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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 05-088322- -00003-0000

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Tr. 1 PATENTE/EXE Eje 1578 FASE NACIONAL  
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Señor

SUPERINTENDENTE DE INDUSTRIA

DIVISION DE NUEVAS CREACIONES

E. \_\_\_\_\_ S. \_\_\_\_\_ D. \_\_\_\_\_

Re: P1.-WYETH HOLDINGS CORPORATION Y INHIBITEX,  
INC.-Solicitud de Patente de Invención en Fase Nacional PCT  
para "CONJUGADOS DE POLISACARIDO-PROTEINA  
PORTADORA ADHESINA SUPERFICIAL DE  
ESTAFILOCOCO PARA INMUNIZACION CONTRA  
INFECCIONES NOSOCOMIALES".-Clase A61K 47/48.-  
Expediente número 05-088.322.-

1. Me refiero a mi memorial de 23 de Diciembre de 2005.
2. De conformidad con lo dispuesto en el Artículo 5.2.12 de la Circular Externa No. 03 de 28 de Abril de 2003, y estando dentro del plazo de seis meses contados desde la fecha de la publicación de la solicitud en la Gaceta de la Propiedad Industrial número **574** de 30 de Marzo de 2007, solicito a ese Despacho proceder a realizar el examen de patentabilidad de la presente invención.
3. Por otro lado, atentamente me permito manifestar a usted que en el extracto de la solicitud arriba nombrada, publicado en la Gaceta de la Propiedad Industrial número **574** de 30 de Marzo de 2007, página 527, el título de la invención aparece publicado en forma equivocada, el título correcto es como sigue a continuación:

**"CONJUGADOS DE POLISACARIDO-PROTEINA PORTADORA ADHESINA**  
**SUPERFICIAL DE ESTAFILOCOCO PARA INMUNIZACION CONTRA**  
**INFECCIONES NOSOCOMIALES"**

4. En vista de lo anterior, muy comedidamente solicito a usted que las aclaraciones correspondientes sean incluidas en la Fe de Erratas de la próxima Gaceta de la Propiedad Industrial.
5. Me permito recordar a usted, que tal como se informó en el Petitorio de la invención, la presente solicitud corresponde a Capítulo I.
6. Anexo me permito presentar:
- (a) Recibo número 07-72.197, expedido por la Superintendencia de Industria y Comercio, que acredita el pago de la tasa que dicho examen causa; y
  - (b) Copia del extracto de la solicitud, tal como fue presentado a ese Despacho con mi solicitud de patente de 2 de Septiembre de 2005.
7. Ruego a usted tomar atenta nota de lo anterior y proceder de conformidad.

Señor Superintendente,

  
ALICIA LLORED A RICAURTE  
T.P. 53.215

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. \_\_\_\_\_

221 220



**Industria y Comercio**  
SUPERINTENDENCIA

**EXTRACTO PARA PUBLICACIÓN**  
**SOLICITUD DE PATENTE DE INVENCION**

No. SOLICITUD INTERNACIONAL : **PCT/US2004/006661** (51) INT. CL.: **A61K 47/48**

(21) No. SOLICITUD:

(22) FECHA DE PRESENTACIÓN: **02-09-05**

(71) SOLICITANTE: **WYETH HOLDINGS CORPORATION y INHIBITEX, INC.**

(30) PRIORIDAD:

(31) No. DE SOLICITUD: **60/452,728**

(72) INVENTOR/ES: **Pavliak Viliam; Baker Steven Morris; Pillai Subramonia Padmanaba; Joseph M. Patti y Bradley D. Prater.**

(32) FECHA DE PRESENTACIÓN: **07-03-03**

(33) PAÍS: **US**

(74) REPRESENTANTE LEGAL: **ALICIA LLOREDA RICAURTE**

(54) TÍTULO:

**"CONJUGADOS DE POLISACARIDO-PROTEINA PORTADORA ADHESINA SUPERFICIAL DE ESTAFILOCOCO PARA INMUNIZACION CONTRA INFECCIONES NOSOCOMIALES"**

(57) RESUMEN:

**Reivindicación número 1.-**

Un conjugado inmunogénico polisacárido-proteína, caracterizado porque comprende: al menos un antígeno polisacárido derivado de un patógeno nosocomial y al menos una proteína portadora adhesina superficial de estafilococo, en donde el conjugado genera anticuerpos específicos para el antígeno polisacárido y la proteína portadora adhesina superficial de estafilococo.

**Reivindicación número 17.-**

Una composición inmunogénica, caracterizada porque comprende el conjugado de conformidad con cualquiera de las reivindicaciones 1 a 16, en un portador o diluyente inmunológicamente aceptable.

**DIVISIÓN DE NUEVAS CREACIONES**

RADICACIÓN EXPEDIENTE No. 05-88322

EL EXTRACTO DE LA PRESENTE SOLICITUD DE **PATENTE DE INVENCION**

FUE PUBLICADO EN LA GACETA DE PROPIEDAD INDUSTRIAL No **574** DE

FECHA **30 DE MARZO DE 2007** EL TÉRMINO HÁBIL SEÑALADO POR LA LEY

EXPIRA EL **03 DE JULIO DE 2007**

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

SE PRESENTARON OPOSICIONES POR PARTE DE TERCEROS.

SI \_\_\_\_\_

NO       X

## Immunogenicity of Alpha-Toxin, Capsular Polysaccharide (CPS) and Recombinant Fibronectin-Binding Protein (r-FnBP) of *Staphylococcus aureus* in Rabbit

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**ABSTRACT.** This study was conducted to evaluate the antibody levels of alpha-toxin, capsular polysaccharides (CPS) and fibronectin-binding protein (FnBP) in rabbits immunized with an experimental vaccine against *Staphylococcus aureus* and to develop the bovine mastitis subunit vaccine in the future. Enzyme immunoassay was used for detection of IgG antibodies against staphylococcal CPS, alpha-toxin and FnBP. The levels of specific antibodies against CPS, alpha-toxin and FnBP in immunized rabbits were significantly increased after first immunization compared with control animals ( $p < 0.05$ ). Of three antigen used in vaccine, immunogenicity of CPS was relatively lower, compared with those of alpha toxin and fibronectin binding protein. Numbers of *S. aureus* in blood of immunized groups were lower than those of control group after bacterial challenge. But the bacterial numbers among immunized groups were not significantly different. *S. aureus* counts in excised organs were significantly lower in all immunized rabbits than in PBS-control group ( $p < 0.05$ ). The present study showed that alpha-toxin, capsular polysaccharide and fibronectin binding protein included in a subunit vaccine were protective.—**KEY WORDS:** alpha-toxin, capsular polysaccharide, r-FnBP, *Staphylococcus aureus*.

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Mastitis is one of the major economic problems in dairy industry [17]. Although the disease is multifactorial, *Staphylococcus aureus* has been considered as the primary agent and it produces several virulence factors such as capsular polysaccharides [5], alpha-toxin [9] and fibronectin binding protein [16].

Of the virulence factors, attention has been paid to capsular polysaccharide since it can inhibit phagocytosis by neutrophils [20]. An earlier study [19] reported that encapsulated *S. aureus* isolated from milk of cows with mastitis inhibited phagocytosis by neutrophils and capsule production was enhanced by serial passage through bovine mammary glands. The capsular polysaccharide has been considered as a potential candidate for a vaccine to minimize mastitis in cows [18].

Alpha-toxin is an exotoxin produced by many strains of *S. aureus* of both human and animal origins. As described by Wood [21], alpha-toxin has specific cytotoxic activity only on neutrophils and macrophages. Although the majority of *S. aureus* strains isolated from bovine mastitic milk samples produce hemolysin, the role of this toxin in the pathogenesis of bovine mastitis for *S. aureus* is not clear. Removal of *S. aureus* from the mammary gland is accomplished mainly via phagocytosis; thus, the capability of alpha-toxin to kill phagocytes may prolong the infection by reducing the bacterial clearance rate. *In vitro* studies have shown that specific anti-alpha-toxin antibodies were able to neutralize the toxin and prevented cytolytic effects on phagocytes [4]. Therefore, the presence of specific antibodies in milk may be useful in preventing the killing of phagocytes by alpha-toxin.

Fibronectin-binding proteins are thought to mediate

attachment of *S. aureus* to host cells which may initiate disease [3]. Most isolated *S. aureus* specifically bind to extracellular matrix fibronectin [11]. FnBP has been shown to mediate binding of *S. aureus* to soluble fibronectin [15]. It has been reported that FnBP promotes adhesion of *S. aureus* to host tissue and may act as virulence factors. Thus, FnBP on *S. aureus* involved in the pathogenesis could be important in the development of subunit vaccine against bovine mastitis [14].

The purpose of this report was to evaluate the antibody levels of alpha-toxin, capsular polysaccharide and FnBP in immunized rabbit serum with an experimental vaccine against *S. aureus* which contributes to developing the bovine mastitis subunit vaccine in the future.

### MATERIALS AND METHODS

**Bacterial strains:** *Staphylococcus aureus* Wood 46 and Smith strains which produce large amount of alpha toxin and type 2 capsular polysaccharide respectively were used.

**Experimental animals:** New Zealand white rabbits weighing 1.5 to 2.0 kg were used.

**Alpha-toxin preparation:** *S. aureus* Wood 46 strain was incubated at 37°C in the optimal growth medium [7]. Briefly, the supernatant was filtered through a 0.45- $\mu$ m filter unit (Nalgene Co., Rochester, NY). The total protein concentration of the crude toxin was determined by BCA method (Pierce Inc., Rockford, Illinois, U.S.A). The crude alpha-toxin was analysed by 12% (w/v) sodium dodecyl sulfate-polyacrylamide gel electrophoresis [12]. The hemolytic assay is a serial two-fold dilution of 50% hemolysis and point assay described by Bernheimer and

Schwartz [2] and Harshman *et al.* [8].

**Capsular polysaccharide preparation:** *S. aureus* Smith strain was grown in nutrient broth supplemented with 10% (v/v) bovine milk whey for 24 hr at 37°C with agitation at 300 rpm. The production of capsular polysaccharide was confirmed by direct examination of the bacteria with transmissible electron microscopy (Hitachi Ltd., Tokyo, Jpn). Capsular materials were purified by the same method described by Poutrel *et al.* [20].

**Preparation of Fibronectin-binding protein (FnBP):** FnBP was prepared by the same method described by Kim *et al.* [10]. Briefly, the FnBP gene was amplified from the chromosomal DNA of *S. aureus* Wood 46 strain using the polymerase chain reaction, and cloned into pGEX-4T-2. Then the recombinant FnBP fused with glutathione-S-transferase was produced from *E. coli* by affinity chromatography, and examined its antigenicity by Western immunoblotting method.

**Vaccination protocol:** Forty rabbits were allocated to 8 groups 5 rabbits each (Table 1). The vaccine was administered intramuscularly twice in two-week interval.

**Determination of Alpha-toxin, CPS and FnBP antibody:** Blood from individual rabbits were sampled aseptically one week before vaccination, and weekly thereafter. Sera were stored at -20°C until use.

Alpha-toxin (0.05 µg/ml) and CPS (0.05 µg/ml) were diluted in sodium citrate buffer (0.6 M, pH 9.6) and coated in wells of a polystyrene plate overnight at 4°C. Uncoated antigen was removed by rinsing with phosphate-buffered saline solution (0.01 M, pH 7.2) with 0.5% Tween-20. After 1-hr incubation with serially diluted rabbit sera, peroxidase-conjugated goat anti-rabbit IgG (diluted 1:1000, Sigma, St. Louis, Missouri, U.S.A.) was added, followed by a 30-min incubation. Finally, 100 µl of substrate (30 mg of O-phenylenediamine; Sigma) was added to each well, and the plate was read by using an ELISA automatic analyzer (Skan soft I, U.S.A.) at 5 min after addition of substrate. Mean values of readings from duplicate samples placed in wells on opposite sides of the cuvette were calculated to obtain an optical density value for each serum sample.

**Quantitation of anti GST-FnBP antibody:** Recombinant GST-FnBP was diluted to a concentration of 1.25 µg/ml in carbonate-bicarbonate buffer (pH 9.6) and then incubated in wells of polystyrene cuvette at 37°C for 2 hr. Wells were

rinsed with PBS and PBST with 1% bovine serum albumin (BSA) was used as a block at 37°C for 30 min. Serum was diluted (1:200) with PBST with 0.5 BSA (electrophoresis grade, Sigma, St. Louis, Missouri, U.S.A.) and dispensed in 50 µl aliquots to each well at 37°C for 1 hr and then rinsed four times. Fifty microliter of substrate including O-phenylenediamine (P5412, Sigma, St. Louis, Missouri, U.S.A.) was added to each well at room temperature for 10 min and the plate was read 5 min later using an ELISA reader (ANTHOS HT<sup>III</sup>).

**Challenge experiments:** *S. aureus* challenge for the subsequent experiments was prepared as follows. *S. aureus* Smith strain was grown overnight (18 to 24 hr) in tryptic soy broth (Difco Laboratories, Detroit, MI, U.S.A.). *S. aureus* suspension was adjusted to 10<sup>8</sup> cells/ml with phosphate-buffered saline (PBS, pH 7.2). The bacteria was inoculated intravenously through jugular vein. Blood samples were collected at 1, 3, 6, 12, and 24 hr. blood samples obtained each time, 100 µl. were inoculated on the tryptic soy agar plates and the number of bacterial colonies were counted.

Rabbits in each group were exsanguinated, and a sample of their blood and urine was cultured for bacterial counts. Rabbits exsanguinated at 24 hr for bacterial counts in blood were also evaluated for the presence of bacteria in liver and spleen. Organs were excised, weighed, washed with absolute ethyl alcohol to eliminate surface-attached organisms, homogenized with sterile PBS (pH 7.2), and cultured on columbia agar plates. The plates were counted 24 hr later and expressed as CFU per gram of tissue.

**Statistical analyses:** The Multiple ANOVA test was used for statistical evaluation of differences in optical density readings between each group of rabbits for pre- and postvaccination serum samples. Also the same test was used to determine the difference of bacterial clearance rate between each group of rabbits for pre- and postvaccination. A value of  $p < 0.05$  was considered to be significant.

## RESULTS

Results of the enzyme immunoassay against alpha-toxin are shown in Fig. 1. Each of groups showed increased antibody levels specific for alpha toxin without significant variation when compared with PBS control group

Table 1. Components of vaccine administered in each group

| Groups  | Antigens used as vaccine                                                     |
|---------|------------------------------------------------------------------------------|
| Group 1 | CPS (600 µg)                                                                 |
| Group 2 | Alpha toxin (10 HU/ml)                                                       |
| Group 3 | Fibronectin binding protein (200 µg)                                         |
| Group 4 | Alpha toxin (10 HU/ml) + CPS (600 µg)                                        |
| Group 5 | Alpha toxin (10 HU/ml) + fibronectin binding protein (200 µg)                |
| Group 6 | Fibronectin binding protein (200 µg) + CPS (600 µg)                          |
| Group 7 | Fibronectin binding protein (200 µg) + alpha toxin (10 HU/ml) + CPS (600 µg) |
| Group 8 | Phosphate buffered saline (1 ml)                                             |

CPS: Capsular polysaccharide.

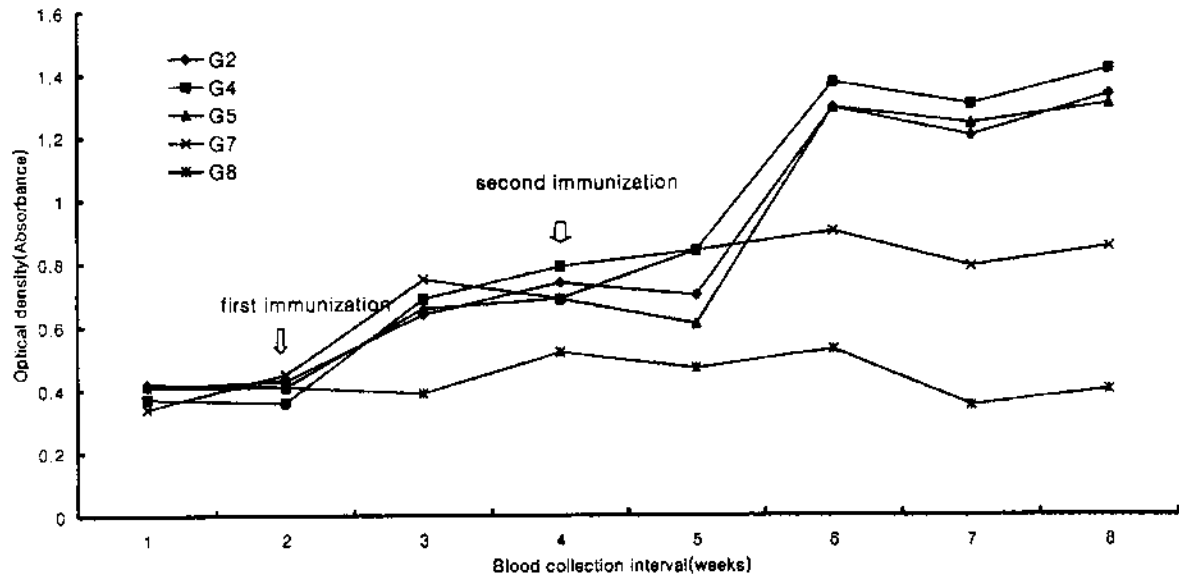


Fig. 1. ELISA results for IgG specific for staphylococcal alpha toxin in rabbits immunized with subunit vaccine twice in 2-week apart (G2: alpha toxin, G4: alpha toxin + CPS, G5: alpha toxin + FnBP, G7: alpha toxin + FnBP - CPS, G8: PBS).

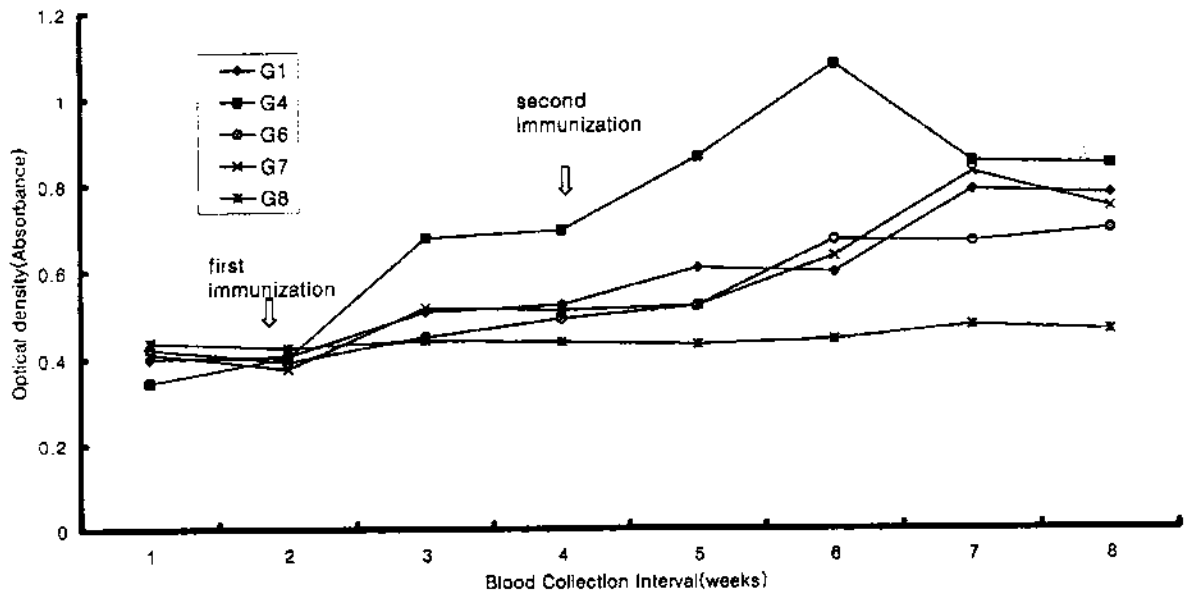


Fig. 2. ELISA results for IgG specific for staphylococcal capsular polysaccharide subjected to bacterin-toxoid stimulated twice in 2-week apart (G1: CPS, G4: alpha toxin + CPS, G6: CPS + FnBP, G7: alpha toxin + CPS + FnBP, G8: PBS).

noted 2 weeks following immunization and, particularly the levels of antibody significantly increased following second immunization ( $p < 0.001$ ).

Immune responses against CPS was analysed by ELISA (enzyme immunoassay) (Fig. 2). Each of groups shows increased CPS specific antibody levels in serum when compared with PBS control group. However, antibody level of group 4 (alpha toxin + CPS) was the highest. Peak levels reached 4 weeks following primary immunization and,

particularly the level of antibody increased significantly ( $p < 0.001$ ).

Anti-FnBP levels of formalized FnBP groups were significantly higher than those of PBS-control group from 1 weeks after first immunization ( $p < 0.05$ ) (Fig. 3). There were no significant differences in antibody levels between formalized-FnBP groups. The levels were highest at 5 weeks through the study and, particularly the levels of antibody increased significantly following second boosting

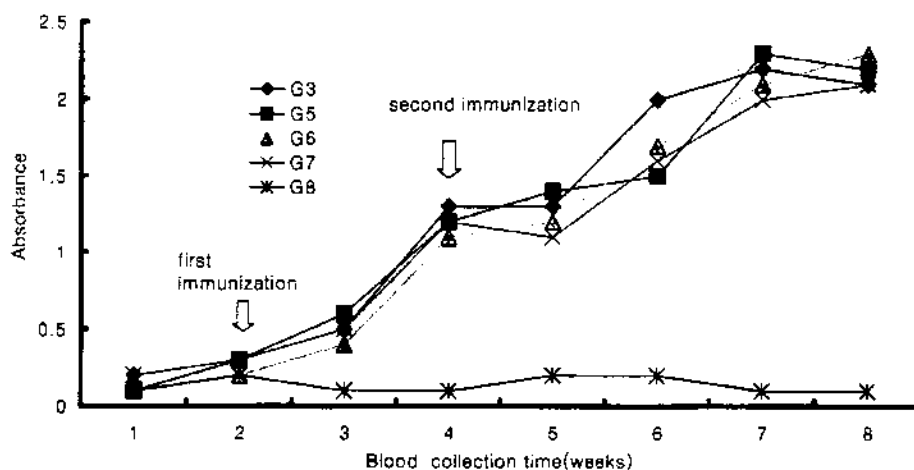


Fig. 3. ELISA results for IgG specific for formalin treated GST-FnBP in rabbits immunized with subunit vaccine (G3: FnBP, G5: alpha-toxin - FnBP, G6: FnBP - CPS, G7: alpha-toxin - FnBP - CPS, G8: PBS).

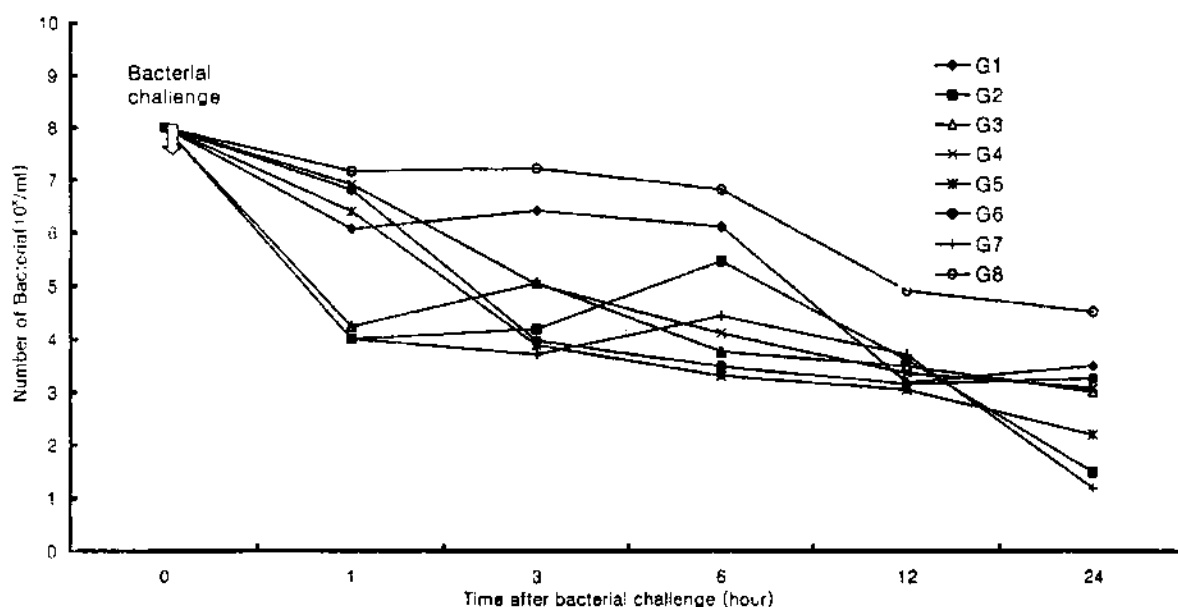


Fig. 4. Effect of IM immunization on the numbers of *S. aureus* in blood of rabbits challenged with  $10^6$  cfu/ml of *S. aureus* Wood 46 (G1: CPS, G2: alpha toxin, G3: FnBP, G4: alpha toxin - CPS, G5: alpha toxin - FnBP, G6: CPS - FnBP, G7: alpha toxin - FnBP - CPS, G8: PBS).

( $p < 0.001$ ).

In the challenge study, *S. aureus* counts from blood samples was performed at the different time points after challenge. The trends of bacterial counting are shown in Fig. 4. The number of bacteria in blood immunized groups was lower than that of control group after *S. aureus* challenge. Of all groups, group 7 (alpha toxin - CPS - FnBP) showed the lowest bacterial count in blood 24 hr after challenge ( $p < 0.001$ ). However, the bacterial counts between the vaccinated groups were not significantly different.

Spleen and liver were excised, washed with 70% ethanol, homogenized in 1 ml of PBS, and cultured for bacterial counts. Mean bacterial counts for all animals are expressed in common logarithmic scale (Fig. 5). Generally, the bacterial number recovered from excised organs showed no significant difference among the vaccinated groups. However vaccinated groups had significantly lower bacterial counts than that of PBS-control group in both spleen and liver ( $p < 0.05$ ).

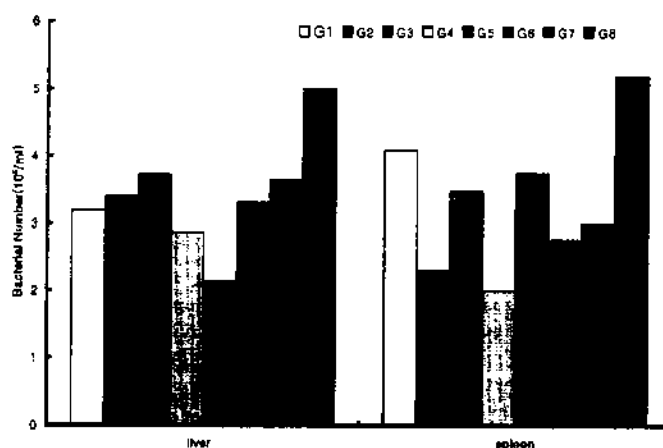


Fig. 5. Effect of IM immunization on bacterial counts from liver and spleen of rabbits challenged with  $10^8$  cfu/ml of *S. aureus* Wood 46 (G1: CPS, G2: alpha toxin, G3: FnBP, G4: alpha toxin + CPS, G5: alpha toxin + FnBP, G6: CPS + FnBP, G7: alpha toxin + CPS + FnBP, G8: PBS).

## DISCUSSION

The increased level of serum antibody against alpha-toxin reflected the increased systemic protective ability against alpha-toxin in rabbits immunized with the vaccine. This finding agrees with reports by Balaban *et al.* [1], who showed that serum antibody levels to RAP (RNAIII activating protein) increased in mouse with *S. aureus* infections. It, thus, seemed that alpha toxin produced in blood reached systemic lymphoid tissue in sufficient concentrations to generate a systemic immune response.

The alpha toxin preparation can be used as vaccine subunit, although only partially purified. These results indicated, however, that if alpha toxin were included in a vaccine to reduce the frequency of *S. aureus*-induced mastitis, sufficient toxin should be included to neutralize minimal amounts of toxin.

The level of serum antibody against CPS, as determined by ELISA, was closely related with systemic protective ability in the rabbit immunized with the vaccine. However, serum antibody levels was relatively lower than those of alpha-toxin and FnBP. Therefore further study of this vaccine was needed to increase levels of serum antibody against capsular polysaccharide through conjugation with alpha-toxin or FnBP.

The CPS prepareate could be used as a subunit vaccine, although only partially purified. However, these results indicated that if CPS were included in a vaccine with the intent to reduce the new infection rate of *S. aureus*-induced mastitis, sufficient CPS should be included in vaccine to produce sufficient anti-CPS level.

The lower number of *S. aureus* recovered from blood suggests that opsono-phagocytosis may play an important role. In antibodies against FnBP may directly block the adherence of the bacteria to host tissue through a fibronectin mediated mechanism.

The number of bacteria in excised organs were significantly lower in immunized group than in control group. This result showed consistency with the study results of Fattom *et al.* [6] other than the use of different antigen for immunization.

The results of the present study showed that active immunization elicited by *S. aureus* alpha toxin, capsular polysaccharide and fibronectin binding protein are protective in rabbit models. Also, the study confirmed the roles of alpha toxin, capsular polysaccharide and fibronectin binding protein as a virulence factor and provided a promising prospective of the vaccine for the prevention of *S. aureus* infection in cows.

The efficacy of this vaccine and of antibodies derived from vaccinated rabbits will determine their usefulness and the validity and application of protective immunity in *S. aureus* pathogens and infections. And it is expected that it will be possible to demonstrate protective immunity in mastitic cows with *S. aureus* infections after determination of the route of administration, the optimal immunized dose and the effective adjuvant.

The antiphagocytic properties of CPS, *S. aureus* isolated from the mastitic the essential role of FnBP in the adhesion to host cells, and anti-toxin effects of alpha-toxin should be considered for development of efficient vaccines against bovine mastitis. Also further studies on bovine *S. aureus* subunit vaccine are required. Although most studies were focused on alpha-toxin and capsular polysaccharide subunit vaccine, we will study the subunit vaccine including alpha-toxin, capsular polysaccharide and fibronectin binding protein to overcome the disadvantages.

**ACKNOWLEDGMENTS.** We are grateful to staffs in Kangwon National University. And we thank the Ministry of Agriculture and Forestry for financial support.

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## USE OF A STAPHYLOCOCCUS AUREUS CONJUGATE VACCINE IN PATIENTS RECEIVING HEMODIALYSIS

HENRY SHINEFIELD, M.D., STEVEN BLACK, M.D., ALI FATTOM, Ph.D., GARY HORWITH, M.D., SCOTT RASGON, M.D., JUAN ORDONEZ, M.D., HOCK YECH, M.D., DAVID LAW, M.D., JOHN B. ROBBINS, M.D., RACHEL SCHNEERSON, M.D., LARRY MUENZ, Ph.D., AND ROBERT NASO, Ph.D.

## ABSTRACT

**Background** In patients with decreased resistance to infection, *Staphylococcus aureus* is a major cause of bacteremia and its complications. The capsular polysaccharides are essential for the pathogenesis of and immunity to *S. aureus* infection and are targets for vaccines.

**Methods** In a double-blind trial involving patients with end-stage renal disease who were receiving hemodialysis, we evaluated the safety, immunogenicity, and efficacy of a vaccine with *S. aureus* type 5 and 8 capsular polysaccharides conjugated to nontoxic recombinant *Pseudomonas aeruginosa* exotoxin A. Between April 1998 and August 1999, 1804 adult patients at 73 hemodialysis centers were randomly assigned to receive a single intramuscular injection of either vaccine or saline. IgG antibodies to *S. aureus* type 5 and 8 capsular polysaccharides were measured for up to two years, and episodes of *S. aureus* bacteremia were recorded. Efficacy was estimated by comparing the incidence of *S. aureus* bacteremia in the patients who received the vaccine with the incidence in the control patients.

**Results** Reactions to the vaccine were generally mild to moderate, and most resolved within two days. The capsular polysaccharides elicited an antibody response of at least 80  $\mu$ g per milliliter (the estimated minimal level conferring protection) in 80 percent of patients for type 5 and in 75 percent of patients for type 8. The efficacy during weeks 3 to 54 was only 26 percent ( $P=0.23$ ). However, between weeks 3 and 40 after vaccination, *S. aureus* bacteremia developed in 11 of 892 patients in the vaccine group who could be evaluated for bacteremia, as compared with 26 of 906 patients in the control group (estimate of efficacy, 57 percent; 95 percent confidence interval, 10 to 81 percent; nominal  $P=0.02$ ).

**Conclusions** In patients receiving hemodialysis, a conjugate vaccine can confer partial immunity against *S. aureus* bacteremia for approximately 40 weeks, after which protection wanes as antibody levels decrease. (N Engl J Med 2002;346:491-6.)

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**S**TAPHYLOCOCCUS *aureus* is a major cause of nosocomial and community-acquired infections, including bacteremia, metastatic abscesses, septic arthritis, endocarditis, osteomyelitis, and wound infections.<sup>1-3</sup> A study of hospitalized patients in 1995 found that the death rate, the length of stay, and the medical costs were twice as high for *S. aureus*-associated hospitalizations as for other hospitalizations.<sup>4</sup> Among patients receiving hemodialysis, *S. aureus* bacteremia is a prominent cause of complications and death, with an annual incidence of 3 to 4 percent.<sup>5</sup> An increasing percentage of isolates are resistant to methicillin<sup>6</sup> and show relative resistance to vancomycin.<sup>7</sup> Hence, immunoprophylaxis against *S. aureus* is a worthwhile objective.

The capsular polysaccharides of *S. aureus* are virulence factors in systemic infections. *S. aureus* capsular polysaccharides confer invasiveness by inhibiting opsonophagocytic killing by polymorphonuclear neutrophils; this pattern is similar to that in other encapsulated bacteria, such as *Streptococcus pneumoniae*.<sup>8</sup> Of the 12 known types of *S. aureus*, types 5 and 8 account for approximately 85 percent of all clinical isolates.<sup>9-12</sup> Antibodies to the capsular polysaccharides of *S. aureus* types 5 and 8 induce type-specific opsonophagocytic killing by human polymorphonuclear neutrophils in vitro and confer protection in animals.<sup>13,14</sup>

*S. aureus* capsular polysaccharides alone are poor immunogens. Their immunogenicity is enhanced by conjugation with carrier proteins.<sup>15-17</sup> Monovalent vaccines containing *S. aureus* type 5 or type 8 capsular polysaccharide bound to recombinant exoprotein A, a nontoxic variant of *Pseudomonas aeruginosa* exotoxin A expressed in *Escherichia coli*, are immunogenic and well tolerated in healthy adults and in patients with end-stage renal disease.<sup>18</sup> In a prior study

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of patients with end-stage renal disease who were receiving peritoneal dialysis, a low dose of a conjugate antistaphylococcal vaccine was not efficacious. Therefore, we evaluated a higher dose of a bivalent conjugate vaccine composed of type 5 and 8 capsular polysaccharides covalently bound to recombinant exoprotein A. The goal of this controlled study was to evaluate the vaccine in terms of safety, immunogenicity, and ability to prevent bacteremia in patients receiving hemodialysis.

## METHODS

### Patients

Patients were recruited at 73 hemodialysis centers in California from April 1998 to August 1999. Enrolled patients were 18 years of age or older, had end-stage renal disease, had received hemodialysis with vascular access through a native-vessel fistula or a synthetic or heterologous graft for at least eight weeks before enrollment, had a Karnofsky score of at least 50 at entry, and were expected to complete the required follow-up visits.

Criteria for exclusion were symptoms or signs consistent with an infection within two weeks before vaccination, a history of infection with the human immunodeficiency virus, hypersensitivity or previous anaphylaxis caused by polysaccharides or polysaccharide conjugate vaccines, drug abuse within one year before vaccination, use of immunosuppressive or immunomodulatory drugs, and cancer or treatment for cancer within six months before vaccination. The study protocol was approved by the institutional review boards of the Kaiser Foundation Research Institute, Southern California Kaiser Permanente, and El Camino Hospital and by the Food and Drug Administration. The study was designed by the investigators, an independent data and safety monitoring board, and the manufacturer. The data were held by the data and safety monitoring board and analyzed by a group of the authors. The manuscript was a collaborative effort of all the listed authors. All patients gave written informed consent.

### Study Design

Eligible patients were randomly assigned to receive a single injection of vaccine or saline placebo. Randomization was stratified according to the type of vascular access (native-vessel fistula or synthetic or heterologous graft) and the presence or absence of persistent nasal carriage of *S. aureus*.

The vaccine (StaphVAX, Nabi, Rockville, Md.) is composed of *S. aureus* type 5 and 8 capsular polysaccharides (100 µg per milliliter of each type) conjugated to an equal weight of recombinant exoprotein A in 0.01 percent polysorbate 80 and sodium phosphate-buffered saline (pH 7.4). The dose was selected on the basis of our previous studies in patients with end-stage renal disease (unpublished data). Vaccine and placebo (sodium phosphate-buffered saline) were supplied as 1 ml of clear liquid in identical vials, each bearing a unique code.

In two screening visits that took place approximately one week apart, subjects were evaluated for eligibility, and the anterior nares were cultured for *S. aureus*. Carriage was defined by two positive cultures. The vaccine or placebo was administered by intramuscular injection into the deltoid muscle or the anterior thigh.

Patients were evaluated 30 minutes after the injection and instructed to record local reactions (e.g., redness, swelling, aching, burning, tenderness, and heat) and systemic reactions (e.g., fever, general discomfort, muscle aches, headache, nausea, and vomiting) each day for one week. One week after injection, patients returned to the dialysis center, and reactions to the vaccine were recorded. Patients were evaluated for adverse events for up to six weeks after the injection. Deaths and all episodes of bacteremia

were recorded until the study ended or the patient withdrew. The primary outcome measure was the first occurrence of *S. aureus* bacteremia in a patient. Blood cultures were obtained before antibiotic therapy was begun.

Serum samples were obtained before and 6, 26, 54, and 67 weeks after vaccination. Antibodies to the *S. aureus* type 5 and 8 capsular polysaccharides were measured by enzyme-linked immunosorbent assay.<sup>13,19</sup> A response to the vaccine was defined as an antibody level of at least 25 µg per milliliter and at least twice the level before vaccination.

### Determination of Sample Size

Surveys in the United States and Europe suggested an incidence rate of *S. aureus* bacteremia of 0.03 to 0.04 per patient-year in those receiving hemodialysis.<sup>5,20-22</sup> A sample size of 900 patients per group was determined to be sufficient to detect, with 80 percent power, a 60 percent reduction in the incidence of *S. aureus* bacteremia in the vaccine group during an observation period from 3 to 54 weeks after vaccination. However, since the antibody correlate of protection was not known before the study, and since antibody levels decline rapidly in patients with end-stage renal disease, other observation periods were evaluated.

### Statistical Analysis

Evaluation of efficacy was based on data obtained two weeks after vaccination. The rate of *S. aureus* bacteremia was compared between the vaccine and control groups by an exact, stratified analysis with the use of StatXact software.<sup>23,24</sup> Four groups were created by the stratification of patients according to nasal-carriage status at base line and type of vascular access. The time to the first episode of *S. aureus* bacteremia was determined by the Kaplan-Meier method and compared by a stratified log-rank test.

Since the potential duration of protection that might be provided by the staphylococcal conjugate vaccine was not known a priori, the effectiveness of the vaccine was evaluated in a post hoc analysis, and nominal P values have been reported. To compute a valid P value for this analysis, a permutation analysis based on the observed data was performed. This analysis, based on a two-sample permutation test, was used to determine the time elapsed since vaccination with the highest log-rank statistic — a measure of the difference between the vaccine and placebo groups in the time to *S. aureus* bacteremia. A total of 2000 simulated data sets were generated from all 1798 subjects who received an injection and could be evaluated for bacteremia to examine the log-rank statistic in weeks 3 to 4, weeks 3 to 5, and so on up to weeks 3 to 54. The P value for the null hypothesis of no vaccine effect was calculated as the proportion of simulated data sets that yielded a log-rank statistic greater than the greatest statistic yielded by the observed data set.

The numbers of patients who had reactions to the injection and who died in the vaccine and placebo groups were compared by Fisher's exact test. No adjustments were made for the multiplicity of tests of safety. To estimate the level of antibody that conferred protection (the correlate of protection), the antibody level was interpolated from the data generated before and 6, 26, and 54 weeks after immunization to estimate the mean antibody level at the point beyond which protective efficacy was no longer observed.

## RESULTS

A total of 1804 of 1991 screened patients recruited at the 73 hemodialysis centers were enrolled; 894 were randomly assigned to receive vaccine and 910 to receive placebo. Among 187 screened patients who were not immunized, 81 did not meet the eligibility criteria or did not comply with the protocol, 71 withdrew consent, 22 had a change in health status,

and 13 were not included for other reasons. The study period was from April 1998 to April 2000. The patients who received the vaccine and the control patients participated in the study for a median of 75 weeks and 74 weeks, respectively; 76 percent of the patients in each group participated in the study for at least 54 weeks. Six patients were excluded from the study after randomization; three control patients died within the first two weeks, and two patients who received the vaccine and one control patient had infections within two weeks before the injection. No patient was excluded from the analysis of safety.

The two groups were similar with regard to pretreatment demographic and clinical characteristics and were representative of the diverse population of California. Thirty-three percent of the patients were white, 31 percent were Hispanic, 23 percent were black, and 13 percent were Asian. Among the 894 patients who received the vaccine and the 910 control patients, 46 percent and 44 percent were women and 52 percent and 51 percent had diabetes, respectively. At vaccination, 69 percent of the patients in both groups had vascular access through a graft, and 22 percent had nasal carriage of *S. aureus*. The mean age in both groups was 58.3 years.

#### Reactions to the Vaccine and Other Adverse Events

There were no statistically significant differences in the number of deaths between the vaccine and control groups, and none of the deaths were considered to be related to the vaccine. The incidence of local reactions, malaise, and myalgia was significantly higher in patients who received the vaccine than in control patients (Table 1). Local reactions were generally mild or moderate and resolved within two days. A causal or temporal relation between *S. aureus* bacteremia and death was identified for 9 of the 152 deaths (5.9 percent) in the vaccine group and 11 of the 146 deaths (7.5 percent) in the control group ( $P=0.65$  according to Fisher's exact test).

#### *S. aureus* Bacteremia

The overall trend in the incidence of infection is shown in Figure 1. In weeks 1 and 2 after vaccination and before the start of follow-up to assess the efficacy of the vaccine, there was one patient with bacteremia in the vaccine group and none in the placebo group. In terms of the primary hypothesis that the vaccine would protect against infection during weeks 3 to 54, there were no statistically significant differences between the groups; the efficacy of the vaccine for this period was 26 percent (95 percent confidence interval, -24 to 57 percent;  $P=0.23$ ) (Table 2). We then investigated the data for other intervals during which the effect of the vaccine was more substantial. We found that during weeks 3 to 40, when

TABLE 1. SUMMARY OF REACTIONS TO THE VACCINE.\*

| REACTION                    | VACCINE GROUP<br>(N=893) | PLACEBO GROUP<br>(N=907) | P VALUE |
|-----------------------------|--------------------------|--------------------------|---------|
|                             | no. (%)                  |                          |         |
| Local                       |                          |                          |         |
| Induration                  | 121 (13.5)               | 40 (4.4)                 | <0.001  |
| Erythema                    | 93 (10.4)                | 44 (4.9)                 | <0.001  |
| Pain at the injection site† | 290 (32.5)               | 128 (14.1)               | <0.001  |
| Heat                        | 85 (9.5)                 | 33 (3.6)                 | <0.001  |
| Any local reaction          | 338 (37.8)               | 179 (19.7)               | <0.001  |
| Systemic                    |                          |                          |         |
| Headache                    | 243 (27.2)               | 227 (25.0)               | 0.31    |
| Myalgia                     | 253 (28.3)               | 199 (21.9)               | 0.002   |
| Malaise                     | 226 (25.3)               | 188 (20.7)               | 0.02    |
| Nausea                      | 168 (18.8)               | 141 (15.5)               | 0.07    |
| Vomiting                    | 64 (7.2)                 | 73 (8.0)                 | 0.53    |
| Fever                       | 41 (4.6)                 | 42 (4.6)                 | 1.00    |
| Any systemic reaction       | 431 (48.3)               | 393 (43.3)               | 0.04    |

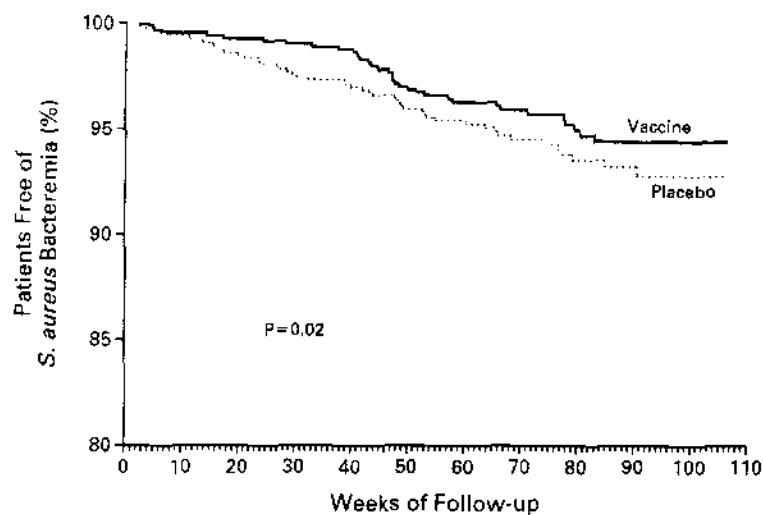
\*Some patients had more than one reaction. Data were not recorded for one patient in the vaccine group and three patients in the placebo group. Fisher's exact test was used to calculate the P values.

†Pain at the injection site included ache, burning, and tenderness.

11 events occurred during 618.9 person-years in patients who received the vaccine and 26 events occurred during 627.0 person-years in control patients, the vaccine reduced the incidence of bacteremia by 57 percent (95 percent confidence interval, 10 to 81 percent; nominal  $P=0.02$ ). With the permutation test used to account for the lack of an a priori hypothesis for a shorter interval of protection, the highest log-rank statistic was 7.03 for the interval of 3 to 32 weeks with a P value of 0.05.

Tests for homogeneity of efficacy during weeks 3 to 91 showed that efficacy was not significantly different across the four groups created by stratification ( $P=0.15$  for an exact test of homogeneity). However, our study had limited power to evaluate this interaction. In both groups, patients with vascular access through a graft rather than a fistula at the start of the study tended to be at increased risk for bacteremia (Table 3). Nasal carriage of *S. aureus* also tended to be associated with an increased risk of bacteremia in control patients (7.6 vs. 3.1 per 100 person-years,  $P=0.06$  according to an exact comparison of person-time rates) but not in patients who received the vaccine.

The vaccine and control groups had a similar distribution of types of *S. aureus* among patients with bacteremia. It was possible to retrieve 24 of 37 isolates from the vaccine group and 37 of 49 isolates from the control group for typing. In the vaccine group, 8 isolates (33 percent) were type 5 and 11 (46 percent)



|         | No. AT RISK |     |     | Week |     |     |     |
|---------|-------------|-----|-----|------|-----|-----|-----|
|         | 0           | 13  | 26  | 52   | 54  | 78  | 104 |
| Vaccine | 892         | 870 | 843 | 705  | 667 | 380 | 15  |
| Placebo | 906         | 889 | 850 | 710  | 661 | 369 | 10  |

Figure 1. Kaplan-Meier Survival Curves for *Staphylococcus aureus* Bacteremia after Week 2.

The P value is for the difference between the two groups at 40 weeks. Six patients were excluded from the study after randomization (three died and three had infections within two weeks before injection).

were type 8. Five (21 percent) were type 336, a recently described surface polysaccharide.<sup>25,26</sup> In the placebo group, 10 isolates (27 percent) were type 5, 20 (54 percent) were type 8, and 7 (19 percent) were type 336. Methicillin resistance was found in 7 of 37 *S. aureus* isolates in the vaccine group and 12 of 48 in the placebo group (1 isolate from a control patient was not tested). Between weeks 3 and 40, there were 37 episodes of *S. aureus* bacteremia (11 in the vaccine group and 26 in the placebo group). It was possible to retrieve 9 of 11 isolates from the vaccine group and 20 of 26 from the placebo group for typing. In the vaccine group, there were five type 5, three type 8, and one type 336 isolates. In the placebo group, there were 6 type 5, 11 type 8, and 3 type 336 isolates ( $P=0.50$  according to the exact chi-square test). Between weeks 3 and 54, there were two patients who received the vaccine and six control patients who had more than one episode of bacteremia ( $P=0.11$  according to the exact Cochran-Mantel-Haenszel test).

#### Relation between Antibody Levels and *S. aureus* Bacteremia

In our analysis, vaccine efficacy was observed for 40 weeks after receipt of the vaccine. We estimated

TABLE 2. CUMULATIVE NUMBER OF PATIENTS IN WHOM *STAPHYLOCOCCUS AUREUS* BACTEREMIA DEVELOPED AND EFFICACY OF THE VACCINE.\*

| Weeks After Injection | Vaccine Group |              | Placebo Group |              | Efficacy (95% CI) | P Value |
|-----------------------|---------------|--------------|---------------|--------------|-------------------|---------|
|                       | No. Infected  | Person-Years | No. Infected  | Person-Years |                   |         |
|                       |               |              |               |              | %                 |         |
| 10                    | 4             | 135.2        | 5             | 138.0        | 18 (-28 to 84)    | 1.0     |
| 20                    | 6             | 300.6        | 13            | 306.6        | 53 (-33 to 85)    | 0.17    |
| 30                    | 8             | 461.9        | 22            | 469.7        | 63 (14 to 86)     | 0.02    |
| 40                    | 11            | 618.9        | 26            | 627.0        | 57 (10 to 81)     | 0.02    |
| 50                    | 25            | 766.5        | 34            | 775.3        | 26 (-28 to 58)    | 0.30    |
| 54                    | 27            | 818.4        | 37            | 827.4        | 26 (-24 to 57)    | 0.23    |
| 91                    | 37            | 1165.0       | 49            | 1161.6       | 25 (-18 to 52)    | 0.24    |

\*Data are for the first episodes of bacteremia among the 1798 patients in whom the efficacy of the vaccine was evaluated. Episodes of bacteremia during weeks 1 and 2 after injection are excluded. The efficacy of the vaccine is calculated as  $100 \times (1 - [\text{incidence of } S. aureus \text{ bacteremia in the vaccine group} / \text{incidence of } S. aureus \text{ bacteremia in the placebo group}])$ . An exact test of the incidence ratio of 1 in comparisons between the vaccine and placebo groups was used to calculate the P values. CI denotes confidence interval.

# STAPHYLOCOCCUS AUREUS CONJUGATE VACCINE IN PATIENTS RECEIVING HEMODIALYSIS

**TABLE 3. INCIDENCE OF STAPHYLOCOCCUS AUREUS BACTEREMIA DEVELOPED DURING WEEKS 3 TO 54, ACCORDING TO TYPE OF VASCULAR ACCESS, NASAL-CARRIAGE STATUS, AND TREATMENT GROUP.**

| TYPE OF VASCULAR ACCESS AND NASAL-CARRIAGE STATUS | VACCINE GROUP (N=892) |                   | PLACEBO GROUP (N=906) |                   |
|---------------------------------------------------|-----------------------|-------------------|-----------------------|-------------------|
|                                                   | TOTAL NO. OF PATIENTS | PATIENTS INFECTED | TOTAL NO. OF PATIENTS | PATIENTS INFECTED |
|                                                   |                       | no. (%)           |                       | no. (%)           |
| Graft and no nasal carriage                       | 493                   | 18 (3.7)          | 496                   | 20 (4.0)          |
| Graft and nasal carriage                          | 123                   | 6 (4.9)           | 129                   | 10 (7.8)          |
| Fistula and no nasal carriage                     | 209                   | 3 (1.4)           | 214                   | 2 (0.9)           |
| Fistula and nasal carriage                        | 67                    | 0                 | 67                    | 5 (7.5)           |

**TABLE 4. GEOMETRIC MEAN LEVELS OF ANTIBODIES SPECIFIC FOR STAPHYLOCOCCUS AUREUS TYPE 5 AND 8 CAPSULAR POLYSACCHARIDES.\***

| TIME OF EVALUATION | VACCINE GROUP   |        |                  | PLACEBO GROUP   |        |                  |
|--------------------|-----------------|--------|------------------|-----------------|--------|------------------|
|                    | NO. OF PATIENTS |        |                  | NO. OF PATIENTS |        |                  |
|                    | TYPE 5          | TYPE 8 | $\mu\text{g/ml}$ | TYPE 5          | TYPE 8 | $\mu\text{g/ml}$ |
| Before injection   | 892             | 892    | 5.9 8.6          | 910             | 910    | 5.7 8.6          |
| Week 6             | 884             | 884    | 230.0 206.0      | 900             | 900    | 5.6 8.6          |
| Week 26            | 838             | 838    | 120.0 100.0      | 859             | 859    | 5.8 8.9          |
| Week 54            | 763             | 763    | 74.2 64.5        | 776             | 776    | 5.7 8.9          |
| Week 67            | 507             | 507    | 78.1 65.8        | 512             | 512    | 6.2 9.4          |

\* Pretreatment values were missing for two patients in the vaccine group because the serum was drawn after the vaccine was administered. The numbers of patients decreased over time in both groups because of attrition.

the geometric mean antibody level in patients who received the vaccine to be approximately 80  $\mu\text{g}$  per milliliter at 40 weeks, and this is a crude estimate of the correlate of protection in our population. For the vaccine and placebo groups, the peak geometric mean levels of antibodies to type 5 and type 8 capsular polysaccharides were not significantly different between those patients with and those without bacteremia.

## Antibody Levels

There were no statistically significant differences in antibody levels before vaccination between the vaccine and placebo groups. Antibodies remained at prevaccination levels in the placebo group. In the vaccine group, the geometric mean antibody levels were 230.0  $\mu\text{g}$  per milliliter for type 5 capsular polysaccharide and 206.0  $\mu\text{g}$  per milliliter for type 8 capsular polysaccharide at week 6 (the first time point evaluated), and they declined thereafter (Table 4). The percentages of patients with a peak antibody level of at least 80  $\mu\text{g}$  per milliliter (the estimated minimal protective level) were 80 percent for type 5 capsular polysaccharide and 75 percent for type 8 capsular polysaccharide. Included among the patients with no response were 27 patients (3 percent) for whom data were not available.

## DISCUSSION

This randomized, double-blind, placebo-controlled study demonstrates that a single injection of a conjugate vaccine containing *S. aureus* type 5 and type 8 capsular polysaccharides is safe and immunogenic and provides some protection for approximately 40 weeks against *S. aureus* bacteremia in patients with end-stage renal disease, an immunocompromised population at especially high risk for *S. aureus* bacteremia.<sup>5,20-22</sup>

Nearly 90 percent of the patients receiving hemodialysis had a response to the vaccine, and in over 75 percent antibody levels reached at least 80  $\mu\text{g}$  per milliliter (the estimated minimal level for protection). The decrease in vaccine efficacy after week 40 paralleled the decrease in levels of specific antibodies in the patient population. Antibody levels decline rapidly in patients receiving hemodialysis, and booster doses of vaccine should be evaluated.

In previous studies, vaccination with 25  $\mu\text{g}$  of a monovalent conjugated *S. aureus* type 5 capsular polysaccharide induced a geometric mean level of antibody to type 5 capsular polysaccharide of 318  $\mu\text{g}$  per milliliter in healthy subjects<sup>27</sup> and 180  $\mu\text{g}$  per milliliter in patients receiving hemodialysis.<sup>18</sup> Since this vaccine was more immunogenic in immunocompetent subjects, it could in theory provide equal or better protection against systemic infection with *S. aureus* for patients about to undergo elective surgery.

We estimated the minimal level at which protection was conferred by antibodies in patients with end-stage renal disease to be 80  $\mu\text{g}$  per milliliter, which is 2 to 3 logs higher than the levels at which protection is conferred by antibodies to *Haemophilus influenzae* type b capsular polysaccharide and *S. pneumoniae* capsular polysaccharides (0.15 and 1  $\mu\text{g}$  per milliliter, respectively).<sup>27,28</sup> The differences in the protective level of antibodies may be attributable to impaired phagocyte function and underlying disease in patients with end-stage renal disease.<sup>29</sup> Thus, identification of a protective level of antibodies for this patient population provides a surrogate for clinical efficacy of this vaccine in other at-risk patients. For immunocompetent adults, the protective levels of antibodies may be lower than those required for patients with end-stage renal disease.

The distribution of the types of *S. aureus* isolates from patients with bacteremia in this study was consistent with results reported by others.<sup>9,12</sup> The similar distribution of methicillin resistance among isolates from both the vaccine and placebo groups is consistent with in vitro data showing that both antibiotic-resistant and antibiotic-sensitive strains of *S. aureus* are killed by antibody-mediated opsonophagocytosis.

For 40 weeks after vaccination, the bivalent vaccine induced protection against *S. aureus* bacteremia. In addition, the efficacy of the vaccine was statistically significant during a 32-week period in the simulation model that took into account the lack of an a priori hypothesis for the duration of the vaccine effect. It is possible that efficacy will increase after the addition of the newly described type 336 to the vaccine.

The results of this study show that conjugate-induced antibodies to *S. aureus* capsular polysaccharides can provide partial protection against *S. aureus* bacteremia. Because patients receiving hemodialysis are among the least likely to have a response to immunoprophylaxis, the efficacy of the vaccine may be similar or perhaps greater in other patient populations.

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279  
228

**SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO  
DIRECCIÓN DE NUEVAS CREACIONES**

2020  
Bogotá, D.C.,

Abogado  
**ALICIA LLOREDA RICAURTE**  
(Apoderado)

|        |            |          |
|--------|------------|----------|
| ASUNTO | Radicación | 05-88322 |
|        | Trámite    | 002      |
|        | Evento     | 001      |
|        | Actuación  | 519      |
|        | Oficio     | 1386     |
|        | Folios     | 05       |

|                                |                                     |                               |                                                                      |
|--------------------------------|-------------------------------------|-------------------------------|----------------------------------------------------------------------|
| Patente de Invención           | <input checked="" type="checkbox"/> | Patente de Modelo de Utilidad | <input type="checkbox"/>                                             |
| Vía Nacional                   | <input type="checkbox"/>            |                               |                                                                      |
| Vía PCT (fase nacional)        | <input checked="" type="checkbox"/> | Cap. I                        | <input type="checkbox"/> Cap. II <input checked="" type="checkbox"/> |
| No. Sol. Internacional         | <u>PCT/US2004/006661</u>            |                               |                                                                      |
| Fecha de Presentación Interna. | <u>04 de marzo de 2004</u>          |                               |                                                                      |

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

**1. Documentos estudiados**

|                          |                    |                             |
|--------------------------|--------------------|-----------------------------|
| <u>Descripción:</u>      | Folios: 72 a 142   | como se radicó inicialmente |
| <u>Reivindicaciones:</u> | Folios: 143 a 148  | como se radicó inicialmente |
| <u>Figuras:</u>          | Folios: 149 a 182  | como se radicó inicialmente |
| <u>Prioridad:</u>        | Folio: 1           |                             |
|                          | Fecha: 07/03/2003  |                             |
|                          | País: US           |                             |
|                          | Número: 60/452,728 |                             |

## 2. Objeto de la invención

- Título de la solicitud (folio 1): "Conjugados de polisacárido-proteína portadora adhesina superficial de estafilococo para inmunización contra infecciones nosocomiales".
- La presente solicitud se refiere a conjugados inmunogénicos de polisacárido-proteína que tienen un antígeno polisacárido derivado de un conjugado nosocomial conjugado a una proteína portadora adhesina superficial de estafilococo, que se utilizan en composiciones inmunogénicas para producir respuestas de anticuerpo hacia el antígeno polisacárido y hacia la proteína portadora adhesina superficial de estafilococo.
- El problema planteado en esta solicitud es la necesidad de proporcionar composiciones inmunogénicas mas eficientes contra enfermedades nosocomiales.
- Para solucionar este problema técnico la solicitud en estudio proporciona un conjugado inmunogénico polisacárido-proteína, caracterizado porque comprende: al menos un antígeno polisacárido derivado de un patógeno nosocomial y al menos una proteína portadora adhesina superficial de estafilococo, en donde el conjugado genera anticuerpos específicos para el antígeno polisacárido y la proteína portadora adhesina superficial de estafilococo.
- La Clasificación Internacional de Patentes (IPC) es: A61K 47/48, A61K 39/385.

## 3. De la solicitud de Patente

### Reivindicaciones (artículo 30 D 486)

El capítulo reivindicatorio de la solicitud en estudio presenta los siguientes defectos:

- El capítulo reivindicatorio en general presenta una serie de conjugados que en su totalidad no tienen soporte en la descripción, así mismo, se realiza una extrapolación (reivindicación 1 y dependientes) que permite realizar n cantidad de composiciones sobre los cuales no se han determinado su acción terapéutica. Por tanto se sugiere restringir los conjugados a los evaluados y que puedan definirse por sus características técnicas y no por su función o su resultado. De esta forma, los "antígenos poliscaridos" y las proteínas portadoras adhesinas" pueden presentar un numero de combinaciones no determinado en la composición por lo que es necesario definirlas.
- Existen caracterizaciones determinadas por un resultado a alcanzar que no permiten definir la materia que se requiere proteger. Este es el caso de la reivindicación 2 que define el conjugado por que éste "genera anticuerpos específicos para el antígeno polisacárido y para la proteína portador adhesina superficial de estafilococo".

Por lo anterior, el capítulo reivindicatorio NO cumple con el requisito de definición, claridad, concisión o sustento por la descripción de acuerdo al art. 30 de la Decisión 486 y/o art. 22 Tratado PCT.

#### 4. No es una invención (artículo 15 D 486 literal a)-f))

*El artículo 15 en su literal b) señala que:*

*No se consideran invenciones el todo o parte de los seres vivos tal como se encuentran en la naturaleza, los procesos biológicos naturales, el material biológico existente en la naturaleza o aquel que pueda ser aislado inclusive genoma o germoplasma de cualquier ser vivo natural.*

- La reivindicación 28 enseña una globulina hiperinmune caracterizada porque contiene anticuerpos dirigidos contra el antígeno polisacárido y la proteína portadora adhesina superficial de estafilococo del conjugado de conformidad con la reivindicación 1.

Este despacho considera que dicha reivindicación no obedece a una invención debido a que se trata de una globulina natural, producida espontáneamente por el organismo gracias a que encuentra los antígenos necesarios para replicarse. El hecho de que dichos fragmentos provengan de compuestos artificiales o no, es independiente de la capacidad que tiene dicha globulina de reconocerlos.

De esta forma se establece que la reivindicación 28 no puede ser considerada una invención.

#### 5. Excepción a la patentabilidad (artículo 20 D486 literal a)-d))

*El literal c del artículo 20 de la decisión 486 establece que "no serán patentables:*

- c) las plantas, los animales los procedimientos esencialmente biológicos para la producción de plantas o animales q no sean procedimientos no biológicos o microbiológicos".*
- d) Los métodos terapéuticos o quirúrgicos para el tratamiento humano o animal, así como los métodos de diagnóstico aplicados a los seres humanos o a animales".*

- Las reivindicaciones 25 – 26 y 29 implican un método para inducir inmunidad activa contra infecciones nosocomiales en un sujeto. Dado que el método contempla un método terapéutico para el tratamiento humano o animal, esta oficina es de la opinión que las reivindicaciones mencionadas se encuentran dentro de la excepción a la patentabilidad y no pueden ser consideradas patentables.

De lo anterior la(s) reivindicaciones 25 – 26 y 29 no pueden ser consideradas patentables.

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

- Las reivindicaciones 30 a 31 obedecen al uso de los conjugados reivindicados para la producción de una composición para el tratamiento de una infección nosocomial.

Por dicha razón, las reivindicaciones analizadas no se pueden considerar

## 7. Determinación del Estado de la Técnica

132

La fecha que se ha tenido en cuenta para determinar el Estado de la Técnica es:

Fecha de presentación de la solicitud prioritaria: 07 de marzo de 2003

Teniendo en cuenta la fecha indicada se realizó la búsqueda en el estado de la técnica de documentos relacionados con la solicitud encontrándose los siguientes documentos más relevantes:

| Nº | Documento         | Título                                                                                                                                                      | Fecha de publicación* |
|----|-------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| D1 | Park et al.       | Immunogenicity of alpha-toxin, capsular polysaccharide (CPS) and recombinant fibronectin-binding protein (r-FnBP) of <i>Staphylococcus aureus</i> in Rabbit | 1999                  |
| D2 | Shinefield et al. | Use of a staphylococcus aureus conjugate vaccine in patients receiving hemodialysis                                                                         | 14 febrero 2002       |

## 8. Análisis de patentabilidad (artículo 14 D486)

### 8.1. Nivel inventivo (artículo 18 D486)

- La reivindicación 1 y sus dependientes divulgan un conjugado inmunogénico polisacárido-proteína, caracterizado porque comprende: **1)** al menos un antígeno polisacárido derivado de un patógeno nosocomial y **2)** al menos una proteína portadora adhesina superficial de estafilococo, en donde el conjugado genera anticuerpos específicos para el antígeno polisacárido y la proteína portadora adhesina superficial de estafilococo.

El documento del Estado de la Técnica más próximo es D1 (ver folios: 223 – 225) que enseña respuestas positivas a vacunas producidas por conjugados de 1) polisacáridos capsulares de estafilococos y 2) proteínas FnBp, que promueven la adhesión de *S. aureus* al tejido hospedero y por consiguiente pueden considerarse adhesinas (ver página 995).

La única diferencia con D1 es que solicitud menciona proteínas adhesinas diferentes, pero este compuesto no concede un efecto técnico inesperado porque estos compuestos siguen sugiriendo un efecto sobre la producción de anticuerpos para el reconocimiento de estos antígenos.

Igualmente el documento del estado de la técnica D2 enseña el uso de vacunas conjugadas de polisacárido tipo 5 y 8 (los cuales son los únicos que fueron probados en la solicitud) con una proteína, exoproteína A (página 491, segunda columna, tercer párrafo).

El problema técnico planteado en esta solicitud que era de proporcionar

transportadora más eficientes contra enfermedades nosocomiales se había resuelto previamente mediante el mismo tipo de compuestos ya conocidos. Por esta razón se considera que las sustituciones de las proteínas transportadoras como las adhesinas son evidentes para una persona medianamente versada en la materia.

De esta forma se establece que las reivindicaciones 1 a 31 no cumplen con el requisito de Nivel Inventivo.

## 9. Conclusión:

Los requisitos de patentabilidad de la presente solicitud se encuentran afectados así:

| Nº | Documento N°      | Reivindicaciones afectadas | Requisito que afecta |
|----|-------------------|----------------------------|----------------------|
| D1 | Park et al.       | 1 - 31                     | Nivel Inventivo      |
| D2 | Shinefield et al. | 1 - 31                     | Nivel Inventivo      |

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de 60 días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

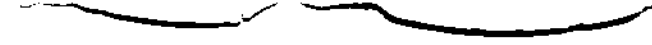
Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única N° 10 de 2001.



**ALIX CARMENZA CÉSPEDES DE VERGEL**  
Directora de Nuevas Creaciones (C)

El anterior requerimiento fue notificado por fijación en lista, de fecha

|                                    |                                                |
|------------------------------------|------------------------------------------------|
| <b>CONCEPTO TÉCNICO</b><br>No. 196 | <b>EXAMINADOR TÉCNICO</b><br>Código No. 202089 |
|------------------------------------|------------------------------------------------|



1/2

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