*	*0.25 (0.22, 0.27)*	*1.22 (1.09, 1.35)*	*100*	*99.0*	*176.07*	*96*	*100.0*	*504.66*
501 [a]	*13vPnC*	*0.97 (0.88, 1.08)*	-	*1.07 (0.95, 1.20)*						

a. Subjects received 3 infant doses plus 1 toddler dose.
b. 7vPnC/13vPnC: 3 infant doses of 7vPnC plus 1 toddler dose of 13vPnC.
c. 13vPnC/13vPnC: 3 infant doses plus 1 toddler dose of 13vPnC.
d. Subjects received 2 infant doses plus 1 toddler dose.

Booster Responses to Prevnar 13 Following a Three-Dose Primary Infant Series of Prevnar (7-valent) or Prevnar 13

In a randomized, double-blind, active control study in France (008) infants were randomly assigned to three groups in a 2:1:1 ratio: (1) Prevnar 13 at 2, 3, 4 and 12 months or (2) Prevnar

(7-valent) at 2, 3, 4 months followed by Prevnar 13 at 12 months or (3) Prevnar (7-valent) at 2, 3, 4 and 12 months. Geometric mean concentrations of anti-capsular polysaccharide IgG antibody responses to each of the 13 serotypes in the 3 groups are shown in Table 7.

Table 7: ***Pneumococcal Anti-capsular Polysaccharide IgG Antibody Geometric Mean Concentrations (µg/mL), OPA percentage of subject ≥1:8 and OPA Geometric Mean Titers One Month After Toddler Booster Dose***

Serotype	*13v/13v Post Toddler N=233-236*	*7v/13v Post Toddler N=108-113*	*7v/7v Post Toddler N=111-127*	*13v/13v Post Toddler*			*7v/13v Post Toddler*		
				N	*OPA ≥ 1:8*	*OPA GMT*	*N*	*OPA ≥ 1:8*	*OPA GMT*
4	*4.20*	*4.04*	*4.85*	-	-	-	-	-	-
6B	*8.99*	*10.33*	*9.63*	-	-	-	-	-	-
9V	*2.59*	*2.29*	*3.24*	-	-	-	-	-	-
14	*9.52*	*7.81*	*10.83*	-	-	-	-	-	-
18C	*2.30*	*2.43*	*2.81*	-	-	-	-	-	-
19F	*5.18*	*3.73*	*4.11*	-	-	-	-	-	-
23F	*3.01*	*3.12*	*3.69*	-	-	-	-	-	-
1	*4.08*	*1.83*	*0.04*	*88*	*100.0*	*126.00*	*90*	*98.9*	*61.58*
3	*0.99*	*1.32*	*0.10*	*88*	*100.0*	*345.33*	*90*	*97.8*	*428.88*
5	*3.30*	*1.14*	*0.53*	*88*	*100.0*	*244.18*	*90*	*97.8*	*130.99*
6A	*6.14*	*2.60*	*1.54*	*86*	*100.0*	*1346.83*	*90*	*98.9*	*891.44*
7F	*4.52*	*3.71*	*0.05*	*86*	*100.0*	*8126.24*	*89*	*100.0*	*17034.59*
19A	*9.50*	*5.33*	*3.98*	*86*	*98.8*	*804.06*	*90*	*97.8*	*1072.43*

Table 7 shows that GMCs to the seven Prevnar (7-valent) serotypes did not differ in the 3 groups. Although the GMCs to the 6 additional serotypes in the Prevnar (7-valent)/ Prevnar 13 recipients were lower than those observed with the four dose Prevnar 13 regimen (except for serotype 3), they were at least comparable to those of a three dose primary series of Prevnar 13 in infants. In both groups that received Prevnar 13, ≥98.7% of subjects had OPA titers ≥1:8 and OPA GMTs were comparable in both groups.

Previously Unvaccinated Older Infants and Children

In an open label study of Prevnar 13 in Poland (3002), children 7 to 11 months of age, 12 to 23 months and ≥24 months through 5 years of age (prior to the 6th birthday) who were naïve to pneumococcal conjugate vaccine, were given 3, 2 or 1 dose of Prevnar 13 according to their age at first dose (see Table 2). Serum IgG concentrations were measured one month after the final

dose in each age group and the data are shown in Table 8.

The age appropriate catch-up immunization schedules result in levels of anti-capsular polysaccharide IgG antibody responses to each of the 13 serotypes that are at least comparable to those of a three dose primary series in infants.

Table 8: Pneumococcal Anti-capsular Polysaccharide IgG Antibody Geometric Mean Concentrations (µg/mL) One Month After the Final Dose by Age Group in Study 3002, Compared with One Month Post 3 Infant Doses in study 004 and study 3005

Serotype	*7 to 11 months of age*	*12 to 23 months of age*	*≥24 months through 5 years of age*	*Post-infant (Study 004)*	*Post-infant (Study 3005)*
	(N= 83-84)	*(N=104-110)*	*(N=135-152)*	*(N=249-252)*	*(N=1172-1213)*
1	*2.88*	*2.74*	*1.78*	*2.03*	*1.78*
3	*1.94*	*1.86*	*1.42*	*0.49*	*0.56*
4	*3.63*	*4.28*	*3.37*	*1.31*	*1.46*
5	*2.85*	*2.16*	*2.33*	*1.33*	*1.24*
6A	*3.72*	*2.62*	*2.96*	*2.19*	*2.21*
6B	*4.77*	*3.38*	*3.41*	*2.10*	*2.51*
7F	*5.30*	*5.99*	*4.92*	*2.57*	*2.57*
9V	*2.56*	*3.08*	*2.67*	*0.98*	*1.09*
14	*8.04*	*6.45*	*2.24*	*4.74*	*5.09*
18C	*2.77*	*3.71*	*2.56*	*1.37*	*1.37*
19A	*4.77*	*4.94*	*6.03*	*2.07*	*1.91*
19F	*2.88*	*3.07*	*2.53*	*1.85*	*2.15*
23F	*2.16*	*1.98*	*1.55*	*1.33*	*1.18*

Simultaneous Administration With Other Vaccines

In studies 004, 3005 and 3008 routine pediatric vaccines were administered at the same visit as Prevnar 13. Immune responses to selected concomitant vaccine antigens were compared in infants receiving Prevnar (7-valent) and Prevnar 13. The proportion of responders at pre-specified antibody levels are shown in Table 9. Responses to all antigens in Prevnar 13 recipients were similar to those in Prevnar (7-valent) recipients and met formal criteria for non-inferiority. Varicella responses as measured by a commercial whole cell ELISA kit, designed to detect immunity after natural infection, were low in both groups, but there was no evidence of interference with the immune response by concomitantly administered Prevnar 13.

Table 9: Subjects Achieving a Pre-specified Antibody Level for Concomitant Vaccines Antigens

Concomitant Vaccine Name/Vaccine Antigen (Prespecified Antibody Level)	Administered with Prevnar 13 % Responders (n^a/N^b)	Administered with Prevnar (7-valent) % Responders (n^a/N^b)
Pediarix (DTaP-IPV-HepB) Responses After the Three-dose Infant Series		
Dip (≥0.1 IU/mL)	95.7 (223/233)	96.1 (221/230)
Tet (≥0.1 IU/mL)	98.4 (181/184)	98.5 (193/196)
PT ≥16.5 EU/mL	94.1 (225/239)	95.0 (228/240)
FHA ≥40.5 EU/mL	96.7 (231/239)	95.0 (228/240)
PRN ≥26 EU/mL	93.7 (224/239)	95.8 (230/240)
Polio Type 1 (titer ≥1:8)	100.0 (183/183)	100.0 (187/187)
Polio Type 2 (titer ≥1:8)	98.9 (181/183)	99.5 (186/187)
Polio Type 3 (titer ≥1:8)	100.0 (182/182)	99.5 (186/187)
HBV ≥10.0 mIU/mL	100.0 (153/153)	100.0 (173/173)
ActHIB (PRP) Responses After the Infant Series		
Hib (PRP) (≥0.15 µg/mL)	97.9 (232/237)	97.8 (225/230)
Hib (PRP) (≥1.0 µg/mL)	77.6 (184/237)	78.3 (180/230)
Pentacel (DTaP-IPV-Hib) Responses After the Infant Series		
Hib (PRP) (≥0.15 µg/mL)	97.8 (266/272)	99.6 (265/266)
Hib (PRP) (≥1.0 µg/mL)	81.6 (222/272)	84.6 (225/266)
PT ≥12.00 EU/mL	98.6 (278/282)	96.0 (266/277)
FHA ≥20.00 EU/mL	99.3 (281/283)	95.7 (266/278)
PRN ≥7.00 EU/mL	96.8 (274/283)	96.0 (266/277)
FIM ≥4.00 EU/mL	93.6 (264/282)	95.3 (262/275)

Table 9: Subjects Achieving a Pre-specified Antibody Level for Concomitant Vaccines Antigens (Cont'd)

Concomitant Vaccine Name/Vaccine Antigen (Prespecfied Antibody Level)	Administered with Prevnar 13 % Responders (n^a/N^b)	Administered with Prevnar (7-valent) % Responders (n^a/N^b)
PedvaxHIB (PRP-OMP) Responses at 12 to 15 Months Following Infant Series with ActHIB		
Hib (PRP) (≥0.15 µg/mL)	100.0 (230/230)	100.0 (214/214)
Hib (PRP) (≥1.0 µg/mL)	90.4 (208/230)	92.1 (197/214)
ProQuad (MMR-Varicella) Responses at 12 to 15 Months		
Measles (≥1.10 I.V.)	96.4 (213/221)	97.1 (204/210)
Mumps (≥1.10 I.V.)	76.5 (169/221).	72.9 (153/210)
Rubella (≥15 IU/mL)	91.9 (192/209)	90.7 (185/204)
Varivax (Varicella) Responses at 12 to 15 Months		
Varicella (≥1.25 gpELISA units/mL)	100 (163/163)	100 (173/173)
Varicella (≥5.00 gpELISA units/mL)	98.8 (161/163)	97.7 (169/173)

a. Number of subjects achieving the pre-specified antibody level
b. Number of subjects in the evaluable immunogenicity population

Efficacy against otitis media and pneumonia

Efficacy of Prevnar 13 against otitis media and pneumonia has not been established. However, the efficacy of the comparator Prevnar (7-valent) has been established since its licensure in 2001 for invasive disease, pneumonia and otitis media.

Effectiveness of Prevnar (7-valent) using a four dose schedule (three doses in the first year of life with a booster dose in the second year) has also been observed against acute otitis media and pneumonia since its introduction in a national immunization programme. In a retrospective evaluation of a large US insurance database, AOM visits were reduced by 42.7 % (95 % CI, 42.4-43.1 %), and prescriptions for AOM by 41.9 % in children younger than 2 years of age, compared with a pre-licensure baseline (2004 vs. 1997-99).[73] In a similar analysis, hospitalisations and ambulatory visits for all-cause pneumonia were reduced by 52.4 % and 41.1 %, respectively.[74] For those events specifically identified as pneumococcal pneumonia, the observed reductions in hospitalisations and ambulatory visits were 57.6 % and 46.9 %, respectively, in children younger than 2 years of age, compared with a pre-licensure baseline (2004 vs. 1997-99).[74]

Effectiveness of Prevnar (7-valent) against AOM and pneumonia has also been observed in Quebec after the implementation of a publicly funded program in all children using a three dose immunization schedule (two doses in the first year of life with a booster dose in the second year). In the three years following vaccine introduction, there was a 13.2% reduction in otitis media claims attributable to Prevnar (7-valent) and an estimated 100,000 otitis media visits averted in children under five years of age.[20] Similarly, a 13% reduction in hospital admissions for all-cause pneumonia was observed with no increase in cases of empyema.[19]

While direct cause-and-effect cannot be concluded from observational analyses of this type, these findings suggest that Prevnar (7-valent) plays an important role in reducing the burden of mucosal disease (AOM and pneumonia) in the target population.

TOXICOLOGY

A repeated dose intramuscular (5 IM doses) rabbit toxicity study of pneumococcal 13-valent conjugate vaccine resulted in the generation of serotype-specific antibody responses and did not demonstrate any significant local or systemic adverse effects. In addition, there were no significant adverse findings in a single-dose IM local tolerance study in rabbits.

In single-dose subcutaneous (SC) safety pharmacology studies of pneumococcal 13-valent conjugate vaccine in rats or monkeys, there were no effects on central nervous, respiratory, or cardiovascular systems. In repeated dose (7 SC doses) toxicity studies in rats and monkeys, no significant adverse effects were observed. In addition, in a repeated dose (5 SC doses) toxicity study in juvenile rats, no significant adverse effects were observed.

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PART III: CONSUMER INFORMATION

Prevnar® 13

Pneumococcal 13-valent Conjugate Vaccine (Diphtheria CRM_{197} Protein)

This leaflet is part III of a three-part "Product Monograph" published when Prevnar® 13 was approved for sale in Canada and is designed specifically for Consumers. This leaflet is a summary and will not tell you everything about Prevnar® 13. Contact your doctor, nurse or pharmacist if you have any questions about the drug.

ABOUT THIS MEDICATION

What the medication is used for:
Prevnar 13 is a pneumococcal vaccine that helps protect against 13 strains of Streptococcus pneumoniae bacteria (Prevnar 13 contains the 7 strains that are included in Prevnar (7-valent), which has been marketed since 2001. Prevnar 13 helps protect your child against diseases such as meningitis, sepsis or bacteraemia (bacteria in blood stream), and bacteraemic pneumonia caused by thirteen strains of the bacteria Streptococcus pneumoniae.

What it does:
The vaccine works by helping the body to make its own antibodies, which protect your child against diseases such as meningitis, sepsis or bacteraemia (bacteria in blood stream), and bacteraemic pneumonia caused by thirteen types of the bacteria Streptococcus pneumoniae.

When it should not be used:
If your child is allergic (hypersensitive) to the active substances, to any other ingredients, or to any other vaccine that contains diphtheria toxoid.

What the medicinal ingredient is:
The active substances are:
- 2.2 µg of saccharide for serotypes 1, 3, 4, 5, 6A, 7F, 9V, 14, 18C, 19A, 19F and 23F
- 4.4 µg of saccharide for serotype 6B

Conjugated to CRM197 carrier protein and adsorbed on aluminum phosphate (0.125 mg Aluminum).

What the important nonmedicinal ingredients are:
Sodium chloride, succinic acid, Polysorbate 80 and water for injection.

What dosage forms it comes in:
The vaccine is a white suspension for injection and provided in a single-dose, pre-filled syringe (0.5 mL). Pack size of 10, without needle.

WARNINGS AND PRECAUTIONS

Serious Warnings and Precautions

Take special care with Prevnar 13:
- If your child has any present or past medical problems after any dose of Prevnar (7-valent) or Prevnar 13.
- If your child is sick with a high fever
- If your child has any bleeding problems

Prevnar 13 will only protect against disease caused by the types of Streptococcus pneumoniae in the vaccine.

As with any vaccine, Prevnar 13 will not protect 100% of those who receive the vaccine.

BEFORE you use Prevnar 13 talk to your doctor or pharmacist:

Please tell your doctor, nurse or pharmacist if your child is taking, has recently taken any other medicines, including medicines obtained without prescription, or has recently received any other vaccine.

INTERACTIONS WITH THIS MEDICATION

The vaccine is not to be mixed with other vaccines/products in the same syringe.

Different injectable vaccines should always be given at different injection-sites.

PROPER USE OF THIS MEDICATION

Usual dose:
The doctor or nurse will inject the recommended dose (0.5 mL) of the vaccine into your child's arm or leg muscle.
Typically, your child should receive four doses of the vaccine. According to official recommendations in your country, an alternative schedule may be used by your healthcare provider. Each dose will be given on a separate occasion. It is important to follow the instructions from the doctor/nurse so that your child completes the course of injections.
Prevnar 13 can be given at the same time as other childhood vaccines; in this case, different injection sites should be used. Prevnar 13 should not be mixed with any other vaccines in the same syringe.

Overdose:
Overdose with Prevnar 13 is unlikely due to it being in a pre-filled syringe.

Missed Dose:

If you forget to go back to the doctor or nurse at the scheduled time, ask the doctor or nurse for advice.

If you have any further questions on the use of Prevnar 13, ask your doctor.

SIDE EFFECTS AND WHAT TO DO ABOUT THEM

Like all vaccines, Prevnar 13 can cause side effects, although not everybody gets them.

The following side effects include those reported for Prevnar 13.

The most common side effects reported in at least 1 in 10 children are:
- Decreased appetite
- Irritability
- Drowsiness/increased sleep, restless sleep/decreased sleep
- Fever; any pain, tenderness, redness, swelling or hardness at the injection-site

Common side effects reported in at least 1 in 100, but less than 1 in 10 children are:
- Diarrhea, vomiting
- Rash
- Fever > 39°C, tenderness at the injection-site interfering with movement

Uncommon side effects reported in at least 1 in 1,000 children, but less than 1 in 100 children are:
- Crying
- Seizures (including febrile seizures)
- Urticaria or urticaria-like rash
- Rash
- Redness, swelling, or hardness at the injection-site > 7.0 cm

Rare side effects reported in at least 1 in 10,000 children, but less than 1 in 1,000 children are:
- Hypersensitivity reaction including swelling of the face and/or lips, difficulty in breathing

Other side effects have been seen with Prevnar (7-valent) because it has been available for a longer period of time. These side effects may be reported in the future with Prevnar 13:

Very rare side effects reported in less than 1 in 10,000 children are:
- Enlarged lymph nodes (lymphadenopathy) in the region of the injection-site
- Hypotonic-hyporesponsive episode (collapse or shock-like state)
- Anaphylactic/anaphylactoid reaction including shock (cardiovascular collapse)
- Angioneurotic edema, erythema multiforme
- Injection-site dermatitis, injection-site urticaria, injection-site pruritus

In babies born very prematurely (at or before 28 weeks before gestation), longer gaps than normal between breaths may occur for 2-3 days after vaccination.

Please speak with your doctor or pharmacist should you have any questions or concerns. If any of the side effects gets serious, or if you notice any side effects not listed in this leaflet, please tell your doctor or pharmacist.

SERIOUS SIDE EFFECTS, HOW OFTEN THEY HAPPEN AND WHAT TO DO ABOUT THEM

Symptom / effect		Talk with your doctor or pharmacist	
		Only if severe	In all cases
Common	Diarrhea; vomiting	✓	
	Rash	✓	
	Fever greater than 39°C		✓
	Injection Site swelling	✓	
Uncommon	Seizures		✓
Rare	Hypersensitivity reaction including facial swelling, difficulty breathing		✓

This is not a complete list of side effects. For any unexpected effects while taking Prevnar 13, contact your doctor or pharmacist.

HOW TO STORE IT

Keep out of the reach and sight of children.

Store in a refrigerator (2°C – 8°C).

Do not freeze.

Do not use Prevnar 13 after the expiry date stated on the carton and label. The expiry date refers to the last day of that month. Medicines should not be disposed of via wastewater or household waste. Ask your pharmacist how to dispose of medicines no longer required. These measures will help to protect the environment.

REPORTING SUSPECTED SIDE EFFECTS

To monitor vaccine safety, the Public Health Agency of Canada collects information on serious and unexpected adverse events following vaccination. If you suspect you have had a serious or unexpected event following receipt of a vaccine you may notify the Public Health Agency of Canada:

By toll-free telephone: 866-844-0018
By toll-free fax: 866-844-5931
By web: http://www.phac-aspc.gc.ca/im/vs-sv/index-eng.php

By regular mail:
The Public Health Agency of Canada
Vaccine Safety Section
130 Colonnade Road
Ottawa, ON K1A 0K9
A/L 6502A

NOTE: Should you require information related to the management of the side effect, please contact your health care provider before notifying the Public Health Agency of Canada. The Public Health Agency of Canada does not provide medical advice.

MORE INFORMATION

This document plus the full product monograph, prepared for health professionals can be found at:
http://www.wyeth.ca/en
or by contacting the sponsor, Wyeth Canada, at:
1-800-461-8844.

This leaflet was prepared by Wyeth Canada.

Last revised: November 9, 2009

Industria y Comercio
SUPERINTENDENCIA

Espacio reservado para el adhesivo de radicación

DIRECCIÓN DE NUEVAS CREACIONES
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1. Identificación del Tramite

[ ] Conversión [X] División [ ] Fusión

[X] Patente de Invención
[ ] Patente de Modelo de Utilidad
[ ] Registro de Diseño Industrial
[ ] Esquema de Trazado de Circuitos Integrados

2. Datos del solicitante

Persona Natural [ ] Persona Jurídica [X]

Wyeth
Nombre o denominación / Nombre o razón social

Nombre del representante legal

Tipo de empresa Micro [ ] Pequeña [ ] Mediana [ ] Otra [ ]

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Nacionalidad/País de constitución	Dirección del titular	Ciudad
Estados Unidos de América	Five Giraldas Farms, Madison, NJ 07940, Estados Unidos	Nueva Jersey
Dirección electrónica	**No. Fax**	**Número telefónico**
Alvaro.correa@bakermckenzie.com	3762211	6341500

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3. Datos del apoderado:

Correa Ordoñez Alvaro	79.143.366	20.281
Apellidos y Nombre	Documento de identificación	Tarjeta profesional

Dirección y domicilio		Ciudad
Av 82 No. 10 62 Piso 6		Bogotá
Dirección electrónica	**No. Fax**	**Número telefónico**
alvaro.correa@bakermckenzie.com	3762211	6341500

En caso de haber presentado poder general para asuntos que se adelanten ante la Delegatura de Propiedad Industrial, sírvase indicar el número de su radicación o protocolo de poder general _____

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5. Comprobante de Pago No 12-0085736 **Fecha** 22 / 08 / 2012

6. Anexos

x	Comprobante de pago de la tasa para la presentación de la corrección y/o modificación
	Poder, si fuere el caso con el que se acredita la representación
	Número de documento que ordena la comisión o fusión o división
x	Documento que contiene la conversión, división o fusión de la solicitud
	Copia de la solicitud y sus anexos en formato magnético

7. Firma	
Nombre y apellidos Alvaro Correa Ordoñez	**Firma**
C.C. 79.143.366	**Tarjeta Profesional 20.281**

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TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	VR PAGO
CONSIGNACION	BANCO DE BOGOTA	062754387	450027491	21/08/2012	73.321.000.00

*** Conceptos Pagados ***

CANT.	RENTISTICO	CONCEPTO	Vr.UNDITARIO	Vr.CONCEPTO
1	50005-01-01 SOLICITUDES	1 TRAMITES DE SOL. DE PATENTE DE INVENCION	590.000.00	590.000.00
				$590.000.00

SON: **QUINIENTOS NOVENTA MIL PESOS MONEDA CORRIENTE***

Responsable: _____
****

Recibo de Caja Aplicado al Expediente No. _____

COMPOSICIÓN CONJUGADA DE POLISACÁRIDO-PROTEÍNA NEUMOCÓCICA MULTIVALENTE

CAMPO DE LA INVENCIÓN

La presente invención se relaciona de manera general con el campo de la medicina, y específicamente con microbiología, inmunología, vacunas y la prevención de infección por patógeno bacteriano mediante inmunización.

ANTECEDENTES DE LA INVENCIÓN

El *Streptococcus pneumoniae* es la causa principal de meningitis, neumonía, y enfermedad invasiva severa en niños y recién nacidos en todo el mundo. Las vacunas de polisacárido neumocócicas múltivalentes se han licenciado durante muchos años y han probado ser valiosas para evitar la enfermedad neumocócica en adultos mayores y en pacientes de alto riesgo. Sin embargo, los recién nacidos y los niños jóvenes responden pobremente a la mayoría de los polisacáridos neumocócicos. La vacuna de conjugado neumocócica hepta-valente (7vPnC, Prevnar®) fue la primera de su clase que demostró ser altamente inmunogénica y efectiva contra la enfermedad invasiva y otitis media en niños y recién nacidos. Esta vacuna está ahora aprobada en muchos países alrededor del mundo. La Prevnar contiene los polisacáridos capsulares de los serotipos 4, 6B, 9V, 14, 18C, 19F y 23F, cada una conjugada a una proteína portadora designada CRM_{197}. La

Prevnar cubre aproximadamente 80-90%, 60-80%, y 40-80% de la enfermedad neumocócica invasiva (IPD) en los Estados Unidos, Europa, y otras regiones del mundo, respectivamente [1,2]. Los datos de vigilancia recogidos en los años siguientes a la introducción de la Prevnar han demostrado claramente una reducción de la enfermedad neumocócica invasiva en recién nacidos en los Estados Unidos como se esperaba (FIG. 1) [3,4].

La vigilancia del IPD conducida en recién nacidos en los Estados Unidos antes de la introducción de la Prevnar demostró que una porción significativa de la enfermedad debida a los serogrupos 6 y 19 se debió a los serotipos 6A (aproximadamente un tercio) y 19A (aproximadamente un cuarto) [5,6]. La vigilancia de la enfermedad invasiva neumocócica conducida en los Estados Unidos después de la licencia de la Prevnar sugiere que un gran grupo de enfermedades es aún atribuible a los serotipos 6A y 19A (FIG. 1) [3]. Más aún, estos dos serotipos cuentan con más casos de enfermedad invasiva que los serotipos 1, 3, 5, y 7F combinados (8.2 vs. 3.3 casos/100,000 niños de dos años y menos). Además, los serotipos 6A y 19A están asociados con altas tasas de resistencia a antibiótico (FIG. 2) [7,8,9]. Aunque es posible que la protección cruzada del serogrupo dé como resultado una declinación de la enfermedad por los serotipos 6A y 19A en razón a que más niños están inmunizados, existe evidencia que sugiere que habrá un límite en la declinación, y un grupo

significativo de enfermedad debido a estos serotipos subsistirá (ver adelante).

Dado el grupo y la importancia relativa de la enfermedad neumocócica invasiva debido a los serotipos 1, 3, 5, 6A, 7F, y 19A, agregar estos serotipos a la formulación Prevnar incrementaría el cubrimiento para la enfermedad invasiva a > 90% en los Estados Unidos y Europa, y tan alta como el 70%-80% en Asia y América Latina. Esta vacuna expandiría significativamente el cubrimiento más allá de la Prevnar, y suministraría cubrimiento para 6A y 19A que no es dependiente de las limitaciones de la protección cruzada del serogrupo.

RESUMEN DE LA INVENCIÓN

De acuerdo con esto, la presente invención suministra de manera general una composición inmunogénica multivalente que comprende 13 diferentes conjugados de polisacárido-proteína, en donde cada uno de los conjugados contiene un polisacárido capsular proveniente de un serotipo diferente de *Streptococcus pneumoniae* conjugado a una proteína portadora, junto con un vehículo fisiológicamente aceptable. De manera opcional, un adyuvante, tal como un adyuvante a base de aluminio, está incluido en la formulación. Más específicamente, la presente invención suministra una composición de conjugado neumocócico 13-valente (13vPnC) que comprende los siete serotipos en la vacuna 7vPnC (4, 6B, 9V,

14, 18C, 19F y 23F) más seis serotipos adicionales (1, 3, 5, 6A, 7F y 19A).

La presente invención también suministra una composición inmunogénica multivalente en donde los polisacáridos capsulares son de los serotipos 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, y 23F del *Streptococcus pneumoniae* y la proteína portadora es CRM_{197}.

La presente invención suministra además una composición inmunogénica multivalente, en donde los polisacáridos capsulares son de los serotipos 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F y 23F de *Streptococcus pneumoniae*, la proteína portadora es CRM_{197}, y el adyuvante es un adyuvante a base de aluminio, tal como fosfato de aluminio, sulfato de aluminio e hidróxido de aluminio. En una modalidad particular de la invención, el adyuvante es fosfato de aluminio.

La presente invención también suministra una composición inmunogénica multivalente, que comprende conjugados de polisacárido-proteína junto con un vehículo fisiológicamente aceptable, en donde cada uno de los conjugados comprende un polisacárido capsular proveniente de un serotipo diferente de *Streptococcus pneumoniae* conjugado a una proteína portadora, y los polisacáridos capsulares se preparan del serotipo 3 y por lo menos un serotipo adicional.

En una modalidad de esta composición inmunogénica multivalente, el serotipo adicional se selecciona del grupo que consiste de los serotipos 1, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F y 23F. En otra modalidad, la proteína portadora es CRM_{197}. En aún otra modalidad, la composición comprende un adyuvante, tal como un adyuvante basado en aluminio seleccionado de fosfato de aluminio, sulfato de aluminio e hidróxido de aluminio. En una modalidad particular, el adyuvante es fosfato de aluminio.

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La presente invención también suministra un método para inducir una respuesta inmune a un conjugado de polisacárido capsular de *Streptococcus pneumoniae*, que comprende administrar a un humano una cantidad inmunológicamente efectiva de cualquiera de las composiciones inmunogénicas ya descritas.

La presente invención suministra además que cualquiera de las composiciones inmunogénicas administrada en una dosis simple formulada de 0.5 mL contenga: 2 µg de cada sacárido, excepto para 6B en 4 µg; aproximadamente 29 µg de proteína portadora CRM_{197}; 0.125 mg de adyuvante de aluminio elemental (0.5 mg de fosfato de aluminio); y amortiguador de cloruro de sodio y succinato de sodio como excipientes.

BREVE DESCRIPCIÓN DE LOS DIBUJOS

La FIG. 1 describe los cambios en las tasas IPD del serotipo en los niños de los Estados Unidos < 2 años de edad de la línea base (1998/1999) a 2001.

La FIG. 2 describe la distribución de aislados neumocócicos con resistencia a la penicilina (PCN) en niños < 5 años de edad (1998).

La FIG. 3 describe las curvas de distribución acumulada inversas (RCDC) de los resultados de OPA post-tercera dosis provenientes del ensayo D118-P16 Prevnar.

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DESCRIPCIÓN DETALLADA DE LA INVENCIÓN

Inclusión de los Serotipos Prevnar 4, 6B, 9V, 14, 18C, 19F, 23F

Los datos provenientes de la vigilancia IPD entre 1995-1998 estimaron que los siete serotipos en Prevnar fueron responsables por alrededor del 82% del IPD en niños < 2 años de edad [5]. En el norte de California, el sitio del ensayo de eficacia, los serotipos Prevnar contaron con el 90% de todos los casos de IPD en recién nacidos y niños jóvenes [10]. Desde la introducción de la vacuna Prevnar en el 2000, ha habido un decrecimiento significativo en las tasas de IPD totales debido a la disminución de la enfermedad debido a los serotipos de vacuna [3,4]. Por lo tanto, no existe justificación en este momento para remover cualquiera de los serotipos Prevnar provenientes de la siguiente generación de las vacunas de conjugado neumocócica sino por el contrario agregar serotipos para obtener un cubrimiento más amplio.

Inclusión de los Serotipos 1, 3, 5 y 7F

En los Estados Unidos, la tasa de IPD causada por el serotipo 1 en niños menores de 5 años es < 2%, aproximadamente lo mismo que para cada uno de los tipos 3 y 7F [1,6]. Los serotipos 1 y 5 cuentan con tasas superiores de IPD en poblaciones de los Estados Unidos en alto riesgo de enfermedad neumocócica invasiva. Específicamente, el serotipo

1 origina 3.5% de IPD en niños nativos de Alaska < 2 años de edad, y 18% en niños de 2-4 años de edad [11]. Tanto el serotipo 1 como el serotipo 5 causan significativamente enfermedad en otras partes del mundo y en poblaciones indígenas en países en desarrollo [12,13,14].

El serotipo 1 también se puede asociar con enfermedad más severa comparada con otros serotipos neumocócicos [15]. Esta observación se basa en la diferencia en las tasas de identificación de caso entre los Estados Unidos y Europa, y la diferencia asociada en la práctica médica. En total, la incidencia del IPD es inferior en Europa que en los Estados Unidos. Sin embargo, el porcentaje de IPD causado por el serotipo 1 en Europa es desproporcionadamente mayor que en los Estados Unidos (6-7%, vs. 1-2%, respectivamente). En Europa, cultivos de sangre se obtienen predominantemente de niños hospitalizados. En los Estados Unidos, es una práctica médica de rutina obtener cultivos de sangre en un paciente externo que se ajusta a niños que presentan fiebre ≥ 39°C y conteos de células sanguíneas blancas elevados. Dada la diferencia en la práctica médica, se postula que el porcentaje inferior de enfermedad causada por el serotipo 1 en los Estados Unidos se puede diluir por tasas superiores de otros serotipos que causan la enfermedad media, mientras que el alto porcentaje en Europa refleja enfermedad más seria. Además, los estudios de seroepidemiología de los niños con neumonía complicada demuestran que el serotipo 1 está desproporcionadamente representado [16,17,18]. Esto sugiere

que la inclusión del serotipo 1 puede reducir la cantidad de enfermedad neumocócica severa, así como también, contribuir a al reducción total en enfermedad neumocócica invasiva.

La adición de los serotipos 3 y 7F incrementará el cubrimiento contra el IPD en la mayoría de las áreas del mundo en aproximadamente 3%-7%, y en Asia en alrededor del 9%. Así, una vacuna 11-valente cubriría el 50% en Asia y alrededor del 80% de IPD en todas las otras regiones [1,2]. Estos serotipos son también importantes con respecto al cubrimiento de otitis media [19]. En un estudio multinacional de los serotipos neumocócicos que originan otitis media, Hausdorff et al encontraron que el serotipo 3 era el octavo aislado de fluido de oído medio más común en total [20]. El serotipo 3 contaba con hasta el 8.7% de los serotipos neumocócicos asociados con otitis media. Así, la importancia de los tipos 3 y 7F en otitis media, así como también en IPD, garantiza su inclusión en una vacuna de conjugado neumocócica.

Sin embargo, no han sido exitosos los intentos de producir una vacuna de conjugado neumocócica multivalente que exhiba inmunogenicidad significativa con respecto a los polisacáridos del serotipo 3. Por ejemplo, en un estudio de inmunogenicidad y seguridad de una vacuna de conjugado de proteína D neumocócica 11-valente (11-Pn-PD), no se observó ningún efecto cebador para el serotipo 3 en recién nacidos que habían recibido tres dosis de la vacuna luego de una

dosis de refuerzo de la misma vacuna o una vacuna de polisacárido neumocócica (Nurkka et al. (2004) Ped. Inf. Dis. J., 23:1008-1014). En otro estudio, los resultados del ensayo opsonofagocítico (OPA) proveniente de niños recién nacidos que habían recibido dosis de 11-Pn-PD no mostró respuestas de anticuerpo para el serotipo 3 a niveles comparables con otros serotipos ensayados (Gatchalian et al., 17th Annual Meeting of the Eur. Soc. Paed. Inf. Dis. (ESPID), Poster No. 4, P1A Poster Session 1, Istanbul Turkey, Mar. 27, 2001). En aún otro estudio, que evaluó la eficacia de un 11-Pn-PD en la prevención de otitis media aguada, la vacuna no suministró protección contra episodios causados por el serotipo 3 (Prymula et al. www.thelancet.com, Vol. 367: 740-748 (marzo 4, 2006). De acuerdo con esto, una vacuna de conjugado neumocócica que comprende polisacáridos capsulares provenientes del serotipo 3 y capaz de elicitar una respuesta inmunogénica al los polisacáridos del serotipo 3 suministra una mejora significativa sobre el estado existente de la técnica.

Inclusión de los Serotipos 6A y 19A

a. Epidemiología de los Serotipos 6A y 19A

Los datos de vigilancia en la literatura sugieren que los serotipos 6A y 19A cuentan con más enfermedad neumocócica invasiva en niños de los Estados Unidos < 2 años de edad que los serotipos 1, 3, 5, y 7F combinados (FIG. 1)[1,5]. Además,

estos serotipos están comúnmente asociados con resistencia a antibiótico (FIG. 2) y juegan un papel importante en otitis media [6,19,20]. La capacidad de la vacuna Prevnar habitual a proteger contra la enfermedad debido a 6A y 19A no es clara. Lo racional para la inclusión de los componentes 6A y 19A en una vacuna 13vPnC se discute adelante.

b. Respuestas a 6A y 19A Inducidas por los Polisacáridos 6B y 19F

Las vacunas de polisacárido neumocócica no conjugadas licenciadas (para uso en personas de por lo menos dos años de edad) ha contenido el polisacárido capsular 6A o 6B pero no ambos [21]. Los datos de inmunogenicidad generados en el momento de la formulación de la vacuna de polisacárido neumocócica 23-valente demostraron que la vacuna 6B monovalente indujo anticuerpo a ambas cápsulas 6A y 6B. Los datos provenientes de varios ensayos que evalúan las respuestas del ensayo IgG y opsonofagocíticas (OPA) en una variedad de poblaciones con polisacárido libre y con vacunas de conjugado neumocócicas sugieren que las respuestas IgG a 6A se inducen por antígenos 6B, pero las respuestas son generalmente inferiores, y la actividad OPA con organismos 6A es diferente que con organismos 6B [22,23,24,25]. Además, los sujetos que responden con el anticuerpo 6B alto pueden tener poca o ninguna actividad contra 6A.

En contraste a la composición química de los polisacáridos capsulares 6A y 6B donde subsiste un alto grado de similitud, las cápsulas 19A y 19F son muy diferentes debido a la presencia de dos cadenas laterales adicionales en el polisacárido 19A. De manera no sorprendente, las respuestas inmunes medidas en voluntarios humanos inmunizados con vacuna de polisacárido 19F mostraron que las respuestas a 19F fueron inducidas en el 80% de los sujetos, pero solamente el 20% de los sujetos tuvo una respuesta a 19A [26]. Los bajos niveles de las respuestas a IgG y OPA reactiva cruzada al serotipo 19A después de inmunización con el polisacárido 19F también han estado documentados en ensayos con vacunas de conjugado [24,26].

Los datos internos en las respuestas OPA reactivas cruzadas a 6A y 19A se han generado del ensayo de puente 7vPnC (D118-P16) conducidas en recién nacidos en los Estados Unidos (FIG. 3). Estos estudios son consistentes con los hallazgos de otros, y demuestran inducción de anticuerpo funcional reactivo cruzado a polisacárido 6A después de inmunización con polisacárido 6B, aunque a un nivel inferior, y muy poco anticuerpo funcional a 19A después de inmunización con 19F.

Impacto de Inmunización 6B y 19F sobre 6A y 19A en Modelos de Animal

Los modelos de animal se han utilizado para evaluar el potencial para la protección cruzada con inmunización de polisacárido. En un modelo de otitis media desarrollado por Giebink et al., se inmunizaron chinchillas con una vacuna de conjugado de proteína de membrana exterior de polisacárido tetravalente (OMP) (que contiene los sacáridos 6B, 14, 19F, 23F) o placebo [27]. En este ensayo apareció alguna reactividad cruzada para 6A; sin embargo este no alcanzó significado estadístico y el nivel de protección fue inferior de aquel con 6B contra otitis media. En este mismo modelo hubo 100% de protección contra otitis media 19F, pero solamente 17% de protección contra otitis media 19A.

Saeland et al. utilizaron suero proveniente de recién nacidos inmunizados con una vacuna de conjugado de tétano neumocócica 8-valente (que contiene 6B y 19F) para inmunizar pasivamente ratones antes de una exposición intranasal con organismos 6A, en un modelo de infección de pulmón [28]. De las 59 muestras de suero, 53% protegieron a los ratones contra bacteremia con 6B y 37% protegieron contra 6A. Los ratones inmunizados pasivamente con suero proveniente de recién nacidos inmunizados con cuatro dosis de una vacuna de conjugado neumocócica 11-valente (que contiene 19F conjugado a toxoide de tétano) les fue dada una exposición intranasal con organismos 19A en el mismo modelo [29]. De los 100 ratones inmunizados pasivamente y luego expuestos, a 60 ratones no se les detectaron organismos 19A en el tejido de pulmón, mientras que los organismos fueron identificados en

todos los ratones a los que se les dio placebo de solución salina. Sin embargo, la inmunización pasiva no protegió contra exposición con organismos 19F en este modelo; por lo tanto, la relevancia del modelo para el serogrupo 19 es cuestionable. En general estos modelos suministran evidencia de algún impacto biológico de inmunización 6B sobre organismos 6A aunque el efecto del serotipo heterólogo no fue tan grande como aquel observado con el serotipo homólogo. El impacto de la inmunización 19F sobre los organismos 19A no se entiende bien de estos modelos.

Impacto de Inmunización de Conjugado de Polisacárido 6B y 19F sobre Enfermedad 6A y 19A en Ensayos de Eficacia/Efectividad

El número de casos de enfermedad debido a los serotipos 6B, 6A, 19F y 19A en ensayos de eficacia 7vPnC y 9vPnC (7vPnC más los serotipos 1 y 5) se anota en la Tabla 1 [30,10,31]. Los números de los casos de enfermedad invasiva son demasiado pequeños para permitir que cualquier conclusión sea extraída para los serotipos 6A y 19A. Sin embargo, el ensayo terminado para otitis media generó un gran número de aislados neumocócicos [32]. En el análisis por protocolo 7vPnC fue el 84% (95% Cl 62%, 93%) eficaz contra otitis media debido al serotipo 6B y 57% (95% Cl 24%, 76%) eficaz contra otitis media debido al serotipo 6A (Tabla 1). En contraste, la eficacia específica del serotipo con 7vPnC no se demostró para otitis media debido a 19F o 19A.

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Tabla 1. Casos de Enfermedad Neumocócica Debido a los Serotipos 6B, 6A, 19F y 19A en Ensayos de Eficacia con las Vacunas 7vPnC y 9vPnC

	6B		6A		19F		19A	
	PnC	Contr.	PnC	Contr.	PnC	Contr.	PnC	Contr.
Ensayo de Eficacia Kaiser - 7vPnC (ITT)	1	7	0	1	2*	13	0	1
Ensayo de Eficacia Navajo - 7vPnC (ITT)	0	5	1	0	1	1	1	0
Ensayo de Eficacia Surafricana - 9vPnC HIV (-) (ITT)	1	2	1	0	0	1	3	1
Ensayo de Eficacia Surafricana - 9vPnC HIV (+) (ITT)	1	7	3	10	2	3	2	3
Ensayo Terminado de Otitis Media 7vPnC (PP)	9*	56	19*	45	43	58	17	26

* Eficacia significativa estadísticamente demostrada
De las referencias 30, 10 y 33, y comunicaciones personales
Contr = control
ITT = Intención para tratar análisis
PP = análisis por protocolo

Los datos de vigilancia IPD post-comercialización también están disponibles de de un ensayo de control de caso conducido por los Centros de Control de Enfermedad para evaluar la efectividad de Prevnar [33]. Los casos de enfermedad invasiva neumocócica que ocurren en niños de 3 a 23 meses de edad se identificaron en laboratorios de vigilancia y coincidieron con tres casos de control por edad y código zip. Después de obtener consentimiento, la historia clínica y de inmunización (los sujetos se consideraron

inmunizados si ellos habían recibido por lo menos una dosis de Prevnar) se obtuvo de padres y proveedores médicos para casos y controles. Los resultados preliminares se presentaron en la reunión 2003 ICAAC y un resumen de los hallazgos de la enfermedad 6B, 19F, 19A y 6A está presente en la Tabla 2. Estos datos indican que Prevnar es capaz de evitar la enfermedad debido a 6A, aunque a un nivel que puede ser un poco inferior que la enfermedad para el serotipo 6B. Estos datos también indican que la protección cruzada para enfermedad invasiva debido a 19A es limitada.

Tabla 2. Resultados preliminares de un Ensayo de Control de Caso Desarrollado por CDC (presente en ICAAC, 2003)

Serotipo	**Conjuntos Informativos, n**	**VE* (95% Cl)**
Tipo de Vacuna, AII=todas	115	94 (87, 97)
Vacuna Relacionad, AII	36	70 (38, 86)
Tipo sin vacuna, AII	43	-4 (-106, 48)
6B	27	94 (72, 99)
19F	19	73 (16, 92)
6A	15	87 (53, 97)
19A	16	40 (-87, 80)

* La efectividad de la vacuna que compara vacunado (≥1 dosis) vs. no vacunado, y ajustado para condiciones subyacentes
Referencia 40 y comunicación personal/confidencial

Un análisis publicado [3] del uso de Prevnar también indicó que los serotipos 6B y 19F conferían una reducción moderada en IPD causada por los serotipos 6A y 19A entre niños menores de dos años de edad (Tabla 1 en [3]). Las tasas

de la enfermedad entre adultos inmunizados originada por los serotipos 6A, 9A, 9L, 9N, 18A, 18B, 18F, 19A, 19B, 19C, 23A y 23B ("todos los serotipos relacionados con vacuna") se redujeron un poco (Tabla 2 en [3]). Estos datos establecen que la inmunidad grupal proveniente del uso de Prevnar en niños de menos de dos años de edad fue modesta para los serotipos 6A y 19A, y suministra una base para la inclusión de los serotipos 6A y 19A en la vacuna 13vPnC de esta invención.

Conclusión para la adición de 6A y 19A

Los datos de vigilancia post-comercialización y los resultados del estudio de control por caso anotados en la FIG. 1 y la Tabla 2 con la vacuna 7vPnC sugieren que, consistente con la otra información sobre las respuestas inmunes y el desempeño en los modelos animales descritos anteriormente, puede haber alguna protección cruzada contra la enfermedad 6A, pero en una menor proporción que con la enfermedad 6B. Adicionalmente, parece que la protección contra 19A es limitada. Por lo tanto, una vacuna 13vPnC que contiene los serotipos 6A y 19A suministra cubrimiento que no es dependiente de las limitaciones de la protección cruzada del serogrupo mediante los serotipos 6B y 19F.

De acuerdo con esto, la presente invención suministra una composición inmunogénica multivalente que comprende 13 diferentes conjugados de polisacárido-proteína, en donde cada

uno de los conjugados contiene un polisacárido capsular diferente conjugado a una proteína portadora, y en donde los polisacáridos capsulares se preparan de los serotipos 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F y 23F de *Streptococcus pneumoniae*, junto con un vehículo fisiológicamente aceptable. Una de tales proteínas portadora es el toxoide de difteria designado CRM_{197}. La composición inmunogénica puede comprender además un adyuvante, tal como un adyuvante basado en aluminio, tal como fosfato de aluminio, sulfato de aluminio e hidróxido de aluminio.

Los polisacáridos capsulares se preparan mediante técnicas estándar conocidas por aquellos expertos en el arte. En la presente invención, los polisacáridos capsulares se preparan de los serotipos 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F y 23F de *Streptococcus pneumoniae*. Estos conjugados neumocócicos se preparan mediante procesos separados y se formulan en una formulación de dosis simple. Por ejemplo, en una modalidad, cada serotipo de polisacárido neumocócico crece en un medio a base de soya. Los polisacáridos individuales son entonces purificados a través de centrifugación, precipitación, ultra-filtración, y cromatografía de columna. Los polisacáridos purificados son químicamente activados para hacer los sacáridos capaces de reaccionar con la proteína portadora.

Una vez activado, cada polisacárido capsular es separadamente conjugado a una proteína portadora para formar

un glicoconjugado. En una modalidad, cada polisacárido capsular se conjuga a la misma proteína portadora. En esta modalidad, la conjugación se efectúa mediante aminación reductiva.

La activación química de los polisacáridos y la subsecuente conjugación a la proteína portadora se logra por medios convencionales. Ver, por ejemplo, Patentes U.S. Nos. 4,673,574 y 4,902,506 [34,35].

Las proteínas portadoras son preferiblemente proteínas que son no tóxicas y no reactogénicas y obtenibles en cantidades y pureza suficientes. Las proteínas portadoras deben ser adecuadas a los procedimientos de conjugación estándar. En una modalidad particular de la presente invención, el CRM_{197} se utiliza como la proteína portadora.

El CRM_{197} (Wyeth, Sanford, NC) es una variante no tóxica (es decir, toxoide) de toxina de difteria aislada de cultivos de la cepa de *Corynebacterium diphtheria* C7 (β197) crecida en ácidos casamino y medio a base de extracto de levadura. El CRM_{197} se purifica a través de ultra-filtración, precipitación de sulfato de amonio, y cromatografía de intercambio de ión. Alternativamente, el CRM_{197} se prepara recombinantemente de acuerdo con la Patente U.S. No. 5,614,382, que se incorpora aquí como referencia. Otros toxoides de difteria son también adecuados para uso como proteínas portadoras.

Otras proteínas portadoras adecuadas incluyen toxinas bacterianas inactivadas tales como el toxoide de tétano, el toxoide de pertussis, el toxoide de cólera (por ejemplo, como se describe en la Solicitud de Patente Internacional WO2004/083251 [38]), *E. coli* LT. *E. coli* ST, y exotoxina A proveniente de *Pseudomonas aeruginosa*. Las proteínas de la membrana exterior bacteriana tal como el complejo c de membrana exterior (OMPC), porinas, proteínas que se unen a transferrina, pneumolisina, proteína A de superficie neumocócica (PspA), proteína de adhesina neumocócica (PsaA), peptidaza C5a proveniente del Grupo A o el Grupo B de *Streptococcus*, o proteína D de *Haemophilus influenzae*, también se puede utilizar. Otras proteínas, tales como ovalbúmina, hemocianina de lapa ojo de cerradura (KLH), seroalbúmina bovina (BSA) o derivado de proteína purificado de tuberculina (PPD) también se puede utilizar como proteínas portadoras.

Después de la conjugación del polisacárido capsular a la proteína portadora, los conjugados de polisacárido-proteína se purifican (enriquecidos con respecto a la cantidad de conjugado de polisacárido-proteína) por una variedad de técnicas. Esas técnicas incluyen operaciones de concentración/diafiltración, precipitación/elusión, cromatografía de columna, y filtración profunda. Ver los ejemplos adelante.

Después de que los glicoconjugados individuales se purifican, ellos son compuestos para formular la composición inmunogénica de la presente invención, lo cual se puede utilizar como una vacuna. La formulación de la composición inmunogénica de la presente invención se puede lograr utilizando métodos reconocidos en la técnica. Por ejemplo, los 13 conjugados neumocócicos individuales se pueden formular con un vehículo fisiológicamente aceptable para preparar la composición. Ejemplos de tales vehículos incluyen, pero no están limitados a, agua, solución salina amortiguada, polioles (por ejemplo, glicerol, propilenglicol, polietilenglicol líquido) y soluciones de dextrosa.

En ciertas modalidades, la composición inmunogénica comprenderá uno o más adyuvantes. Como se define aquí, un "adyuvante" es una sustancia que sirve para mejorar la inmunogenicidad de una composición inmunogénica de esta invención. Así, los adyuvantes son dados a menudo para reforzar la respuesta inmune y son bien conocidos para el experto. Los adyuvantes adecuados para mejorar la efectividad de la composición incluyen, pero no están limitados a:

(1) sales de aluminio (alumbre), tales como hidróxido de aluminio, fosfato de aluminio, sulfato de aluminio, etc.;

(2) formulaciones de emulsión de aceite en agua (con o sin otros agentes inmunoestimulantes específicos tales como

péptidos de muramilo (definidos adelante) o componentes de pared celular bacteriana), tales como, por ejemplo,

(a) MF59 (Publicación PCT No. WO 90/14837), que contiene 5% de Squaleno, 0.5% de Tween 80, y 0.5% de Span 85 (que contiene opcionalmente varias cantidades de MTP-PE (ver adelante, aunque no se requiere) formulada en partículas submicrónicas que utilizan un microfluidizador tal como el microfluidizador Modelo 110Y (Microfludics, Newton, MA),

(b) SAF, que contiene 10% de Squaleno, 0.4% de Tween 80, 5% de polímero bloqueado plurónico L121, y thr-MDP (ver adelante) microfluidizado en una emulsión submicrónica o centrifugado para generar una emulsión de tamaño de partícula mayor, y

(c) Sistema de adyuvante Ribi™ (RAS), (Corixa, Hamilton, MT) que contiene 2% de Squaleno, 0.2% de Tween 80, y uno o más componentes de pared celular bacteriana proveniente del grupo que consiste de monofosforilípido A 3-O-desailado (MPL™) descrito en la Patente U.S. No. 4,912,094 (Corixa), dimicolato de trehalosa (TDM), y esqueleto de pared celular (CWS), preferiblemente MPL + CWS (Detox™);

(3) Adyuvantes de saponina, tales como Quil A o ESTIMULON™ QS-21 (Antigenics, Framingham, MA) (Patente U.S. No. 5,057,540) se puede utilizar o partículas generadas de éstos tales como ISCOM (complejos inmunoestimulantes);

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(4) Lipopolisacáridos bacterianos, análogos de lípido A sintético tales como los compuestos de fosfato de aminoalquilo glucosalina (AGP), o derivados o análogos de éstos, que están disponibles de Corixa, y que se describen en la Patente U.S. No. 6,113,918; uno de tales AGP es 2-[(R)-3-Tetradecanoiloxitetradecanoilamino]etil 2-Deoxi-4-O-fosfono-3-O-[(R)-3-tetradecanoiloxitetradecanoil]-2-[(R)-3-tetradecanoiloxitetradecanoilamino]-b-D-glucopiranosido, que también es conocido como 529 (anteriormente conocido como RC529), que se formula como una forma acuosa o como una emulsión estable, polinucleótidos sintéticos tales como oligonucleótidos que contienen motivo(s) CpG (Patente U.S. No. 6,207,646);

(5) citoquinas, tales como interleuquinas (por ejemplo, IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, IL-15, IL-18, etc.), interferones (por ejemplo, interferón gama), factor estimulante de colonia de macrófago de granulosito (GM-CSF), factor estimulante de colonia de macrófago (M-CSF), factor de necrosis tumoral (TNF), moléculas coestimuladoras B7-1 y B7-2, etc;

(6) mutantes destoxificados de una toxina ribosilante de ADP bacteriano tal como una toxina de cólera (CT) en forma de tipo silvestre o mutante, por ejemplo, donde el ácido glutámico en la posición 29 del amino ácido se reemplaza por otro amino ácido, preferiblemente una histidina, de acuerdo con el número de la solicitud de patente internacional

publicada WO 00/18434 (ver también WO 02/098368 y WO 02/098369), una toxina pertussis (PT), o una toxina lábil al calor de *E. coli* (LT), particularmente LT-K63, LT-R72, CT-S109, PT-K9/G129 (ver, por ejemplo, WO 93/13302 y WO 92/19265); y

(7) otras sustancias que actúan como agentes inmunoestimulantes para mejorar la efectividad de la composición.

Los péptidos de muramina incluyen, pero no están limitados a, N-acetil-muramil-L-treonil-D-isoglutamina (thr-MDP), N-acetil-normuramil-L-alanina-2-(1'-2' dipalmitoil-sn-glicero-3-hidroxifosforiloxi)-etilamina (MTP-PE), etc.

Las formulaciones de vacuna de la presente invención se pueden utilizar para proteger o tratar un humano susceptible a infección neumocócica, por medio de administrar la vacuna por vía de una ruta sistémica o de mucosa. Estas administraciones pueden incluir inyección por vía de las rutas intramuscular, intraperitoneal, intradérmica o subcutánea; o por vía de administración de mucosa a los tractos orales/alimentario, respiratorio o genitourinario. En una modalidad, la administración intranasal se utiliza para el tratamiento de neumonía u otitis media (como vehículo nasofaringeo de neumococo puede ser más efectivamente evitado, atenuando así la infección en su etapa más temprana).

La cantidad de conjugado en cada dosis de vacuna se selecciona como una cantidad que induce una respuesta inmune sin efectos significativos adversos. Tal cantidad puede variar dependiendo del serotipo neumocócico. En general, cada dosis comprenderá 0.1 a 100 µg de polisacárido, particularmente 0.1 a 10 µg, y más particularmente 1 a 5 µg.

Las cantidades óptimas de los componentes para una vacuna particular se pueden establecer mediante estudios estándar que involucran la observación de las respuestas inmunes apropiadas en sujetos. Luego de una vacunación inicial, los sujetos pueden recibir una o varias inmunizaciones de refuerzo adecuadamente espaciadas.

En una modalidad particular de la presente invención, la vacuna 13vPnC es una formulación líquida estéril de polisacáridos capsulares neumocócicos de los serotipos 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, y 23F conjugada individualmente a CRM_{197}. Cada dosis de 0.5 mL se formula para contener: 2 µg de cada sacárido, excepto para 6B a 4 µg; aproximadamente 29 µg de proteína portadora CRM_{197}; 0.125 mg de adyuvante de aluminio elemental (0.5 mg de fosfato de aluminio); y amortiguador de cloruro de sodio y succinato de sodio como excipientes. El líquido se llena en jeringas de dosis simple sin un preservativo. Después de agitar, la vacuna es una suspensión homogénea, blanca lista para administración intramuscular.

La elección del nivel de dosis de la vacuna 13vPnC es similar a aquella vacuna 7vPnC comercializada (Prevnar). El nivel de dosis de sacárido de 2 µg se selecciona para todos los serotipos, excepto para 6B, que está a 4 µg por dosis. La vacuna 7vPnC ha mostrado una seguridad, inmunogenicidad, y eficacia deseable contra IPD en el nivel de dosis de 2 µg de sacárido para los serotipos 4, 9V, 14, 18C, 19F y 23F, y en la dosis de 4 µg para 6B.

El programa de inmunización puede seguir aquel diseñado para la vacuna 7vPnC. Por ejemplo, el programa de rutina para recién nacidos y niños que están aprendiendo a caminar contra la enfermedad invasiva causada por *S. pneumoniae* debido a que los serotipos incluidos en la vacuna 13vPnC es 2, 4, 6 y 12-15 meses de edad. Las composiciones de esta invención también son adecuadas para uso con otros niños, adolescentes y adultos.

Las composiciones de esta invención pueden además incluir uno o más antígenos adicionales para uso contra otitis media causada por infección con otras bacterias. Tales bacterias incluyen *Haemophilus influenza, Moraxella catarrhalis* (anteriormente conocida como *Branhamella catarrhalis*) y *Alloiococcus otitidis*.

Ejemplos de antígenos de *Haemophilus influenza* sin tipo adecuados para la inclusión incluye la proteína P4, también conocida como proteína "e" (Patente U.S. No. 5,601,831;

Solicitud de Patente Internacional WO03/078453), la proteína P6, también conocida como el PAL o la proteína PBOMP-1 (Patente U.S. No. 5,110,908; Solicitud de Patente Internacional WO0100790), la proteína P5 (Patente Republicada U.S. No. 37,741), la proteína de adhesión y penetración de *Haemophilus* (Patentes U.S. Nos. 6,245,337 y 6,676,948), la proteína de adhesina de punta LKP (Patente U.S. No. 5,643,725) y la proteína NucA (Patente U.S. No. 6,221,365).

Ejemplos de antígenos de *Moraxella catarrhalis* adecuados para la inclusión incluyen la proteína UspA2 (Patentes U.S. Nos. 5,552,146, 6,310,190), la proteína CD (Patente U.S. No. 5,725,862), la proteína E (Patente U.S. No. 5,948,412) y la proteína de membrana exterior de 74 kilodalton (Patente U.S. No. 6,899,885).

Ejemplos de antígenos de *Alloiococcus otitidis* adecuados para la inclusión incluyen aquellos identificados en la Solicitud de Patente Internacional WO03/048304.

Las composiciones de esta invención también pueden incluir una o más proteínas de *Streptococcus pneumoniae*. Ejemplos de proteínas de *Streptococcus pneumoniae* adecuadas para la inclusión incluyen aquellas identificadas en la Solicitud de Patente Internacional WO02/083855, así como también aquella descrita en la Solicitud de Patente Internacional WO02/053761.

Las composiciones de esta invención pueden además incluir una o más proteínas provenientes de *Neisseria meningitidis* tipo B. Ejemplos de proteínas de *Neisseria meningitidis* tipo B adecuadas para la inclusión incluyen aquellas identificadas en las Solicitudes de Patente Internacional WO03/063766, WO2004/094596, WO01/85772, WO02/16612 y WO01/87939.

La descripción anterior describe de manera general la presente invención. Un entendimiento completo se puede obtener mediante referencia a los siguientes ejemplos específicos. Estos ejemplos se describen solamente con el propósito de ilustración y no pretenden limitar el alcance de la invención.

EJEMPLOS

Ejemplo 1

Preparación de Serotipo 1 de Polisacárido Capsular de *S. Pneumoniae*

Preparación de Bancos de Célula Maestra y de Trabajo

El serotipo 1 de *S. pneumoniae* se obtuvo del American Type Culture Collection, ATCC, cepa 6301. Varias generaciones de reservas de semilla se crearon con el fin de expandir la cepa y remover los componentes de origen animal (generaciones F1, F2, y F3). Dos generaciones adicionales de reservas de

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semilla se produjeron. La primera generación adicional se hizo de un frasco F3, y la generación posterior se hizo de un frasco de la primera generación adicional. Los frascos de semilla se almacenaron congelados (<-70°C) con glicerol sintético como criopreservativo. Además de los frascos congelados, los frascos liofilizados se prepararon para la generación F4. Para la preparación del banco de célula, todos los cultivos crecieron en un medio a base de soya. Antes de congelamiento, las células se concentraron mediante centrifugación, el medio de gasto fue removido, y las plaquetas de célula fueron resuspendidas en un medio fresco que contiene un criopreservativo, tal como glicerol sintético.

Fermentación y Cosecha

Los cultivos del banco de célula de trabajo utilizado para inocular las botellas de semilla contenían un medio a base de soya. Las botellas se incubaron a 36°C ± 2°C sin agitación hasta que se cumplieron los requisitos de crecimiento. Una botella de semilla se utilizó para inocular un fermentador de semilla que contiene un medio a base de soya. Un pH de aproximadamente 7.0 se mantuvo con una solución de carbonato de sodio estéril. Después de que se alcanzó la densidad óptica objetivo, el fermentador de semilla se utilizó para inocular el fermentador de producción que contiene medio a base de soya. El pH se mantuvo con solución de carbonato de sodio estéril. La fermentación se

terminó después de cesar el crecimiento o cuando el volumen de trabajo del fermentador se alcanzó. Una cantidad apropiada de desoxicolato de sodio al 12% estéril se agregó al cultivo para lisar las células bacterianas y liberar el polisacárido asociado a la célula. Después de lisar, se enfriaron los contenidos del fermentador. El pH del caldo de cultivo lisado se ajustó a aproximadamente pH 6.6 con ácido acético. El lisado se clarificó mediante centrifugación de flujo continuo seguido por filtración profunda y microfiltración a 0.45µm.

En un proceso alternativo, el pH de fermentación de aproximadamente 7.0 se mantuvo con NaOH 3N. Después de que se alcanzó la densidad óptica objetivo, el fermentador de semilla se utilizó para inocular el fermentador de producción que contiene medio a base de soya. El pH se mantuvo con NaOH 3N. La fermentación se termino después de cesar el crecimiento o cuando se alcanzó el volumen de trabajo del fermentador. Una cantidad apropiada de deoxicolato sodio al 12% estéril se agregó al cultivo para obtener una concentración de 0.12% en el caldo, para lisar las células bacterianas y liberar el polisacárido asociado a la célula. Después de lisado, los contenidos del fermentador se mantuvieron, con agitación, durante un intervalo de tiempo entre 8 y 24 horas a una temperatura entre 7°C y °3°C, para asegurar que la lisis celular completa y la liberación del polisacárido hubiera ocurrido. La agitación durante este período de mantenimiento evitó que el sedimento del lisado se asentara en las paredes del fermentador y la sonda de pH,

permitiendo de esta manera que se mantuviera la integridad de la sonda de pH. Luego, el pH del caldo de cultivo lisado se ajustó a aproximadamente pH 5.0 con ácido acético al 50%. Después de mantener un tiempo sin agitación, durante un intervalo de tiempo entre 12 y 24 horas a una temperatura entre 15°C y 25°C, una porción significativa de las proteínas previamente solubles desapareció de la solución como un precipitado sólido con poca pérdida o degradación del polisacárido, que permaneció en la solución. La solución con el precipitado se clarificó entonces mediante centrifugación de flujo continua seguida por filtración profunda y microfiltración de 0.45 µm.

Purificación

La purificación del polisacárido neumocócico consistió de varias operaciones de concentración/diafiltración, precipitación/elusión, cromatografía de columna, y etapas de filtración profunda. Todos los procedimientos se desarrollaron a temperatura ambiente a menos que se especifique otra cosa.

El caldo clarificado proveniente de los cultivos del fermentador del serotipo 1 *S. pneumoniae* se concentró y se diafiltró utilizando un filtro MWCO de 100 kDa (valor de corte del peso molecular en kilodalton). La diafiltración se logró utilizando amortiguador de fosfato de sodio a pH neutro. La diafiltración removió los componentes de medio de

peso molecular bajo provenientes de biopolímeros de peso molecular más alto tal como ácido nucleico, proteína y polisacárido.

El polisacárido se precipitó de la solución concentrada y diafiltrada al agregar bromuro de hexadeciltrimetil amonio (HB) proveniente de una solución madre para dar una concentración final de 1% HB (p/v). El precipitado de polisacárido/HB se capturó sobre un filtro profundo y el filtrado se descartó. El precipitado de polisacárido se resolubilizó y se eluyó al recircular una solución de cloruro de sodio a través del filtro profundo que contiene precipitado. Los filtros fueron entonces enjuagados con una solución adicional de cloruro de sodio.

Se agregó yoduro de sodio (NaI) a la solución de polisacárido proveniente de una solución madre de NaI para lograr una concentración final de 0.5% para precipitar HB. El precipitado se removió mediante filtración profunda. El filtrado contiene el polisacárido objetivo. El recipiente de precipitación y el filtro se enjuagaron con una solución de NaCl/NaI y el enjuague se combinó con una la solución de polisacárido parcialmente purificada. El filtro se descartó. El polisacárido fue entonces filtrado a través de un filtro de 0.2 µm.

La solución de polisacárido se concentró sobre un ultrafiltro MWCO de 30 kDa y diafiltrado con una solución de cloruro de sodio.

La solución de polisacárido parcialmente purificado se purificó adicionalmente mediante filtración a través de un filtro profundo impregnado con carbono activado. Después de la filtración, el filtro de carbono se enjuagó con una solución de cloruro de sodio. El enjuague se combinó con la solución de polisacárido, que es luego filtrada a través de un filtro de 0.2 µm.

La solución de polisacárido se concentró sobre un ultrafiltro MWCO de 30 kDa y se ajustó con un amortiguador de fosfato de sodio 1M para lograr una concentración final de fosfato de sodio 0.025 M. El pH se revisó y se ajustó a 7.0 ± 0.2.

La columna de hidroxiapatita cerámïca (HA) se equilibró con amortiguador de fosfato de sodio que contiene cloruro de sodio para obtener la conductividad apropiada (<15 µS). La solución de polisacárido fue entonces cargada sobre la columna. Bajo estas condiciones, las impurezas unidas a la resina y al polisacárido se recuperaron en el flujo a través de la columna. La solución de polisacárido se filtró a través de filtros de línea dentada de 0.2µm localizados antes y después de la columna.

La solución de polisacárido se concentró utilizando un filtro MWCO de 30 kDa. El concentrado fue entonces diafiltrado con Agua para inyección (WFI).

La solución de polisacárido diafiltrado se filtró a través de un filtro de membrana de 0.2 µm en botellas de polipropileno. Las muestras se removieron para liberar las pruebas y el polisacárido purificado se almacenó congelado a -25° ± 5°C.

Caracterización

Los datos 1H-NMR fueron consistentes con la estructura química mediante la asignación de las señales asignadas a los protones de la molécula de polisacárido. El espectro 1H-NMR mostró una serie de señales bien resueltas (protones provenientes del grupo metilo) para la cuantificación del grupo funcional O-acetilo en el polisacárido.

La identidad del polisacárido monovalente se confirmó mediante inmunoelectroforesis de contra corriente utilizando antisuero específico.

La cromatografía de filtración de gel de alto desempeño acoplada con el índice de refracción y detectores de dispersión de luz láser multi-ángulo (MALLS) se utilizo en conjunto con la concentración de la muestra para calcular el peso molecular.

El medio de cromatografía de exclusión de tamaño (CL-4B) se utilizó para perfilar la distribución de tamaño molecular relativa del polisacárido.

Ejemplo 2

Preparación de Conjugado de Sacárido de Pneumococo - CRM_{197}

Serotipo 1

Activación y Conjugación

Los recipientes del polisacárido purificados se descongelaron y se combinaron en un recipiente de reacción. Al recipiente, carbonato de sodio 0.2 M, pH 9.0 se agregó para desacetilación parcial (hidrólisis) durante 3 horas a 50°C. La reacción se enfrió a 20°C y la neutralización se desarrolló mediante ácido acético 0.2 M. La oxidación en la presencia de periodato de sodio se desarrolló mediante incubación a 2-8°C, y la mezcla se agitó durante 15-21 horas.

La mezcla de reacción de activación se concentró y se diafiltró 10x con NaCl al 0.9% utilizando una membrana MWCO de 30 K. El retenido fue filtrado a 0.2 µm. El sacárido activado se llenó en botellas de liofilización de vidrio de 100 mL y se congeló en cubierta a -75°C y se liofilizó.

"Congelado en cubierta" es un método para preparar muestras para liofilización (secado por congelado). Los recipientes son rotados automáticamente por rodillos

impulsados por motor en un baño refrigerado que contiene alcohol o cualquier otro fluido apropiado. Un recubrimiento delgado del producto es congelado homogéneamente alrededor del interior de la "cubierta" del recipiente, que permite que un mayor volumen de material sea procesado de manera segura durante cada corrida de secado por congelamiento. Estas unidades automáticas, refrigeradas suministran unos medios simples y eficientes de pre-congelar muchos recipientes a la vez, produciendo los recubrimientos deseados al interior, y suministrando un área de superficie suficiente para un secado por congelado eficiente.

Las botellas de material liofilizado se llevaron a temperatura ambiente y fueron resuspendidas en solución CRM_{197} a una proporción de sacárido/proteína de 2:1. A la mezcla de sacárido/proteína se agregó un amortiguador de fosfato de sodio 1M a una concentración iónica final de 0.2M y un pH de 7.5, luego se agregó cianoborohidruro de sodio. La reacción se incubó a 23°C durante 18 horas, seguido por una segunda incubación a 37°C durante 72 horas. Luego de las incubaciones de cianoborohidruro, la mezcla de reacción se diluyó con solución salina fría seguida por adición de carbonato de sodio 1M para ajustar la mezcla de reacción a pH 9.0. Los aldehídos no reaccionados fueron apagados por la adición de borohidruro de sodio mediante incubación a 23°C durante 3-6 horas.

La mezcla de reacción se diluyó dos veces con solución salina y se transfirió a través de un prefiltro de 0.45 - 5 µm en un recipiente de retenido. La mezcla de reacción se diafiltró 30x con amortiguador de fosfato 0.15 M, pH 6, y 20x con solución salina. El retenido fue filtrado a través de un filtro de 0.2 µm.

La solución de conjugado se diluyó a un objetivo de 0.5 mg/mL en solución salina 0.9%, y luego se filtró estéril en recipientes de concentración de masa final (FBC) en una caperuza Clase 100. El conjugado se almacenó a 2-8°C.

Caracterización

El medio de cromatografía de exclusión de tamaño (CL-4B) se utilizó para perfilar la distribución de tamaño molecular relativa del conjugado.

La identidad del conjugado se confirmó por el ensayo "slot-blot" utilizando antisuero específico.

Las concentraciones de sacárido y proteína se determinaron mediante los ensayos de ácido urónico y Lowry, respectivamente. La proporción de sacárido a proteína en el complejo de conjugado covalentemente unido se obtuvo mediante el cálculo:

$$\text{Proporción} = \frac{\text{µg/mL sacárido}}{\text{µg/mL proteína}}$$

El contenido de O-acetilo se midió por medio del método Hestrin (Hestrin et al., J. Biol. Chem. 1949, 180, p. 249). La proporción de la concentración de O-acetilo a la concentración de sacárido total dio µmoles de O-acetilo por mg de sacárido.

Ejemplo 3

Preparación del Serotipo 3 de Polisacárido Capsular de *S. Pneumoniae*

Preparación de los Bancos de Célula Maestro y de Trabajo

El serotipo 3 de *S. pneumoniae* se obtuvo del Dr. Robert Austrian, Universidad de Pennsylvania, Filadelfia, Pennsylvania. Para la preparación del sistema de banco de células, ver el Ejemplo 1.

Fermentación y Cosecha

Los cultivos provenientes del banco de célula de trabajo se utilizaron para inocular botellas de semilla que contienen un medio basado en soya. Las botellas se incubaron a 36°C ± 2°C sin agitación hasta que se cumplieron los requisitos de crecimiento. Una botella de semilla se utilizó para inocular un fermentador de semilla que contiene un medio a base de soya. Un pH de aproximadamente 7.0 se mantuvo con una solución de carbonato de sodio estéril. Después de que se alcanzó la densidad óptica objetivo, el fermentador de

semilla se utilizó para inocular un fermentador de semilla intermedio. Después de que se alcanzó la densidad óptica objetivo, el fermentador de semilla intermedio se utilizó para inocular el fermentador de producción. El pH se mantuvo con una solución de carbonato de sodio estéril. La fermentación se terminó después de que se alcanzó el volumen de trabajo del fermentador. Una cantidad apropiada de desoxicolato de sodio al 12% estéril se agregó al cultivo para lisar las células bacterianas y liberar el polisacárido asociado a la célula. Después de lisar, los contenidos del fermentador se enfriaron. El pH del caldo de cultivo lisado se ajustó a aproximadamente pH 6.6 con ácido acético. El lisado se clarificó mediante centrifugación de flujo continuo seguido por filtración profunda y microfiltración de 0.45μm.

Purificación

La purificación del polisacárido neumocócico consistió de varias operaciones de concentración/diafiltración, precipitación/elusión, cromatografía de columna, y etapas de filtración profunda. Todos los procedimientos se desarrollaron a temperatura ambiente a menos que se especifique otra cosa.

El caldo clarificado proveniente de los cultivos del fermentador del serotipo 3 de *S. pneumoniae* se concentró y se diafiltró utilizando un filtro MWCO de 100 kDa. La diafiltración se logró utilizando un amortiguador de fosfato

de sodio a pH neutro. La diafiltración removió los componentes de medio de peso molecular bajo provenientes de los biopolímeros de peso molecular mayor tal como ácido nucleico, proteína y polisacárido.

Antes de la adición de bromuro de hexadeciltrimetil amonio (HB), un volumen calculado de una solución madre de NaCl se agregó a la solución de polisacárido concentrada y diafiltrada para dar una concentración final de NaCl 0.25 M. El polisacárido fue luego precipitado al agregar HB proveniente de una solución madre para dar una concentración final de HB al 1% (p/v). El polisacárido/precipitado HB se capturó sobre un filtro profundo y el filtrado se descartó. El precipitado de polisacárido se resolubilizó y se eluyó al recircular una solución de cloruro de sodio a través del filtro profundo que contiene precipitado. Los filtros fueron entonces enjuagados con una solución de cloruro de sodio adicional.

Se agregó yoduro de sodio (NaI) a la solución de polisacárido proveniente de una solución madre de NaI para lograr la concentración final de 0.5% para precipitar HB. El precipitado se removió mediante filtración profunda. El filtrado contenía el polisacárido objetivo. El recipiente de precipitación y el filtro se enjuagaron con una solución de NaCl/NaI y el enjuagado se combinó con la solución de polisacárido parcialmente purificada. El filtro se descartó.

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El polisacárido fue entonces filtrado a través de un filtro de 0.2 µm.

La solución de polisacárido se concentró sobre un ultrafiltro MWCO de 30 kDa y se diafiltró con una solución de cloruro de sodio.

La solución de polisacárido parcialmente purificada fue purificada además mediante filtración a través de un filtro profundo impregnado con carbono activado. Después de la filtración, el filtro de carbono se enjuagó con una solución de cloruro de sodio. El enjuague se combinó con la solución de polisacárido, que fue entonces filtrada a través de un filtro de 0.2 µm.

La solución de polisacárido se concentró sobre un ultrafiltro MWCO de 30 kDa y se ajustó con un amortiguador de fosfato de sodio 1M para lograr una concentración final de fosfato de sodio 0.025M. El pH se revisó y se ajustó a 7.0 ± 0.2.

La columna de hidroxiapatita cerámica (HA) se equilibró con un amortiguador de fosfato de sodio que contiene cloruro de sodio para obtener la conductividad apropiada (<15 µS). La solución de polisacárido fue entonces cargada sobre la columna. Bajo estas condiciones, las impurezas unidas a la resina y al polisacárido se recuperaron en el flujo pasante desde la columna. El polisacárido fue fluido a través de la

columna con amortiguador y luego filtrado a través de un filtro de 0.2µm.

La solución de polisacárido se concentró utilizando un filtro MWCO de 30 kDa. El concentrado fue entonces diafiltrado con WFI.

La solución de polisacárido diafiltrado se filtró a través de un filtro de membrana de 0.2 µm en recipientes de acero inoxidable. Las muestras se removieron para liberar la prueba y el polisacárido purificado se almacenó congelado a -25° ± 5°C.

Caracterización

Los datos 1H-NMR fueron consistentes con la estructura química mediante la asignación de las señales asignadas a los protones de la molécula de polisacárido.

La identidad del polisacárido monovalente se confirmó mediante inmunoelectrofóresis de contra corriente utilizando antisuero específico.

La cromatografía de filtración de gel de alto desempeño acoplada con el índice de refracción y detectores de dispersión de luz láser multi-ángulo (MALLS) se utilizo en conjunto con la concentración de muestra para calcular el peso molecular.

El medio de cromatografía de exclusión de tamaño (CL-4B) se utilizó para perfilar la distribución de tamaño molecular relativa del polisacárido.

Ejemplo 4

Preparación del Conjugado de Sacárido Neumocócico - CRM_{197}

Serotipo 3

Activación y Conjugación

Los recipientes del sacárido serotipo 3 purificados se descongelaron y se combinaron en un recipiente de reacción. Al recipiente, se agregó WFI y ácido acético 2M a una concentración final de 0.2M y 2mg/mL de sacárido. La temperatura de la solución se elevó a 85°C durante 1 hora para hidrolizar el polisacárido. La reacción se enfrió a ≤25°C y se agregó cloruro de magnesio 1M a una concentración final de 0.1M. La oxidación en la presencia de periodato de sodio se desarrolló mediante incubación durante 16-24 horas a 23°C.

La mezcla de reacción de activación se concentró y se diafiltró 10x con WFI utilizando una membrana MWCO de 100 K. El retenido fue filtrado a través de un filtro de 0.2 µm.

Para componerlo, se agregó fosfato de sodio 0.2M, pH 7.0, al sacárido activado a una concentración final de 10mM y un pH de 6.0-6.5. La proteína portadora CRM_{197} se mezcló con

la solución de sacárido a una proporción de 2g de sacárido por 1g de CRM_{197}. La solución de sacárido/proteína combinada se llenó en botellas de liofilización de vidrio de 100 mL con un llenado objetivo de 50mL, congelada en recipiente a -75°C, y liofilizada.

Las botellas de material de sacárido/proteína co-liofilizadas se llevaron a temperatura ambiente y se resuspendieron en un amortiguador de fosfato de sodio 0.1M, pH 7.0, a una concentración de sacárido final de 20 mg/mL. El pH se ajustó a 6.5 y luego 0.5 de equivalente molar de cianoborohidruro de sodio se agregaron. La reacción se incubó a 37°C durante 48 horas. Luego de la incubación de cianoborohidruro, la mezcla de reacción se diluyó con succinato 5mM/amortiguador de solución salina 0.9%. Los aldehídos no reaccionados fueron apagados mediante la adición de borohidruro de sodio y la incubación a 23°C durante 3-6 horas. La mezcla de reacción se transfirió a través de un pre-filtro de 0.45-5 µm en un recipiente de retenido.

La mezcla de reacción se diafiltró 30x con amortiguador de fosfato 0.1M (pH 9), 20x con amortiguador de fosfato 0.15M (pH 6), y 20x con succinato 5mM/solución salina 0.9%. El retenido se filtró a través de un filtro de 0.2 µm.

La solución de conjugado se diluyó a un objetivo de sacárido de 0.5 mg/mL, y luego se esterilizó filtrada en

recipientes FBC en una caperuza Clase 100. El conjugado se almacenó a 2-8°C.

Caracterización

El medio de cromatografía de exclusión de tamaño (CL-4B) se utilizó para perfilar la distribución de tamaño molecular relativa del conjugado.

La identidad del conjugado se confirmó mediante un ensayo "slot-blot2 utilizando antisuero específico.

Las concentraciones de sacárido y proteína se determinaron mediante los ensayos de Anthrone y Lowry, respectivamente. La proporción del sacárido a proteína en el complejo de conjugado covalentemente unido se obtuvo mediante el cálculo:

$$\text{Proporción} = \frac{\mu g/mL \text{ sacárido}}{\mu g/mL \text{ proteína}}$$

Ejemplo 5

Preparación del Serotipo 5 de Polisacárido Capsular de *S. Pneumoniae*

El serotipo 5 de *S. pneumoniae* se obtuvo del Dr. Gerald Schiffman de la Universidad Estatal de New York, Brooklyn, New York. Para la preparación del sistema de banco celular,

ver el Ejemplo 1. Para la fermentación, cosecha, purificación y caracterización del polisacárido, ver el Ejemplo 1.

Proceso de Fermentación Alternativo

Los cultivos provenientes del banco de célula de trabajo utilizados para inocular las botellas de semilla que contienen un medio basado de soya y una solución de $NaHCO_3$ estéril de 10mM. Las botellas se incubaron a 36°C ± 2°C sin agitación hasta que se cumplieron los requisitos de crecimiento. Una botella de semilla se utilizó para inocular un fermentador de semilla que contiene un medio a base de soya y una solución de $NaHCO_3$ estéril de 10mM. Un pH de aproximadamente 7.0 se mantuvo con NaOH 3N. Después de que se alcanzó la densidad óptica objetivo, el fermentador de semilla se utilizó para inocular el fermentador de producción que contiene un medio a base de soya con una concentración de $NaHCO_3$ de 10mM. El pH se mantuvo con NaOH 3N. La fermentación se terminó luego de cesar el crecimiento y cuando el volumen de trabajo del fermentador se alcanzó. Una cantidad apropiada de desoxicolato de sodio al 12% estéril se agregó al cultivo para obtener una concentración de 0.12% en el caldo, para lisar las células bacterianas y liberar el polisacárido asociado a la célula. Después de lisar, los contenidos del fermentador se mantuvieron, con agitación, durante un intervalo de tiempo entre 8 y 24 horas a una temperatura de entre 7°C y 13°C para asegurar que hubiera ocurrido la lisis celular completa y la liberación del polisacárido. La

agitación durante este período de mantenimiento evitó que el sedimento del lisado se asentara en las paredes del fermentador y la sonda de pH, permitiendo de esta manera que se mantuviera la integridad de la sonda de pH. Luego, el pH del caldo de cultivo lisado se ajustó a aproximadamente un pH de 4.5 con 50% de ácido acético. Después de un tiempo de mantenimiento sin agitación, durante un intervalo de tiempo entre 12 y 24 horas a una temperatura entre 15°C y 25°C, una porción significativa de las proteínas previamente solubles desaparecieron de la solución como un precipitado sólido con poca pérdida o degradación del polisacárido, que permaneció en la solución. La solución con el precipitado fue entonces clarificada mediante centrifugación de flujo continuo seguido por filtración profunda y microfiltración a 0.45 µm.

Ejemplo 6

Preparación del Conjugado de Sacárido Neumocócico Serotipo 5 – CRM_{197}

Activación y Conjugación

Los recipientes del sacárido del serotipo 5 fueron descongelados y combinados en un recipiente de reacción. Al recipiente, se agregaron acetato de sodio 0.1M, pH 4.7, seguido por oxidación en la presencia de periodato de sodio mediante incubación durante 16-22 horas a 23°C.

La mezcla de reacción de activación se concentró y se diafiltró 10x con WFI utilizando una membrana MWCO 100K. El retenido se filtró a través de un filtro de 0.2 µm.

El sacárido activado serotipo 5 se combinó con CRM_{197} a una proporción de 0.8:1. La solución de sacárido/proteína combinada se llenó en botellas de liofilización de vidrio de 100 mL (50 mL llenado de objetivo), congelado en cubierta a -75°C, y co-liofilizado.

Las botellas de material co-liofilizado fueron llevadas a temperatura ambiente y resuspendidas en fosfato de sodio 0.1M, pH 7.5, y se agregó cianoborohidruro de sodio. La reacción se incubó a 30°C durante 72 horas, seguido por una segunda adición de cianoborohidruro e incubada a 30°C durante 20-28 horas.

Luego de las incubaciones de cianoborohidruro, la mezcla de reacción se diluyó dos veces con solución salina y se transfirió a través de un pre-filtro de 0.45-5 µm en un recipiente de retenido. La mezcla de reacción se diafiltró 30x con amortiguador de fosfato 0.01M, pH 8, 20x con amortiguador de fosfato 0.15M, pH 6, y 20x con solución salina. El retenido se filtró a través de un filtro de 0.2 µm.

La solución de conjugado se diluyó a un objetivo de sacárido de 0.5 mg/mL, y luego estéril se filtró en

recipientes FBC en una caperuza Clase 100. El conjugado se almacenó a 2-8°C.

Para la caracterización del conjugado, ver el Ejemplo 2.

Ejemplo 7

Preparación del Serotipo 6A de Polisacárido Capsular de *S. Pneumoniae*

El serotipo 6A de *S. pneumoniae* se obtuvo del Dr. Gerald Schiffman de la Universidad Estatal de New York, Brooklyn, New York. Para la preparación del sistema de banco celular, ver el Ejemplo 1. Para la fermentación, cosecha y purificación del polisacárido, ver el Ejemplo 1, excepto que durante la purificación, la etapa de concentración de MWCO 30 kDa, se omitió antes de la etapa de cromatografía.

Ejemplo 8

Preparación del Conjugado de Sacárido Neumocócico Serotipo 6A - CRM_{197}

Activación y Conjugación

El polisacárido del serotipo 6A es un polímero de alto peso molecular que se había reducido en tamaño antes de la oxidación. Los recipientes del sacárido del serotipo 6A se descongelaron y se combinaron en un recipiente de reacción. Al recipiente, se agregó ácido acético 2 M a una

concentración final de 0.1 M para hidrólisis durante 1.5 horas a 60°C. La reacción se enfrió a 23°C y la neutralización se desarrolló al ajustar la mezcla de reacción con NaOH 1 M a pH 6. La oxidación en la presencia de periodato de sodio se desarrolló mediante incubación a 23°C durante 14-22 horas.

La mezcla de reacción de activación se concentró y se diafiltró 10x con WFI utilizando una membrana MWCO 100K. El retenido se filtró a través de un filtro de 0.2 µm.

El serotipo 6A se compuso con sucrosa y se llenó en botellas de liofilización de vidrio de 100 mL (50mL llenado objetivo) y congeladas en cubierta a -75°C y liofilizadas.

Las botellas de material liofilizado se llevaron a temperatura ambiente y resuspendidas en dimetilsulfóxido (DMSO) a una proporción de sacárido/proteína de 1:1. Después de la adición de cianoborohidruro de sodio, la mezcla de reacción se incubó a 23°C durante 18 horas. Luego de la incubación de cianoborohidruro, la mezcla de reacción se diluyó con solución salina fría. Los aldehídos no reaccionados fueron apagados mediante adición de borohidruro de sodio mediante incubación a 23°C durante 3-20 horas.

La mezcla de reacción diluida se transfirió a través de un pre-filtro de 5 µm en un recipiente de retenido. La mezcla de reacción se diafiltró 10x con NaCl 0.9% y 30x con NaCl

amortiguado con succinato. El retenido se filtró a través de un filtro de 0.2 µm.

La solución de conjugado se diluyó a un objetivo de sacárido de 0.5 mg/mL, y luego estéril se filtró en recipientes FBC en una caperuza Clase 100. El conjugado se almacenó a 2-8°C.

Para la caracterización del conjugado, ver el Ejemplo 2.

Ejemplo 9

Preparación del Serotipo 7F de Polisacárido Capsular de *S. Pneumoniae*

El serotipo 7F de *S. pneumoniae* se obtuvo del Dr. Gerald Schiffman de la Universidad Estatal de New York, Brooklyn, New York. Para la preparación del sistema de banco celular, y para la fermentación y cosecha del polisacárido, ver el Ejemplo 3. Para un proceso de fermentación y de cosecha alternativos, ver el proceso alternativo descrito en el Ejemplo 1.

Purificación

La purificación del polisacárido neumocócico consistió de varias operaciones de concentración/diafiltración, precipitación/elusión, cromatografía de columna, y etapas de filtración profunda. Todos los procedimientos se

desarrollaron a temperatura ambiente a menos que se especifique otra cosa.

El caldo clarificado proveniente de cultivos del fermentador del serotipo 7F de *S. pneumoniae* se concentró y se filtró utilizando un filtro MWCO de 100 kDa. La diafiltración se logró utilizando un amortiguador de fosfato de sodio a pH neutro. La diafiltración removió los componentes medios de bajo peso molecular provenientes de los biopolímeros de peso molecular mayor tal como ácido nucleico, proteína y polisacárido.

El serotipo 7F no forma un precipitado con HB. En su lugar, las impurezas se precipitaron de la solución concentrada y diafiltrada al agregar el HB proveniente de una solución madre a una concentración final de 1% de HB. El precipitado se capturó sobre un filtro profundo y el filtro se descarto. El polisacárido estaba contenido en el filtrado.

Se agregó yoduro de sodio (NaI) a la solución de polisacárido proveniente de la solución de NaI madre para lograr una concentración final de 0.5% para precipitar HB. El precipitado se removió mediante filtración profunda. El filtrado contenía el polisacárido objetivo. El recipiente de precipitación y el filtro se enjuagaron con una solución de NaCl/NaI y los enjuagues se combinaron con la solución de polisacárido parcialmente purificada. El filtro se descartó.

El polisacárido fue entonces filtrado a través de un filtro de 0.2 µm.

La solución de polisacárido se concentró sobre un ultrafiltro MWCO de 30 kDa y se diafiltró con una solución de cloruro de sodio.

La solución de polisacárido parcialmente purificada se purificó adicionalmente mediante filtración a través de un filtro profundo impregnado con carbono activado. Después de la filtración, el filtro de carbono se enjuagó con una solución de cloruro de sodio. El enjuague se combinó con la solución de polisacárido, que luego se filtró a través de un filtro de 0.2 µm.

La solución de polisacárido se concentró sobre un ultrafiltro MWCO de 30 kDa y se ajustó con un amortiguador de fosfato de sodio 1M para lograr una concentración final de fosfato de sodio 0.025M. El pH se revisó y se ajustó a 7.0 ± 0.2.

La columna de hidroxiapatita cerámica (HA) se equilibró con un amortiguador de fosfato de sodio que contiene cloruro de sodio para obtener la conductividad apropiada (15 µS). La solución de polisacárido fue entonces cargada sobre la columna. Bajo estas condiciones, las impurezas unidas a la resina y el polisacárido se recuperaron en el flujo pasante desde la columna. El polisacárido fluyó a través de la

columna con amortiguador y se filtró a través de un filtro de 0.2 µm.

La solución de polisacárido se concentró utilizando un filtro MWCO de 30 kDa. El concentrado fue entonces diafiltrado con WFI.

La solución de polisacárido diafiltrada se filtro a través de un filtro de membrana de 0.2 µm en recipientes de acero inoxidable. Las muestras se removieron para liberar el ensayo y el polisacárido purificado se almacenó a 2° - 8°C.

Para la caracterización del polisacárido, ver el Ejemplo 3.

Ejemplo 10

Preparación del Conjugado de Sacárido Neumocócico Serotipo 7F - CRM_{197}

Activación y Conjugación

La oxidación en la presencia de periodato de sodio se desarrolló mediante la incubación durante 16-24 horas a 23°C.

La mezcla de reacción de activación se concentró y se diafiltró 10x con NaOAc 10mM, pH 4.5, utilizando una membrana MWCO 100K. El retenido se filtró a través de un filtro de 0.2 µm.

El serotipo 7F se lleno en botellas de liofilización de vidrio de 100 mL (50 mL de llenado objetivo) y congeladas en cubierta a -75°C y liofilizadas.

Las botellas del serotipo liofilizado 7F y CRM_{197} se llevaron a temperatura ambiente y fueron resuspendidas en DMSO a una proporción de sacárido/proteína de 1.5:1. Después de la adición de cianoborohidruro de sodio, la reacción se incubó a 23°C durante 8-10 horas. Los aldehídos no reaccionados fueron apagados mediante la adición de borohidruro de sodio mediante incubación a 23°C durante 16 horas.

La mezcla de reacción se diluyó 10 veces con solución salina fría y se transfirió a través de un pre-filtro de 5 µm en un recipiente de retenido. La mezcla de reacción se diafiltró 10x con solución salina 0.9% y 30x con solución salina amortiguada con succinato. El retenido se filtró a través de un filtro de 0.2 µm.

La solución conjugada se diluyó con un objetivo de sacárido de 0.5 mg/mL solución salina 0.9%, y luego estéril se filtró en recipientes FBC en una caperuza Clase 100. El conjugado se almacenó a -8°C.

Para caracterización del conjugado, ver el Ejemplo 4.

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Ejemplo 11

Preparación del Serotipo 19A de Polisacárido Capsular de *S. Pneumoniae*

El serotipo 19A de *S. pneumoniae* se obtuvo del Dr. Gerald Schiffman de la Universidad Estatal de New York, Brooklyn, New York. Para la preparación del sistema de banco celular, ver el Ejemplo 1. Para la fermentación, cosecha y purificación del polisacárido, ver el Ejemplo 7. Para la caracterización, ver el Ejemplo 3.

Ejemplo 12

Preparación del Conjugado de Sacárido Neumocócico Serotipo 19A - CRM_{197}

Activación y Conjugación

Los recipientes del sacárido serotipo 19A fueron descongelados y combinados en un recipiente de reacción. Se agregó acetato de sodio a 10 mM (pH 5.0) y la oxidación se llevó a cabo en la presencia de periodato de sodio mediante incubación durante 16-24 horas a 23°C.

La mezcla de reacción de activación se concentró y se diafiltró 10x con acetato 10mM, pH 5.0, utilizando una membrana MWCO 100K. El retenido se filtró a través de un filtro de 0.2 µm.

El sacárido activado se compuso con sucrosa seguido por la adición de CRM_{197}. El sacárido activado con serotipo 19A y la mezcla CRM_{197} (proporción 0.8:1) se llenó en botellas de liofilización de vidrio 100 mL (50 mL llenado de objetivo), congeladas en cubierta a -75°C, y liofilizadas.

Las botellas de material liofilizado fueron llevadas a temperatura ambiente y resuspendidas en DMSO. A la mezcla de sacárido/proteína, se agregó cianoborohidruro de sodio (100 mg/ml). La reacción se incubó a 23°C durante 15 horas. Luego de la incubación de cianoborohidruro, los aldehídos no reaccionados fueron apagados mediante la adición de borohidruro de sodio mediante incubación a 23°C durante 3-20 horas.

La mezcla de reacción se diluyó 10 veces con solución salina fría y se transfirió a través de un prefiltro de 5 µm en un recipiente de retenido. La mezcla de reacción se diafiltró 10x con 0.9% de NaCl, se filtró 0.45 µm, y 30x con diafiltración utilizando un amortiguador de succinato 5mM/NaCl 0.9%, pH 6. El retenido se filtró a través de un filtro de 0.2 µm.

La solución de conjugado se diluyó a un objetivo de 0.5 mg/mL utilizando succinato 5mM/solución salina 0.9%, y luego filtrada estéril en recipientes FBC en una caperuza Clase 100. El conjugado se almacenó a 2 - 8°C.

Para la caracterización del conjugado, ver el Ejemplo 4.

Ejemplo 13

Preparación de los Serotipos 4, 6B, 9V, 14, 18C, 19F y 23F de Polisacárido Capsular de *S. Pneumoniae*

Preparación del Cultivo de Semilla de *S. pneumoniae*

Los serotipos 4, 6B, 9V, 18C, 19F y 23F de *S. pneumoniae* se obtuvieron del Dr. Gerald Schiffman, Universidad Estatal de New York, Brooklyn, New York. El serotipo 14 *S. pneumoniae* se obtuvo del ATCC, cepa 6314.

Separadamente, un frasco de cada uno de los serotipos deseados de *Streptococcus pneumoniae* se utilizó para iniciar una tanda de fermentación. Dos botellas que contienen un medio a base de soya y rojo fenol se ajustaron a un rango de pH de 7.4 *± 0.2 utilizando carbonato de sodio, y el volumen requerido de la solución de dextrosa al 50%/sulfato de magnesio al 1% se agregaron luego a las botellas. Las dos botellas se inocularon* con diferentes cantidades de semilla. Las botellas se incubaron a 36° ± 2°C hasta que el medio se volvió amarillo. Luego de la incubación, las muestras se removieron de cada botella y se ensayaron para densidad óptica (OD) (0.3 a 0.9) y pH (4.6 a 5.5). Una de las dos botellas se seleccionó para la inoculación del fermentador de semilla.

El medio a base de soya se transfirió al fermentador de semilla y se esterilizó. Luego se agregó un volumen de solución 50% de dextrosa/1% de sulfato de magnesio al fermentador. El pH y la agitación del fermentador de semilla se monitorearon y se controlaron (pH 6.7 a 7.4). La temperatura se mantuvo a 36° ± 2°C. La inoculación de semilla (botella) fue conectada asépticamente al fermentador de semilla y el inóculo fue transferido. El fermentador se mantuvo en un control de pH y las muestras fueron periódicamente removidas y probadas para OD y pH. Cuando el OD deseado de 0.5 a 600 nm se alcanzó, el fermentador intermedio se inoculó con el caldo de fermentación proveniente del fermentador de semilla.

El medio a base de soya se transfirió al fermentador intermedio y se esterilizó. Luego una solución de dextrosa al 50%/sulfato de magnesio 1% volumen se agregó al fermentador. El pH y la agitación del fermentador intermedio se monitorearon y controlaron (pH 6.7 a 7.4). La temperatura se mantuvo a 36° ± 2°C. Los contenidos del fermentador de semilla se transfirieron al fermentador intermedio. El fermentador se mantuvo en control de pH y las muestras se removieron periódicamente y se probaron para OD y pH. Cuando el OD deseado de 0.5 a 600 nm se alcanzó, el fermentador de producción se inoculó con un caldo de fermentación proveniente del fermentador intermedio.

El medio a base de soya se transfirió al fermentador de producción y se esterilizó. Luego se agregó un volumen de 50% de dextrosa/1% de solución de sulfato de magnesio. El pH y la agitación del fermentador de producción se monitorearon y controlaron (pH 6.7 a 7.4). La temperatura se mantuvo a 36° ± 2°C. El fermentador se mantuvo en control de pH y las muestras se removieron periódicamente y se probaron para OD y pH, hasta que se completó la fermentación.

Se agregó desoxicolato de sodio al fermentador a una concentración final de aproximadamente 0.12% p/v. El cultivo se mezcló durante un mínimo de treinta minutos y el punto de ajuste de la temperatura se redujo a 10°C. El cultivo se incubó durante toda la noche y luego de confirmación de inactivación, el pH del cultivo se ajustó a entre 6.4 y 6.8, según fuera necesario, con 50% de ácido acético. La temperatura del fermentador se incrementó a 20° ± 5°C y los contenidos se transfirieron al tanque de mantenimiento de clarificación.

Los contenidos del tanque de mantenimiento de clarificación (incluyendo los desechos celulares) se procesaron a través de una centrifuga a una tasa de flujo entre 25 y 600 litros por hora (excepto el Serotipo 4, en donde los desechos de célula fueron descartados y la tasa de flujo ajustada a entre 25 y 250 litros por hora). Las muestras del sobrenadante se removieron y se ensayaron para OD. El OD deseado durante la centrifugación fue ≤0.15.

Inicialmente, el sobrenadante fue recirculado a través de un montaje de filtro profundo hasta que se logró un OD de 0.05 ± 0.03. Luego el sobrenadante pasó a través del montaje de filtro profundo y a través de un filtro de membrana de 0.45 µm al tanque de mantenimiento de filtrado.

Posteriormente, el producto se transfirió a través de tubos cerrados al área de purificación para procesamiento.

Todas las operaciones anteriores (centrifugación, filtración y transferencia) se desarrollaron entre 10°C a 30°C.

Para una fermentación alternativa y un proceso de cosecha para los serotipos 4 y 6B, ver el proceso alternativo descrito en el Ejemplo 1.

Purificación

La purificación de cada polisacárido neumocócico consistió de varias operaciones de concentración/diafiltración, precipitación/elusión, cromatografía de columna, y etapas de filtración profunda. Todos los procedimientos se desarrollaron a temperatura ambiente a menos que se especifique otra cosa.

El caldo clarificado proveniente de los cultivos del fermentador del serotipo de *S. pneumoniae* deseado se

concentró y se diafiltró utilizando un filtro MWCO de 100 kDa. La diafiltración se logró utilizando amortiguador de fosfato de sodio a pH < 9.0. La diafiltración removió los componentes medios de peso molecular bajo provenientes de biopolímeros de peso molecular mayor tales como ácido nucleico, proteína y polisacárido.

El polisacárido se precipitó de la solución concentrada y diafiltrada al agregar HB proveniente de una solución madre para dar una concentración final de 1% de HB (p/v) (excepto el Serotipo 23F, que tuvo una concentración final de 2.5%). El polisacárido/precipitado HB se capturó sobre un filtro profundo y el filtrado se descartó. (Nota: el Serotipo 14 no se precipitó; por lo tanto el filtrado se retuvo). El precipitado del polisacárido se resolubilizó y se eluyó al recircular una solución de cloruro de sodio a través de un filtro profundo que contiene precipitado. Los filtros fueron entonces enjuagados con una solución de cloruro de sodio adicional.

Se agregó yoduro de sodio (NaI) a la solución de polisacárido proveniente de una solución de NaI madre para lograr una concentración final de 0.5% para precipitar HB (excepto para el Serotipo 6B, que tuvo una concentración final de 0.25%). El precipitado se removió mediante filtración profunda. El filtrado contenía el polisacárido objetivo. El filtro se descartó. El polisacárido fue luego filtrado a través de un filtro de 0.2 µm.

La solución de polisacárido se concentró sobre un ultrafiltro MWCO de 30 kDa y se diafiltró con una solución de cloruro de sodio.

La solución de polisacárido parcialmente purificada se purificó adicionalmente mediante filtración a través de un filtro profundo impregnado con carbono activado. Después de la filtración, el filtro de carbono se enjuagó con una solución de cloruro de sodio. El enjuague se combinó con la solución de polisacárido, la cual fue entonces filtrada a través de un filtro de 0.2 µm.

La solución de polisacárido se concentró sobre un ultrafiltro MWCO de 30 kDa y el filtro se enjuagó con una solución de cloruro de sodio. El pH se revisó y se ajusto a 7.0 ± 0.3.

La columna de hidroxiapatita cerámica (HA) se equilibró con amortiguador de fosfato de sodio que contiene cloruro de sodio hasta que el pH es 7.0 ± 0.3 y la conductividad era 26 ± 4µS. La solución de polisacárido fue entonces cargada sobre la columna. Bajo estas condiciones, las impurezas unidas a la resina y al polisacárido se recuperaron en el flujo pasante desde la columna. La solución de polisacárido se filtró a través de un filtro 0.2 µm.

La solución de polisacárido se concentró utilizando un filtro MWCO de 30 kDa. El concentrado fue entonces diafiltrado con WFI hasta que la conductividad era < 15µS.

La solución de polisacárido diafiltrada fue filtrada a través de un filtro de membrana de 0.2µm en recipientes en masa y almacenados a 2-8°C.

Ejemplo 14

Preparación de Conjugados de Sacárido Neumocócicos – CRM_{197} para los Serotipos 4, 6B, 9V, 14, 18C, 19F y 23F

Proceso de Activación

Los sacáridos de serotipo diferente luego de diferentes sendas para activación (hidrólisis o no hidrólisis antes de activación) y conjugación (reacciones acuosas o DMSO) como se describió en este ejemplo.

El polisacárido se transfirió desde los recipientes de masa al recipiente de reactor. El polisacárido fue entonces diluido en WFI y fosfato de sodio a un rango de concentración final de 1.6 – 2.4 mg/mL.

Etapa 1

Para los serotipos 6B, 9V, 14, 19F y 23F, el pH se ajustó a pH 6.0 ± 0.3.

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Para el serotipo 4, se agregó ácido clorhídrico (concentración de ácido final 0.01 M) y la solución se incubó durante 25 - 35 minutos a 45° ± 2°C. La hidrólisis se detuvo y se enfrió a 21 - 25°C y se agregó fosfato de sodio 1M a un objetivo de pH 6.7 ± 0.2. Un ensayo en proceso fue hecho para confirmar el nivel apropiado de la despiruvilación.

Para el serotipo 18C, se agregó ácido acético glacial (concentración de ácido final 0.2M) y la solución se incubó durante 205 - 215 minutos a 94° ± 2°C. La temperatura fue entonces disminuyendo a 21 - 25°C y se agregó 1 - 2M de fosfato de sodio a un objetivo de pH 6.8 ± 0.2.

Etapa 2: Reacción de Periodato

Los equivalentes molares de periodato de sodio requeridos para la activación de sacárido neumocócico se determinaron utilizando el contenido de sacárido total (excepto para el serotipo 4). Para el serotipo 4, se utilizó una proporción de 0.8-1.2 moles de periodato de sodio por mol de sacárido. Con una mezcla completa, a la reacción de oxidación se le permitió proceder entre 16 a 20 a 21 - 25°C para todos los serotipos excepto 19F para el cual la temperatura fue ≤ 15°C.

Etapa 3: Ultrafiltración

El sacárido oxidado se concentró y se diafiltró con WFI (0.01 M amortiguador de fosfato de sodio pH 6.0 para el serotipo 19F) sobre un ultrafiltro MWCO de 100 kDa (ultrafiltro 5 kDa para 18C). El permeado se descartó y el retenido se filtró a través de un filtro de 0.22 µm.

Etapa 4: Liofilización

Para los serotipos 4, 9V, y 14 el sacárido concentrado se mezcló con una proteína portadora CRM_{197}, se llenó en botellas de vidrio, se congeló en cubierta y se almacenó a ≤ -65°C. El sacárido concentrado congelado - CRM_{197} se liofilizó y luego se almacenó a -25° ± 5°C.

Para los serotipos 6B, 19F, y 23F una cantidad específica de sucrosa se agregó la cual se calculó para lograr una concentración de sucrosa de 5% ± 3% en la mezcla de reacción de conjugación. El Serotipo 18C no requiere adición de sucrosa. El sacárido concentrado fue entonces llenado en botellas de vidrio, congelado en cubierta y almacenado a ≤ -65°C. El sacárido concentrado congelado se liofilizó y luego se almacenó a -25° ± 5°C.

Proceso de Conjugación

Se utilizaron dos procesos de conjugación: conjugación acuosa para los serotipos 4, 9V, 14 y 18C, y la conjugación DMSO para los serotipos 6B, 19F y 23F.

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Conjugación Acuosa

Etapa 1: Disolución

Para los serotipos 4, 9V y 14, la mezcla de sacárido activado liofilizado - CRM_{197} se descongeló y se equilibró a temperatura ambiente. El sacárido activado liofilizado - CRM_{197} fue entonces reconstituido en un amortiguador de fosfato de sodio 0.1M a una proporción típica de:

- 1L de amortiguador por 16 - 24 g de sacárido para el serotipo 4 y 9V
- 1L de amortiguador por 6 - 10 de sacárido para el serotipo 14

La mezcla de reacción se incubó a 37° ± 2°C hasta una disolución total para el serotipo 9V y a 23° ± 2°C para los serotipos 4 y 14.

Para el serotipo 18C, el sacárido liofilizado se reconstituyó en una solución de CRM_{197} en fosfato de sodio dibásico 1M a una proporción típica de 0.11 L de fosfato de sodio por 1 L de solución CRM_{197}. La mezcla de reacción (8-12 g/L concentración de sacárido) se incubó a 23° ± 2°C hasta disolución total.

El pH se probó como un control en proceso en esta etapa.

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Etapa 2: Reacción de Conjugación

Para los serotipos 4 y 9V, la reacción de conjugación se inició al agregar la solución de cianoborohidruro de sodio (100 mg/mL) para lograr 1.0 - 1.4 moles de cianoborohidruro de sodio por mol de sacárido. La mezcla de reacción se incubó durante 44 - 52 horas a 37° ± 2°C. La temperatura se redujo entonces a 23° ± 2°C y se agregó cloruro de sodio 0.9% al reactor. La solución de borohidruro de sodio (100 mg/mL) se agregó para lograr 1.8 - 2.2 equivalentes molares de borohidruro de sodio por mol de sacárido. La mezcla se incubó durante 3 - 6 horas a 23° ± 2°C. La mezcla se diluyó con cloruro de sodio 0.9% y el reactor se enjuagó. La mezcla de conjugación diluida se filtró utilizando un pre-filtro de 1.2 µm en un recipiente de soporte.

Para los serotipos 14 y 18C, la reacción de conjugación se inició al agregar la solución de cianoborohidruro (100 mg/mL) para lograr 1.0 - 1.4 moles de cianoborohidruro de sodio por mol de sacárido. La mezcla de reacción se incubó durante 12 - 24 horas a 23° ± 2°C. La temperatura se incrementó a 37° ± 2°C y la reacción se incubó durante 72 - 96 horas. La temperatura se redujo entonces a 23° ± 2°C y se agregó cloruro de sodio 0.9% al reactor. Se agregó una solución de borohidruro de sodio (100mg/mL) para lograr 1.8 - 2.2 de equivalentes molares de borohidruro de sodio por mol de sacárido. La mezcla se incubó durante 3 - 6 horas a 23° ± 2°C. La mezcla se diluyó con 0.9% de cloruro de sodio y el

reactor se enjuagó. La mezcla de conjugación diluida fue entonces filtrada utilizando 1.2 µm de pre-fitro en un recipiente de soporte.

Etapa 3: Ultrafiltración 100 kDa

La mezcla de conjugación diluida se concentró y se diafiltró sobre un ultrafiltro MWCO de 100 kDa con un mínimo de 15 volúmenes (serotipo 4) o 40 volúmenes (serotipos 9V, 14, y 18C) de cloruro de sodio 0.9%.

El perneado se descartó.

Para el serotipo 4, el retenido se filtró a través de 0.45 µm de filtro.

Un control en proceso (contenido de sacárido) se desarrolló en esta etapa.

Etapa 4: Purificación de Columna HA

Esta etapa fue solamente desarrollada para el conjugado serotipo 4.

La columna HA fue neutralizada primero utilizando amortiguador de fosfato de sodio 0.5M (pH 7.0 ± 0.3) y luego equilibrada con cloruro de sodio 0.9%. El retenido filtrado (serotipo 4) se cargó sobre la columna a una proporción de flujo de 1.0 L/min. La columna se lavó con 0.9% de cloruro de sodio a una tasa de flujo de ≤ 2.0 L/min. El producto fue

luego eluído con amortiguador de fosfato de sodio 0.5M a una tasa de flujo de ≤ 2.0 L/min.

La fracción de HA fue entonces concentrada y diafiltrada sobre una membrana MWCO de 100 kDa con un mínimo de 20 volúmenes de cloruro de sodio 0.9%. El permeado se descartó.

Etapa 5: Filtración de Estéril

El retenido después de diafiltración MWCO de 100 kDa se filtró a través de un filtro de 0.22 µm. Los controles en procesos (contenido de sacárido, proteína libre, sacárido libre y cianuro) se desarrollaron sobre el producto filtrado. Los controles en proceso sobre el retenido filtrado se desarrollaron para determinar si la concentración adicional, diafiltración, y/o dilución eran necesarias para cumplir los objetivos FBC. Estas y pruebas adicionales se repitieron en muestras FBC.

Según fuera necesario, el conjugado filtrado se diluyó con cloruro de sodio 0.9% con el fin de lograr una concentración final de menos de 0.55 g/L. Las pruebas de liberación para el contenido de sacárido, contenido de proteína y proporción de sacárido:proteína se desarrolló en esta etapa.

Finalmente, el conjugado se filtró (0.22 µm) y se llenó en tarros de acero inoxidable de 10 L a una cantidad típica

de 2.64 g/tarro. En esta etapa, el rendimiento, contenido de sacárido, contenido de proteína, pH, proporción sacárido:proteína y contenido de lisina se desarrollaron como controles en proceso. La prueba de liberación (apariencia, proteína libre, sacárido libre, endotoxina, determinación de tamaño molecular, cianuro residual, identidad de sacárido, identidad CRM_{197}) se desarrolló en esta etapa.

Conjugación DMSO

Etapa I: Disolución

Los serotipos de sacárido activado liofilizado 6B, 19F, 23F y la proteína portadora CRM_{197} liofilizada se equilibraron a temperatura ambiente y se reconstituyeron en DMSO. La concentración de disolución típicamente varió desde 2-3 gramos de sacárido (2-2.5 g de proteína) por litro de DMSO.

Etapa II: Reacción de Conjugación

El sacárido activado y la proteína portadora CRM_{197} se mezclaron durante 60 - 75 minutos a 23° ± 2°C a un rango de proporción de 0.6 g - 1.0 g de sacárido/g CRM_{197} para los serotipos 6B y 19F o 1.2 a 1.8 g sacárido/g CRM_{197} para el serotipo 23F.

La reacción de conjugación se inició al agregar la solución de cianoborohidruro de sodio (100mg/mL) a una

proporción de 0.8 - 1.2 equivalentes molares de cianoborohidruro de sodio a un mol de sacárido activado. El WFI se agregó a la mezcla de reacción a un objetivo de 1% (v/v) y la mezcla se incubó durante 40 horas a 23° ± 2°C.

La solución de borohidruro de sodio, 100 mg/mL (típica 1.8 - 2.2 equivalentes molares de borohidruro de sodio por mol de sacárido activado) y WFI (objetivo 5% v/v) se agregaron a la reacción y la mezcla se incubó durante 3 - 6 horas a 23° ± 2°C. Este procedimiento redujo cualquiera de los aldehídos no reaccionados presentes sobre los sacáridos. Luego la mezcla de reacción se transfirió a un tanque de disolución que contiene 0.9% de cloruro de sodio a < 15°C.

Etapa III: Ultrafiltración 100 kDa

La mezcla de conjugado diluida se filtró a través de un filtro de 1.2 µm y se concentró y diafiltró en una membrana MWCO de 100 kDa con un mínimo de 15 volúmenes de cloruro de sodio 0.9% (fosfato de sodio 0.01M/amortiguador de NaCl 0.05M se utilizó para el serotipo 23F). El permeado se descartó. El retenido se filtró a través de un filtro de 0.45 µm. Una muestra de contenido de sacárido en proceso se tomó en esta etapa.

Etapa IV: Purificación de Columna DEAE

Esta etapa se desarrolló solamente para el serotipo 23F.

La columna DEAE se equilibró con fosfato de sodio 0.01M/amortiguador de cloruro de sodio 0.05M. El retenido filtrado (serotipo 23F) se cargó sobre la columna y se lavó con fosfato de sodio 0.01M/amortiguador de cloruro de sodio 0.05M. La columna fue luego lavada con fosfato de sodio 0.01M/amortiguador de NaCl 0.9%. El producto fue entonces eluido con fosfato de sodio 0.01M/amortiguador de cloruro de sodio 0.5M.

Etapa V: Ultrafiltración 100 kDa

El retenido de 6B y 19F se concentró y se diafiltró con por lo menos 30 volúmenes de cloruro de sodio 0.9%. El permeado se descartó.

El eluído del serotipo 23F se concentró y se diafiltró con un mínimo de 20 volúmenes de cloruro de sodio 0.9%. El permeado se descartó.

Etapa VI: Filtración Estéril

El retenido después de diafiltración MWCO de 100 kDa se filtró a través de un filtro de 0.22 µm. Los controles en proceso (contenido de sacárido, proteína libre, sacárido libre, DMSO residual y cianuro residual) se desarrollaron sobre el producto filtrado. Los controles en proceso sobre el retenido filtrado se desarrollaron para determinar si la

concentración adicional, diafiltración, y/o dilución eran necesarias para cumplir con los objetivos FBC. Estas y pruebas adicionales se repitieron en muestras FBC.

Según fuera necesario, el conjugado filtrado se diluyó con cloruro de sodio 0.9% para lograr una concentración final de menos de 0.55 g/L. Las pruebas de liberación para el contenido de sacárido, contenido de proteína y proporción de sacárido:proteína se desarrollaron en esta etapa.

Finalmente, el conjugado se filtró (0.22 µm) y se llenó en tarros de acero inoxidable de 10 L a una cantidad de 2.64 g/tarro. En esta etapa, el rendimiento, contenido de sacárido, contenido de proteína, pH, proporción sacárido:proteína y contenido de lisina se desarrollaron como controles en proceso. La prueba de liberación (apariencia, proteína libre, sacárido libre, endotoxina, determinación de tamaño molecular, cianuro residual, DMSO residual, identidad de sacárido e identidad CRM_{197}) se desarrolló en esta etapa.

Ejemplo 15

Formulación de una Vacuna de Conjugado Neumocócica Multivalente

Los concentrados de masa finales de los 13 conjugados contienen 0.85% de cloruro de sodio. Los concentrados en masa del tipo 3, 6A, 7F y 19A también contienen amortiguador de succinato de sodio 5 mM a pH de 5.8. Los volúmnes requeridos

de los concentrados en masa se calcularon con base en el volumen de tanda y las concentraciones de sacárido en masa. Después del 80% del cloruro de sodio 0.85% (solución salina fisiológica) y la cantidad requerida de amortiguador de succinato se agregaron al recipiente de formulación pre-marcado, se agregaron concentrados de masa. La preparación fue entonces filtrada estéril a través de una membrana de 0.22 µm en un segundo recipiente al utilizar una unidad de filtro de membrana Millipore Durapore. El primer recipiente se lavó con el restante 20% del cloruro de sodio 0.85% y la solución se pasó a través del mismo filtro y se recolectó en el segundo recipiente. La masa formulada se mezcló suavemente durante y luego de la adición del fosfato de aluminio en masa. El pH se revisó y se ajustó si era necesario. El producto de masa formulado se almacenó a 2-8°C.

El producto de masa formulado se llenó en jeringas de vidrio de borosilicato Tipo 1 obtenidas de Becton Dickinson. La vacuna se monitoreó a intervalos regulares para turbidez para asegurar la uniformidad de la operación de llenado. La vacuna llenada (Producto Final) se almacenó a 2-8°C.

Ejemplo 16

Inmunogenicidad de la Vacuna de Conjugado 13-Valente

A la fecha, los estudios preclínicos desarrollados sobre una vacuna 13vPnC han sido en conejos. Los estudios #HT01-0021 y #HT01-0036 se diseñaron para examinar

independientemente el efecto de la conjugación química de los polisacáridos capsulares (PS) de *S. pneumoniae* a CRM_{197} y el efecto del adyuvante de fosfato de aluminio ($AlPO_4$) sobre la respuesta inmune a la vacuna 13vPnC en conejos. Estos efectos se caracterizaron por el ELISA específico de antígeno para las concentraciones IgG de suero y para la función de anticuerpo por ensayo opsonofagocítico (OPA).

Estudio #HT01-0021

El estudio #HT01-0021 examinó la capacidad de la vacuna 13vPnC con el adyuvante $AlPO_4$ para elicitar las respuestas inmunes específicas del serotipo de la vacuna. Los serotipos neumocócicos representados en la vacuna 13vPnC incluyen los tipos 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F y 23F. Los objetivos secundarios incluyeron una evaluación de las cinéticas y la duración de la respuesta del anticuerpo. Conejos Blancos de Nueva Zelanda se inmunizaron intramuscularmente en la semana 0 y en la semana 2 con la dosis clínica humana planeada de cada polisacárido (2 µg de cada PS, excepto 4 µg de 6B) formulada con o sin $AlPO_4$ (100 µg/dosis). Se recolectó suero en varios puntos de tiempo. El IgG específico de serotipo se midió mediante ELISA y se evaluó la actividad funcional mediante OPA.

La Tabla 3 muestra el título medio geométrico (GMT) logrado en muestras de suero agrupadas, luego de dos dosis de la vacuna 13vPnC. Una proporción del IgG GMT se utilizó para

comparar las respuestas durante la semana 4 a la semana 0. Estos datos demuestran que la inclusión del $AlPO_4$ en la formulación 13vPnC elicitó mayores niveles del anticuerpo IgG en comparación con la misma vacuna sin adyuvante. Aunque las respuestas de anticuerpo fueron mayores cuando $AlPO_4$ se incluyó en la formulación, estos incrementos no fueron estadísticamente significativos.

Las respuestas de anticuerpo funcional también se evaluaron en conejo luego de la inmunización con las dos formulaciones 13vPnC (Tabla 4). Cuando se compara las formulaciones de vacuna con o sin adyuvante, los GMT de OPA mayores se observaron en el grupo de tratamiento de vacuna 13vPnC + $AlPO_4$. Los títulos OPA se detectaron en grupos de suero en la semana 4 a todos los serotipos de vacuna en ambos grupos. Para la mayoría de los serotipos, los títulos de OPA medidos en la semana 4 fueron por lo menos 4 veces mayores que aquellos en la semana cero (línea base).

Las respuestas cinéticas de cada uno de los serotipos de vacuna 13vPnC se evaluaron de los grupos de suero de ambos grupos de tratamiento. Los títulos IgG de cada serotipo se midieron de muestras de sangre en la semana 0 y en las semanas 1, 2, 3, 4, 8, 12, 26, y 39 y luego comparadas. Con la excepción del serotipo 1, las respuestas de anticuerpo en animales que recibieron vacuna adyuvada fueron superiores a aquellas que recibieron vacuna no adyuvada y el pico en la semana 2 del programa de inmunización (datos no mostrados).

En total, los datos indicaron que la vacuna 13vPnC formulada con fosfato de aluminio es inmunogénica en conejos, elicitando respuestas de anticuerpo sustanciales a los polisacáridos capsulares neumocócicos contenidos en la vacuna y estas respuestas están asociadas con actividad funcional. Las respuestas observadas a los siete serotipos núcleo luego de inmunización con 13vPnC + $AlPO_4$ son consistentes con las respuestas históricas de los conejos a la formulación heptavalente.

Tabla 3. Respuestas Inmunes IgG en Conejo (GMT) Luego de Inmunización con Dos Dosis de Glicoconjugado Neumocócico 13-Valente

	Diluyente con $ALPO_4$[a]			13vPnC[a]			13vPnC + $ALPO_4$[a]	
Serotipo	Semana 0	Semana 4	Proporción Wk4:Wk0	Semana 0	Semana 4 (95%Cl)	Proporción Wk4:Wk0	Semana 0	Semana 4 (95% Cl)	Proporción Wk4:Wk0
1	< 100	< 100	1.0	50	5,926 (2,758-12,733)	119	50	11,091 (5,327-23,093)	222
3	< 100	< 100	1.0	50	6,647 (2,773-15,932)	133	58	16,443 (7,096-38,106)	284
4	< 100	< 100	1.0	50	13,554 (8,031-22,875)	271	50	29,183 (15,342-55,508)	584
5	134	< 100	0.4	50	5,859 (2,450-14,009)	117	50	16,714 (6,959-40,140)	334
6A	141	< 100	0.4	74	22,415 (11,987-41,914)	303	83	63,734 (21,141-192,146)	768
6B	< 100	< 100	1.0	57	8,108 (3,564-18,444)	142	54	23,505 (11,286-48,955)	435
7F	3,859	579	0.2	171	43,591 (26,931-70,557)	444	143	84,888 (46,445-155,151)	496
9V	289	995	3.4	205	15,780 (7,193-34,616)	125	208	43,331[b] (23,256-71,510)	217
14	437	177	0.4	61	6,906 (3,416-13,962)	113	70	16,076 (9,649-26,785)	322
18C	< 100	< 100	1.0	50	21,283 (15,770-28,725)	426	50	35,040 (24,708-49,692)	701
19A	< 100	< 100	1.0	121	113,599 (54,518-236,707)	939	144	280,976 (119,587-660,167)	1,951
19F	< 100	< 100	1.0	50	14,365 (7,346-28,090)	287	50	24,912 (9,243-67,141)	498
23F	< 100	< 100	1.0	50	5,323 (1,894-14,962)	106	50	15,041 (4,711-48,018)	301

a: Los GMT de suero agrupados consistieron de volúmenes iguales de suero de cada conejo individual con un grupo
b: Estadísticamente diferente (p=0.022) del grupo de tratamiento sin $ALPO_4$

Tabla 4. GMT OPA de *S. pneumoniae* para Grupos de Suero de Conejo NZW luego de Inmunización con Dos Dosis de Glicoconjugado Neumocócico 13-Valente

	13vPnC[a]			13vPnC + $ALPO_4$[a]		
Serotipo	Semana 0	Semana 4	Proporción Wk4:Wk0	Semana 0	Semana 4	Proporción Wk4:Wk0
1	<8	64	16	<8	64	16
3	<8	8	2	<8	16	4
4	<8	16	4	<8	32	8
5	<8	128	32	<8	512	128
6A	8	128	16	8	512	64
6B	<8	256	64	8	1,024	128
7F	8	64	8	8	128	16
169V	8	64	8	8	128	16
14	16	32	2	16	32	2
18C	8	256	32	<8	256	64
19A	<8	256	64	<8	1,024	256
19F	<8	128	32	<8	512	128
23F	8	64	8	<8	256	64

a: Los grupos consistieron de volúmenes iguales de suero provenientes de conejos individuales dentro de un grupo de tratamiento (n=12)

Estudio #HT01-0036

El estudio #HT01-0036 comparó las respuestas inmunes de conejo con los polisacáridos (PS) contenidos en la vacuna, después de inmunización con la vacuna 13vPnC con o sin conjugación a la proteína CRM_{197}. Los conejos Blancos de Nueva Zelanda se inmunizaron intramuscularmente en la semana 0 y la semana 2 con una dosis de 2.2 µg de cada PS (excepto 4.4 µg de 6B). Los animales recibieron una de tres preparaciones de vacuna: (a) 13vPnC (PS directamente conjugado a CRM_{197}), (b) 13vPnC, (PS libre) o (c) 13vPnC + CRM_{197} (PS libre mezclado con CRM_{197}). Todas las preparaciones de vacuna contenían $AlPO_4$ como el adyuvante en 125 µg/dosis.

Las respuestas inmunes específicas de serotipo para todas las preparaciones de vacuna se evaluaron en un ELISA IgG y un anticuerpo funcional que mide el OPA mediado por complemento. Las respuestas inmunes se compararon entre los grupos de tratamiento.

La Tabla 5 presenta los datos GMT obtenidos desde los sangrados en semana 4 analizados en los ELISA de IgG específico de antígeno. Análisis adicionales muestran la proporción de los valores GMT en la semana 4 a la semana 0. Los datos indican que la preparación de vacuna de conjugado elicitó mayores títulos IgG de suero que el PS libre o la vacuna PS + CRM_{197} libre. Con la excepción del *S. pneumoniae* tipo 14, la vacuna 13vPnC fue capaz de inducir los anticuerpos funcionales para las cepas representativas de *S. pneumoniae* en un OPA (Tabla 6). Después de dos inmunizaciones con cualquiera de las vacunas 13vPnPS o 13vPnPS + CRM_{197}, ninguno podría inducir títulos OPA ≥ 8 veces en la semana 4 con relación a la semana 0 para 10 fuera de los 13 serotipos medidos (Tabla 6).

En conclusión, estos resultados indican que la conjugación de los polisacáridos de vacuna neumocócica 13-valente produce títulos IgG de suero mayor y una activiad de anticuerpo funcional mayor total que la vista con el polisacárido libre solo o mezclado con el CRM_{197} no conjugado.

Tabla 5. Respuestas IgG de Conejo (GMT) al PnPs por ELISA Luego de Inmunización con Dos Dosis de Glicoconjugado Neumocócico 13-Valente

	3vPnPS (PS libre)			13vPnPS = CRM_{197} (PS mezclado con CRM_{197})			13vPnC		
Serotipo	Semana 0	Semana 4 (95%CI)	Proporción Wk4:Wk0	Semana 0	Semana 4 (95%CI)	Proporción Wk4:Wk0	Semana 0	Semana 4 (95%CI)	Proporción Wk4:Wk0
1	378	2,290 (843-5,790)	5.8	395	1,959 (809-4,739)	5.0	472	35,970 (29,130-44,417)	76.2
3	57	240 (64-908)	4.2	89	163 (74-358)	1.8	50	10,414 (10,414-16,676)	208.3
4	50	379 (150-959)	7.6	50	607 (313-1,178)	12.1	50	12,890 (9,117-18,224)	257.8
5	343	226 (113-450)	4.5	50	321 (147-701)	6.4	50	35,264 (24,467-50,824)	705.3
6A	154	466 (316-688)	3.0	98	210 (95-464)	2.1	163	234,245 (167,152-328,283)	1,437.1
6B	63	727 (384-1,375)	11.6	62	745 (384-1,440)	12.0	131	33,599 (22,934-49,222)	256.5
7F	50	61 (39-95)	1.2	50	72 (47-111)	1.4	50	35,702 (24,350-52,347)	714.0
9V	50	104 (48-195)	2.1	55	169 (74-390)	3.0	50	50,033[b] (34,765-72,007)	1,000.7
14	66	298 (117-757)	4.5	50	195 (71-535)	3.9	50	20,121 (12,087-32,138)	402.4
18C	89	1,555 (655-3,688)	17.5	66	761 (300-1,935)	11.5	101	71,451 (32,745-124,641)	707.4
19A	50	89 (44-179)	1.8	50	80 (39-163)	1.6	50	23,485 (12,857-42,723)	469.7
19F	61	1,362 (559-3,317)	22.3	61	991 (370-2,654)	16.3	67	19,358 (12,553-33,173)	288.9
23F	73	1,085 (487-2,420)	14.9	121	638 (311-1,311)	5.3	68	45,972 (25,134-84,089)	676.1

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Tabla 6. OPA de *S. pneumoniae* para Grupos de Suero de Conejo luego de Inmunización con Dos Dosis de Vacunas Neumocócicas 13-Valentes

	Títulos OPA						
	Sin tratamiento	13vPnPS (PS libre)		13vPnPS + CRM_{197} (PS libre mezclado con CRM_{197}		13vPnC	
Serotipo	Semana 0[a]	Semana 4	Proporción Wk4:Wk0	Semana 4	Proporción Wk4:Wk0	Semana 4	Proporción Wk4:Wk0
1	4	16	4	16	4	8	32
3	4	4	1	4	1	4	8
4	4	4	1	4	1	4	64
5	4	32	8	16	4	16	64
6A	8	64	8	32	4	32	664
6B	8	64	8	32	4	32	32
7F	16	32	2	16	1	16	16
9V	16	16	1	32	2	32	8
14	16	16	1	16	1	16	2
18C	4	16	4	16	4	8	64
19A	8	8	1	8	1	16	64
19F	4	4	1	4	1	8	64
23F	16	32	2	16	1	32	32

a: Utilizado como valores de semana 0 para todos los grupos

Se debe entender que la discusión anterior y los ejemplos simplemente presentan una descripción detallada de ciertas modalidades. Por lo tanto debe ser evidente para aquellos expertos en la técnica que varias modificaciones equivalentes se pueden hacer sin apartarse del espíritu y alcance de la invención.

Todos los artículos de revista, otras referencias, patentes y solicitudes de patente que se identifiquen en esta solicitud de patente se incorporan como referencia en su totalidad.

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REIVINDICACIONES

1. Una composición inmunogénica multivalente que comprende 13 diferentes de conjugados de polisacárido-proteína junto con un vehículo fisiológicamente aceptable, en donde cada uno de los conjugados comprende un polisacárido capsular de un serotipo diferente de *Steptococcus pneumoniae* conjugado a una proteína portadora y los polisacáridos capsulares se preparan de los serotipos 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F y 23F, en donde la proteína portadora es CRM_{197} y donde la conjugación se afecta por aminación reductiva.

2. La composición inmunogénica de la reivindicación 1, comprende, además un adyuvante.

3. La composición inmunogénica de la reivindicación 2, en donde el adyuvante es fosfato de aluminio.

4. La composición inmunogénica multivalente que comprende 13 diferentes conjugados de polisacárido-proteína, junto con un vehículo fisiológicamente aceptable, en donde cada uno de los conjugados comprende un polisacárido capsular de un serotipo diferente de *Steptococcus pneumoniae* conjugado a una proteína portadora y los polisacáridos capsulares se preparan de los serotipos 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F y 23F, en donde la proteína portadora es CRM_{197} y en donde cada dosis de 0,5 mL se formula para contener: 2,2 µg de cada sacárido, excepto para 6B a 4,4 µg; aproximadamente 29 µg de la proteína portadora CRM_{197}; 0,125 mg de adyuvante de aluminio elemental (0,5 mg de fosfato de aluminio), y cloruro de sodio y amortiguador de succinato de sodio como excipientes.

5. Una jeringa de dosis única llenada con una formulación líquida esteril de polisacáridos capsulares neumocócicos de los serotipos 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F y 23F, individualmente conjugada a CRM_{197}, en donde cada dosis de 0,5 mL se formula para contener: 2,2 µg de cada sacárido, excepto para 6B a 4,4 µg; aproximadamente 29 µg de la proteína portadora CRM_{197}; 0,125 mg de adyuvante de aluminio elemental (0,5 mg de fosfato de aluminio), y cloruro de sodio y amortiguador de succinato de sodio como excipientes.

6. Una jeringa de dosis única llenada con una formulación líquida esteril de polisacáridos capsulares neumocócicos de los serotipos 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F y 23F, individualmente conjugada a CRM_{197}, en donde cada dosis de 0,5 mL se formula para contener: 2 µg de cada sacárido, excepto para 6B a 4 µg; aproximadamente 29 µg de la proteína portadora CRM_{197}; 0,125 mg de adyuvante de aluminio elemental (0,5 mg de fosfato de aluminio), y cloruro de sodio y amortiguador de succinato de sodio como excipientes.

7. La jeringa de dosis única de la reivindicación 5 u 6 para administración intramuscular.

8. Un proceso para preparar una composición inmunogénica que comprende 13 diferentes conjugados de polisacárido-proteína junto con un vehículo fisiológicamente aceptable, en donde cada uno de los conjugados contiene un polisacárido capsular diferente conjugado a la proteína portadora CRM_{197}, en donde:

los polisacáridos capsulares se preparan de los serotipos 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F y 23F de *Steptococcus pneumoniae;*

los polisacáridos individuales se purifican a través de centrifugación, precipitación, ultrafiltración y cromatografía de columna;

los polisacáridos purificados se activan químicamente para hacer los sacáridos capaces de reaccionar con la proteína portadora CRM_{197};

una vez activado, cada polisacárido capsular se conjuga separadamente a la proteína portadora CRM_{197} para formar un glicoconjugado;

los glicoconjugados individuales se purifican y son compuestos para formular la composición inmunogénica.

9. El proceso de la reivindicación 8 en donde la conjugación se afecta por aminación reductiva.

10. Un proceso para preparar una composición inmunogénica multivalente que comprende 13 diferentes conjugados de polisacárido-proteína junto con un vehículo fisiológicamente aceptable, en donde cada uno de los conjugados comprende un polisacárido capsular de un serotipo diferente de *Steptococcus pneumoniae* conjugado a una proteína portadora y los polisacáridos capsulares se preparan de los serotipos 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F y 23F, en donde la proteína portadora es CRM_{197}, en donde después de que son purificados los glicoconjugados individuales ellos son compuestos para formular la composición inmunogénica.