(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
26 February 2004 (26.02.2004)

PCT

(10) International Publication Number
WO 2004/016231 A2

(51) International Patent Classification[7]: A61K

(21) International Application Number:
PCT/US2003/008199

(22) International Filing Date: 17 February 2003 (17.02.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/365,351 15 March 2002 (15.03.2002) US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2004/016231 A2

(54) Title: PROTECTION OF PATIENTS LACKING A FUNCTIONAL SPLEEN AGAINST S. PNEUMONIAE WITH PNEUMOCOCCAL SURFACE PROTEIN A (PspA)

(57) Abstract: The present invention relates to a method of treating *Streptococcus pneumoniae* infection in a subject lacking a functional spleen. This method involves administering to the subject lacking a functional spleen an antibody that recognizes pneumococcal surface protein A (PspA) or a binding portion thereof under conditions effective to treat *Streptococcus pneumoniae* infection in the subject. The present invention also relates to a method of immunization against *Streptococcus pneumoniae* infection in a subject lacking a functional spleen. This method involves administering to the subject lacking a functional spleen PspA or a fragment thereof under conditions effective to elicit immunity that can prevent *Streptococcus pneumoniae* infection in the subject.

PROTECTION OF PATIENTS LACKING A FUNCTIONAL SPLEEN AGAINST S. PNEUMONIAE WITH PNEUMOCOCCAL SURFACE PROTEIN A (PspA)

[0001] This application claims the benefit of U.S. Patent Application Serial No. 60/365,351, filed March 15, 2002, which is hereby incorporated by reference in its entirety.

[0002] This invention arose out of research sponsored by the National Institute of Health (Grant No. P60 HL58418). The U.S. Government may have certain rights in this invention.

FIELD OF THE INVENTION

[0003] The present invention relates to a method of treating *Streptococcus pneumoniae* infection in a subject lacking a functional spleen by administering to the subject an antibody that recognizes pneumococcal surface protein A (PspA) or a binding portion thereof under conditions effective to treat *S. pneumoniae* infection in the subject. The present invention also relates to a method of immunization against *S. pneumoniae* infection in a subject lacking a functional spleen by administering to the subject PspA or a fragment thereof under conditions effective to elicit immunity that can prevent *S. pneumoniae* infection in the subject.

BACKGROUND OF THE INVENTION

[0004] *Streptococcus pneumoniae* is the most common etiology of fatal infections among sickle cell disease patients (Buchanan, "Infection," In Embury, eds., Sickle Cell Disease: Basic Principles and Clinical Practice, New York, New York:Raven Press, p. 567-587 (1994); Landsman et al., "Infections in Children With Sickle Cell Anemia," Am. J. Ped. Hem./Onc. 4:407-415 (1882); Wong et al., "Infection Caused by *Streptococcus pneumoniae* in Children With Sickle Cell Disease: Epidemiology, Immunologic Mechanisms, Prophylaxis, and Vaccination," Clin. Infect. Dis., 14:1124-1136 (1992); Overturf, "Infections and Immunizations of Children With Sickle Cell Disease," Adv. Pediatr. Infect. Dis., 14:191-218 (1999); Overturf, "Pneumococcal Vaccination of Children, " Semin. Pediatr. Infect. Dis.,

13:155-64 (2002)). It is also the most common cause of fatal community acquired pneumonia in the general population (Gillespie, "Aspects of Pneumococcal Infection Including Bacterial Virulence, Host Response and Vaccination," J. Med. Microbiol., 28:237-248 (1989); Musher, "Infections Caused by *Streptococcus pneumoniae*: Clinical Spectrum, Pathogenesis, Immunity, and Treatment," Clin. Infect. Dis., 14:801-809 (1992)). *S. pneumoniae* causes more deaths in the United States (about 40,000/year) than acute infections with any other bacterium (Fraser, "What Are Our Bacterial Disease Problems," in Robbins, eds., Bacterial Vaccines, Vol. IV, New York, New York, pp. xix-xxiv (1982)). In Africa and other parts of the developing world, *S. pneumoniae* is a major cause of serious respiratory infections, and is one of the most common causes of death (20-30% of total deaths) in children $\leq$ 5 years of age.

[0005] In non-sickle cell disease patients in the United States, most of the fatal pneumococcal infections occur in the elderly. Sickle cell disease patients (SCDP) are at an increased risk of fatal pneumococcal infection at all ages, but their greatest risk is during the first 5 years of life. The most common forms of fatal pneumococcal infection in SCDP are bacteremia and meningitis (Buchanan, "Infection," in Embury, eds., Sickle Cell Disease: Basic Principles and Clinical Practice, New York, New York:Raven Press, p. 567-587 (1994); Robinson et al., "Pneumococcal Meningitis in Sickle-Cell Anemia," N. Engl. J. Med., 274:1006-1008 (1966)). Serious pneumococcal infections are several-fold more common in African Americans than in Americans of northern European descent. Part of this difference is due to the high frequency of sickle cell disease (SCD) in the African American population. About 1 in 400 African Americans has clinical SCD (Bunn et al., "Hemoglobin: Molecular, Genetic, and Cellular Aspects," Saunders, Philadelphia (1986)). Prior to prophylaxis by antibiotics, by age 5 years, 30% of SCD patients had at least one bout of serious invasive pneumococcal infection (30 to 100 times the incidence in normal children (Wong et al., "Infection Caused by *Streptococcus pneumoniae* in Children With Sickle Cell Disease: Epidemiology, Immunologic Mechanisms, Prophylaxis, and Vaccination, " Clin. Infect. Dis., 14:1124-1136 (1992)). Mortality is about 35% among children with SCD who have invasive infections with *S. pneumoniae* (Powars et al., "Pneumococcal Septicemia in Children With Sickle Cell Anemia: Changing Trend of Survival," JAMA, 245:1839-1842 (1981); Powars, "Natural History of

Sickle Cell Disease -- The First Ten Years," Sem. Hematol., 12:267-285 (1975); Overturf et al., "Bacterial Meningitis and Septicemia in Sickle Cell Disease," Am. J. Dis. Child., 131:784-787 (1977)). Prior to the use of prophylactic antibiotics, 20% of children with SCD, even in the developed world, died of infection before age 5. Most of these deaths are caused by *S. pneumoniae* (Article, "Sickle Cell Anaemia in Infancy," British Medical Journal, i:1439 (1978)).

[0006] Several studies have demonstrated a higher incidence of pneumococcal infection in SCD than in non-SCD patients (Wong et al., "Infection Caused by *Streptococcus pneumoniae* in Children With Sickle Cell Disease: Epidemiology, Immunologic Mechanisms, Prophylaxis, and Vaccination," Clin. Infect. Dis., 14:1124-1136 (1992); Henneberger et al., "The Descriptive Epidemiology of Pneumococcal Menintitis in New York City," Am. J. Epidemiol., 117:484-491 (1983); Fraser et al., "Risk Factors in Bacterial Meningitis: Charleston County, South Carolina," J. Infect. Dis., 127:271-177 (1973)). The overall rate of meningitis in the African American population is 30 per 100,000 (Fraser et al., "Risk Factors in Bacterial Meningitis: Charleston County, South Carolina," J. Infect. Dis., 127:271-177 (1973)). In Charleston, South Carolina, in the early 1970s, the rate of pneumococcal meningitis among African Americans was 5.5 times that for those of European ancestry. When the data were further analyzed, it was found that the annual incidence of pneumococcal meningitis among African Americans with SCD was 68 times the rate for African Americans without SCD and 147 times the rate for children of European heritage.

[0007] In recent years penicillin prophylaxis and aggressive clinical follow up of infections have significantly reduced rates of fatal pneumococcal infection in SCD patients (Orange et al., "Pneumococcal Serotypes Causing Disease in Children in Alabama," Pediatr. Infect. Dis., 12:244-246 (1993); Zarkowsky et al., "Bacteremia in Sickle Hemoglobinopathies," J. Pediatr., 109:579-585 (1986); Hord et al., "*Streptococcus pneumoniae* Sepsis and Meningitis During the Penicillin Prophylaxis Era in Children With Sickle Cell Disease," J. Pediatr. Hematol. Oncol., 24:470-2 (2002)). Prophylactic antibiotic therapy became common because of nearly uniform sensitivity of pneumococci to penicillin, the low toxicity of penicillin, and its low cost. This life saving prophylaxis, however, has contributed to the increased antibiotic resistance that is now common among *S. pneumoniae* (Appelbaum, "World-

Wide Development of Antibiotic Resistance in Pneumococci," Eur. J. Clin. Microbiol., 6:367-377 (1987); Klugman, "Pneumococcal Resistance to Antibiotics," Clin. Microbiol. Rev., 3:171-196 (1990); Zenni et al., "*Streptococcus pneumoniae* Colonization in the Young Child: Association With Otitis Media and Resistance to Penicillin," J. Pediatr., 127:533-537 (1995); Hofmann et al., "Prevalence of Drug-Resistant *Streptococcus pneumoniae* in Atlanta," N. Engl. J. Med., 333:481-486 (1995); Robinson et al., "Clones of *Streptococcus pneumoniae* Isolated From Nasopharyngeal Carriage and Invasive Disease in Young Children in Central Tennessee," J Infect Dis., 183:1501-7 (2001)). In a report from Louisiana, the incidence of intermediate penicillin resistance among isolates from SCDP was 62%, as compared to 35% for strains from non-SCD patients (Steele et al., "Colonization With Antibiotic-Resistant *Streptococcus pneumoniae* in Children With Sickle Cell Disease," J Pediatr., 128:531-5 (1996)). In Detroit, Michigan, 50% of isolates from SCDP were penicillin resistant, as opposed to 10% resistance for isolates from non-SCD patients (Sakhalkar et al., "Prevalence of Penicillin-Nonsusceptible *Streptococcus pneumoniae* in Nasopharyngeal Cultures From Patients With Sickle Cell Disease," South. Med. J., 94:401-4 (2001)).

[0008] The frequency of penicillin non-susceptible strains has reached about 31% nationwide with about 1/3 showing high resistants (Diekema et al., "Antimicrobial-Drug Use and Changes in Resistance in *Streptococcus pneumoniae*," Emerg. Infect. Dis., 6:552-6 (2000); American Academy of Pediatrics Committee on Infectious Diseases, "Therapy for Children With Invasive Pneumococcal Infections," Pediatrics, 99:289-99 (1997)). The frequency of strains resistant to multiple antibiotics is also increasing (Diekema et al., "Antimicrobial-Drug Use and Changes in Resistance in *Streptococcus pneumoniae*, " Emerg. Infect. Dis., 6:552-6 (2000); Butler et al., "Pneumococcal Drug Resistance: The New 'Special Enemy of Old Age,'" Clin. Infect. Dis., 28:730-5 (1999); Breiman et al., "Emergence of Drug-Resistant Pneumococcal Infections in the United States," JAMA, 271:1831-1835 (1994)). In some locales, penicillin-resistant strains and multi-drug resistant strains have been reported to cause serious problems in treatment of SCD (Steele et al., "Colonization With Antibiotic-Resistant *Streptococcus pneumoniae* in Children With Sickle Cell Disease," J. Pediatr., 128:531-5 (1996); Freeman et al., "Infection in Major Sickle Hemoglobinopathies: Should Management Strategies Change?," Md.

Med. J., 42:1001-4 (1993)) and non-SCD patients (Butler et al., "Pneumococcal Drug Resistance: The New 'Special Enemy of Old Age, '" Clin. Infect. Dis., 28:730-5 (1999)).

[0009] Fatal infections with penicillin-sensitive pneumococci frequently progress so rapidly that by the time the patient seeks medical treatment it is too late for effective antibiotic therapy (Freeman et al., "Infection in Major Sickle Hemoglobinopathies: Should Management Strategies Change?," Md. Med. J., 42:1001-4 (1993); Austrian et al., "Pneumococcal Bacteremia With Special Reference to Bacteremic Pneumococcal Pneumonia," Ann. Internal. Med., 60:759-776 (1964)). SCDP are at especially high risk because of their poor ability to control pneumococcal bacteremia (Buchanan, "Infection," in Embury, eds., Sickle Cell Disease: Basic Principles and Clinical Practice, New York, New York: Raven Press, p. 567-587 (1994)). SCDP must be treated aggressively early in the course of disease if death is to be avoided. Infections with antibiotic resistant strains put SCDP at especially high risk. It is hoped that elevated levels of protective antibodies can compensate for the intrinsic susceptibility of SCDP and protect them from invasive disease. Vaccines that prevent acquisition or invasion could eliminate the need for antibiotic prophylaxis.

[0010] The vaccine presently used to protect adults from pneumococcal infections contains 23 different capsular polysaccharides (Austrian, "Pneumococcal Infections," in Germanier, ed., Bacterial Vaccines, New York, New York:Academic Press, Inc., pp. 257-288 (1984); Filice, "Pneumococcal Vaccines and Public Health Policy; Consequences of Missed Opportunities," Arch. Intern. Med., 150:1373-1375 (1990); Robbins et al., "Considerations for Formulating the Second-Generation Pneumococcal Capsular Polysaccharide Vaccine With Emphasis on the Cross-Reactive Types Within Groups," J. Infect. Dis., 148:1136-1159 (1983)). Of the 90 known pneumococcal capsular types, this vaccine contains those most commonly seen in pneumococcal infections of adults. While this vaccine is very effective in young adults, who are normally at low risk of serious disease, it is no more than 60% effective in the elderly (Shapiro et al., "Protective Efficacy of Polyvalent Pneumococcal Polysaccharide Vaccine," N. Engl. J. Med., 325:1453-1460 (1991)). In children less than 2 years of age, the vaccine is ineffective and is not recommended (Cowan et al., "Pneumococcal Polysaccharide Immunization in Infants and Children,"

Pediatrics, 62:721-727 (1978); Gotschlich et al., "The Immune Response to Bacterial Polysaccharides in Man.," in Haber, eds., Antibodies in Human Diagnosis and Therapy, New York, New York:Raven, pp. 391-402 (1977)). Even in SCD children older than 2 years of age, there is little evidence for its efficacy. This vaccine was also poorly immunogenic in the young SCDP who are those at highest risk of disease (Bjornson et al., "Serotype-Specific Immunoglobulin G Antibody Responses to Pneumococcal Polysaccharide Vaccine in Children With Sickle Cell Anemia: Effects of Continued Penicillin Prophylaxis," J. Pediatr., 129:828-35 (1996)).

[0011] Vaccines composed of immunogenic polysaccharide-protein conjugates have been under development for many years, and one, Prevnar©, is now licensed for use in children. It contains 7 polysaccharide-protein conjugates representative of the capsular types most commonly expressed by pneumococci infecting children in the United States. Each of the 7 different polysaccharides is separately conjugated chemically to mutant diphtheria toxin (Rennels et al., "Safety and Immunogenicity of Heptavalent Pneumococcal Vaccine Conjugated to CRM197 in United States Infants," Pediatrics, 101:604-611 (1998)). This 7-valent conjugate vaccine has been successful at preventing bacteremia and meningitis in young children with strains of the 7 types included in the vaccine (Shinefield et al., "Efficacy of Pneumococcal Conjugate Vaccines in Large Scale Field Trials," Pediatr. Infect. Dis. J., 19:394-7 (2000)). It is immunogenic in children with SCD (Overturf, "Pneumococcal Vaccination of Children," Semin. Pediatr. Infect. Dis., 13:155-64 (2002); Vernacchio et al., "Comparison of an Opsonophagocytic Assay and IgG ELISA to Assess Responses to Pneumococcal Polysaccharide and Pneumococcal Conjugate Vaccines in Children and Young Adults with Sickle Cell Disease," J. Infect. Dis., 181:1162-6 (2000)) and is recommended for use in children with SCD (American Academy of Pediatrics, Committee on Infectious Diseases, "Policy Statement: Recommendations for the Prevention of Pneumococcal Infections, Including the Use of Pneumococcal Conjugate Vaccine (Prevnar), Pneumococcal Polysaccharide Vaccine, and Antibiotic Prophylaxis," Pediatrics, 106:362-6 (2000); Jacobson et al., "The Pneumococcal Conjugate Vaccine Minerva," Pediatr., 54:295-303 (2002)). It is likely that this vaccine will save the lives of many children with SCD. A regimen where Prevnar© is followed by a boost with the 23 valent vaccine has also proven able to elicit antibodies in children over 2 years of age (Vernacchio et

al., "Combined Schedule of 7-Valent Pneumococcal Conjugate Vaccine Followed by 23-Valent Pneumococcal Vaccine in Children and Young Adults with Sickle Cell Disease," J Pediatr., 133:275-8 (1998)). However, an efficacy test is unlikely in SCDP now that the vaccine is recommended for use in that population. The vaccine does not protect against types not included in the vaccine (Shinefield et al., "Efficacy of Pneumococcal Conjugate Vaccines in Large Scale Field Trials," Pediatr Infect Dis J., 19:394-7 (2000)) and capsular type replacement has already been observed in strains recovered from children (Dagan et al., "Reduction of Nasopharyngeal Carriage of Pneumococci During the Second Year of Life by a Heptavalent Conjugate Pneumococcal Vaccine," J. Infect. Dis., 174:1271-1278 (1996)).

[0012] Another problem with a polysaccharide-protein vaccine is that each of the different polysaccharide-protein conjugates must be constructed independently and then combined in appropriate ratios to form the final vaccine where the immunogenicity to the different polysaccharides is balanced. The cost of the present 7-valent vaccine per child is now about $300. This cost will have to be decreased about 1000 fold if it is to be readily availability to those in the developing world at highest risk of pneumococcal infection. To improve coverage, the number of polysaccharides should be increased, but will probably be limited to a maximum of 10 or 11, because of costs and antigen load.

[0013] An alternative approach is to develop a vaccine composed of protection-eliciting pneumococcal proteins. Children generally make good responses to protein antigens. By inclusion of specific proteins, such a vaccine could target more than one virulence mechanism. Recent studies have shown that immunization with mixtures of pneumococcal proteins are often more protective in mice than individual proteins (Briles et al., "Intranasal Immunization of Mice with a Mixture of the Pneumococcal Proteins PsaA and PspA is Highly Protective Against Nasopharyngeal Carriage of *Streptococcus pneumoniae*," Infect Immun., 68:796-800 (2000); Ogunniyi et al., "Immunization of Mice with Combinations of Pneumococcal Virulence Proteins Elicits Enhanced Protection Against Challenge with *Streptococcus pneumoniae*," Infect. Immun., 68:3028-3033 (2000)). A protein vaccine would also have the advantage that the recombinant proteins can be produced inexpensively, thus making the vaccine more affordable worldwide. Pneumococcal proteins might also serve as carriers for conjugation of pneumococcal polysaccharides. This approach

could broaden the protection elicited by the present conjugate vaccine and might also permit the development of a "hybrid" vaccine containing the 3 or 4 most cost-effective polysaccharides conjugated to the 3 or 4 most promising cross-reactive proteins.

[0014] Several pneumococcal proteins, PspA, pneumococcal surface adhesion A (PsaA), pneumolysin, neuraminidase, autolysin, and PspC, have been shown to elicit immunity in mice that is protective against pneumococcal infection (Ogunniyi et al., "Immunization of Mice with Combinations of Pneumococcal Virulence Proteins Elicits Enhanced Protection Against Challenge with *Streptococcus pneumoniae*," Infect. Immun., 68:3028-3033 (2000); McDaniel et al., "Monoclonal Antibodies Against Protease Sensitive Pneumococcal Antigens can Protect Mice from Fatal Infection with *Streptococcus pneumoniae*," J. Exp. Med., 160:386-397 (1984); McDaniel et al., "Use of Insertional Inactivation to Facilitate Studies of Biological Properties of Pneumococcal Surface Protein A (PspA)," J. Exp. Med., 165:381-394 (1987); Tart et al., "Truncated *Streptococcus pneumoniae* PspA Molecules Elicit Cross-Protective Immunity Against Pneumococcal Challenge in Mice," J. Infect. Dis. 173:380-386 (1996); Briles et al., "The Potential for Using Protein Vaccines to Protect Against Otitis Media Caused by *Streptococcus pneumoniae*," Vaccine, 19:S87-S95 (2001); Briles et al., "Immunization of Humans with rPspA Elicits Antibodies, which Passively Protect Mice from Fatal Infection with *Streptococcus pneumoniae* Bearing Heterologous PspA," J Infect Dis., 182:1694-701 (2000); Berry et al., "Contribution of Autolysin to Virulence of *Streptococcus pneumoniae*," Infect. Immun., 57:2324-2330 (1989); Lock et al., "Comparative Efficacy of Pneumococcal Neuraminidase and Pneumolysin as Immunogens Protective Against *Streptococcus pneumoniae*," Microb. Pathog., 5:461-467 (1988)); Paton et al., "Inhibition of Human Polymorphonuclear Leukocyte Respiratory Burst, Bactericidal Activity, and Migration by Pneumolysin," Infect. Immun., 41:1212-1216 (1983); Walker et al., "Molecular Cloning, Characterization, and Complete Nucleotide Sequence of the Gene for Pneumolysin, the Sulfhydryl-Activated Toxin of *Streptococcus pneumoniae*," Infect. Immun., 55:1184-1189 (1987); Balachandran et al., "The Role of Pneumococcal Surface Protein C (PspC) in Nasopharyngeal Carriage and Pneumonia and its Ability to Elicit Protection Against Carriage of *Streptococcus pneumoniae*," Infect Immun., 70:2526-2534 (2002)). Each of these proteins has been

found to be a virulence factor (Briles et al., "Immunity to *Streptococcus pneumoniae*," p. 263-280. *In* M. Cunningham, and R. S. Fujinami (eds), Effect of Microbes on the Immune System, Lippincott-Raven, Philadelphia (2000); Briles et al., "The Potential for Using Protein Vaccines to Protect Against Otitis Media Caused by *Streptococcus pneumoniae*," Vaccine, 19:S87-S95 (2001); Balachandran et al., "The Role of Pneumococcal Surface Protein C (PspC) in Nasopharyngeal Carriage and Pneumonia and its Ability to Elicit Protection Against Carriage of *Streptococcus pneumoniae*," Infect Immun., 70:2526-2534 (2002)). Based on analysis of the genome of the pneumococcus, additional vaccine candidates have been proposed (Adamou et al., "Identification and Characterization of a Novel Family of Pneumococcal Proteins that are Protective Against Sepsis," Infect Immun., 69:949-58 (2001); Wizemann et al., "Use of a Whole Genome Approach to Identify Vaccine Molecules Affording Protection Against *Streptococcus pneumoniae* Infection," Infect. Immun., 69:1593-8 (2001)). Only one pneumococcal protein, PspA, has been used to immunize human volunteers. The human antibody elicited by immunization with PspA was able to protect mice from fatal sepsis with pneumococci (Briles et al., "Immunization of Humans with rPspA Elicits Antibodies, which Passively Protect Mice from Fatal Infection with *Streptococcus pneumoniae* Bearing Heterologous PspA," J Infect Dis., 182:1694-701 (2000)).

[0015] PspA is expressed on the surface of all pneumococci (Crain et al., "Pneumococcal Surface Protein A (PspA) is Serologically Highly Variable and is Expressed by All Clinically Important Capsular Serotypes of *Streptococcus pneumoniae*," Infect. Immun., 58:3293-3299 (1990)). Subcutaneous immunization with full-length PspA and the recombinant N-terminal half of PspA can elicit protection against pneumococcal infection in mice (Briles et al., "The Potential for Using Protein Vaccines to Protect Against Otitis Media Caused by *Streptococcus pneumoniae*," Vaccine, 19:S87-S95 (2001); McDaniel et al., "PspA, a Surface Protein of *Streptococcus pneumoniae*, is Capable of Eliciting Protection Against Pneumococci of More than One Capsular Type," Infect. Immun., 59:222-228 (1991); Briles et al., "The Potential to Use PspA and Other Pneumococcal Proteins to Elicit Protection Against Pneumococcal Infection," Vaccine, 18:1707-1711 (2000)). Monoclonal antibodies or immune serum to PspA from animals or humans can provide passive protection against systemic pneumococcal infection (Briles et al.,

"Antipneumococcal Effects of C-Reactive Protein and Monoclonal Antibodies to Pneumococcal Cell Wall and Capsular Antigens," Infect. Immun., 57:1457–1464 (1989); McDaniel et al., "Monoclonal Antibodies Against Protease Sensitive Pneumococcal Antigens can Protect Mice from Fatal Infection with *Streptococcus pneumoniae*," J. Exp. Med., 160:386-397 (1984); Briles et al., "The Potential to Use PspA and Other Pneumococcal Proteins to Elicit Protection Against Pneumococcal Infection," Vaccine, 18:1707-1711 (2000)). PspA is immunogenic when given intranasally with cholera toxin B subunit, cholera toxin (CT), or IL-12 as an adjuvant (Wu et al., "Intranasal Immunization of Mice with PspA (Pneumococcal Surface Protein A) Can Prevent Intranasal Carriage and Infection with *Streptococcus pneumoniae*," J. Infect. Dis., 175:839-846 (1997); Yamamoto et al., "A Nontoxic Adjuvant for Mucosal Immunity to Pneumococcal Surface Protein A," J. Immunol., 161:4115-4121 (1998); Arulanandam et al., "Intranasal Vaccination with Pneumococcal Surface Protein A and IL-12 Augments Antibody-Mediated Opsonization and Protective Immunity against *Streptococcus pneumoniae* Infection," Infect Immun., 69:6718-24 (2001)), and the immunity is effective at protecting mice from nasal colonization with pneumococci (Briles et al., "Intranasal Immunization of Mice with a Mixture of the Pneumococcal Proteins PsaA and PspA is Highly Protective Against Nasopharyngeal Carriage of *Streptococcus pneumoniae*," Infect Immun., 68:796-800 (2000); Wu et al., "Intranasal Immunization of Mice with PspA (Pneumococcal Surface Protein A) Can Prevent Intranasal Carriage and Infection with *Streptococcus pneumoniae*," J. Infect. Dis., 175:839-846 (1997)). Intranasal immunization with PspA also elicits serum antibody, which is protective against invasive infection (Wu et al., "Intranasal Immunization of Mice with PspA (Pneumococcal Surface Protein A) Can Prevent Intranasal Carriage and Infection with *Streptococcus pneumoniae*," J. Infect. Dis., 175:839-846 (1997); Yamamoto et al., "A Nontoxic Adjuvant for Mucosal Immunity to Pneumococcal Surface Protein A," J. Immunol., 161:4115-4121 (1998); Arulanandam et al., "Intranasal Vaccination with Pneumococcal Surface Protein A and IL-12 Augments Antibody-Mediated Opsonization and Protective Immunity against *Streptococcus pneumoniae* Infection," Infect Immun., 69:6718-24 (2001)).

[0016] PspA (about 70 kDa) consists of 4 distinct domains (McDaniel et al., "Comparison of the PspA Sequence from *Streptococcus pneumoniae* EF5668 to the

Previously Identified PspA Sequence from Strain Rx1 and Ability of PspA from EF5668 to Elicit Protection Against Pneumococci of Different Capsular Types," Infect. Immun., 66:4748-4754 (1998); Hollingshead et al., "Diversity of PspA: Mosaic Genes and Evidence for Past Recombination in *Streptococcus pneumoniae*," Infect. Immun., 68:5889-5900 (2000)). The N-terminal 40% of the molecule is highly charged and is predicted to be an anti-parallel coiled-coil alpha-helix (McDaniel et al., "Comparison of the PspA Sequence from *Streptococcus pneumoniae* EF5668 to the Previously Identified PspA Sequence from Strain Rx1 and Ability of PspA from EF5668 to Elicit Protection Against Pneumococci of Different Capsular Types," Infect. Immun., 66:4748-4754 (1998); Yother et al., "Structural Properties and Evolutionary Relationships of PspA, a Surface Protein of *Streptococcus pneumoniae*, as Revealed by Sequence Analysis," J. Bacteriol., 174:601-609 (1992); Jedrzejas et al., "Production and Characterization of the Functional Fragment of Pneumococcal Surface Protein A," Arch Biochem Biophys., 373:116-125 (2000)). Adjacent to this alpha-helical/charged domain is a proline-rich region, which, in Rx1, consists of an 82 amino acid domain containing 23 prolines grouped into two proline-rich regions, whose predominant motif is pro-ala-pro-ala-pro. C-terminal to the proline regions is a choline-binding domain composed of nine or ten 20 amino acid repeats that binds PspA to phosphocholine (PC) residues on surface lipoteichoic acids (McDaniel et al., "Comparison of the PspA Sequence from *Streptococcus Pneumoniae* EF5668 to the Previously Identified PspA Sequence from Strain Rx1 and Ability of PspA from EF5668 to Elicit Protection Against Pneumococci of Different Capsular Types," Infect. Immun., 66:4748-4754 (1998); Yother et al., "Structural Properties and Evolutionary Relationships of PspA, a Surface Protein of *Streptococcus pneumoniae*, as Revealed by Sequence Analysis," J. Bacteriol., 174:601-609 (1992); Yother et al., "Truncated Forms of PspA that are Secreted from *Streptococcus pneumoniae* and Their Use in Functional Studies and Cloning of the *pspA* Gene," J. Bact., 174:610-618 (1992); Yother et al., "Novel Surface Attachment Mechanism for the *Streptococcus pneumoniae* Protein PspA," J. Bacteriol., 176:2976-2985 (1994)).

[0017] PspA appears to have more than one mechanism of action. It is now clear that PspA interferes with the activation of complement component 3 (C3) at the pneumococcal surface (Tu et al., "Pneumococcal Surface Protein A (PspA) Inhibits Complement Activation by *Streptococcus pneumoniae*," Infect. Immun., 67:4720-

4724 (1999); Ren et al., "Both Family 1 and Family 2 PspAs Can Inhibit Complement Deposition and Confer Virulence to a Capsular 3 Serotype *Streptococcus pneumoniae*," Infect. Immun., 71:75-85 (2003); Neeleman et al., "Resistance to Both Complement Activation and Phagocytosis in Type 3 Pneumococci is Mediated by Binding of Complement Regulatory Protein Factor H," Infect. Immun., 67:4517-4524 (1999)). PspA's inhibition of complement deposition may be particularly important to PspA's virulence role in bacteremia and sepsis. PspA also binds apolactoferrin, and the binding of PspA to apolactoferrin is able to reduce the killing of pneumococci by apolactoferrin (Hammerschmidt et al., "Identification of Pneumococcal Surface Protein A as a Lactoferrin-Binding Protein of *Streptococcus pneumoniae*," Infect. Immun., 67:1683-1687 (1999); Hakansson et al., "Characterization of the Binding of Human Lactoferrin to Pneumococcal Surface Protein A (PspA)," Infect Immun., 69:3372-3381 (2001)). This role of PspA may be particularly important in the colonization of the upper airways.

[0018] The most structurally variable region of PspA is in the alpha-helical region, which contains the immunogenic epitopes (Hollingshead et al., "Diversity of PspA: Mosaic Genes and Evidence for Past Recombination in *Streptococcus pneumoniae*," Infect. Immun., 68:5889-5900 (2000)). Based on its DNA and amino acid sequences, PspAs have been divided into PspA families 1 and 2 (Hollingshead et al., "Diversity of PspA: Mosaic Genes and Evidence for Past Recombination in *Streptococcus pneumoniae*," Infect. Immun., 68:5889-5900 (2000)). At least 97% of the over 2000 PspAs from pneumococci that has been examined from around the world fall into these two families (Hollingshead et al., "Diversity of PspA: Mosaic Genes and Evidence for Past Recombination in *Streptococcus pneumoniae*," Infect. Immun., 68:5889-5900 (2000); Coral et al., "Families of Pneumococcal Surface Protein A (PspA) of *Streptococcus pneumoniae* Invasive Isolates Recovered from Colombian Children," Emerging Infectious Diseases., 7:832-836 (2001)). Twenty six strains of PspAs from SCDP have been examined, where it has been found that the PspAs were all in either PspA family 1 or family 2.

[0019] Although PspA exhibits antigenic variation (Crain et al., "Pneumococcal Surface Protein A (PspA) is Serologically Highly Variable and is Expressed by All Clinically Important Capsular Serotypes of *Streptococcus pneumoniae*," Infect. Immun., 58:3293-3299 (1990)), it is also highly cross-reactive

(Briles et al., "Immunization of Humans with rPspA Elicits Antibodies, which Passively Protect Mice from Fatal Infection with *Streptococcus pneumoniae* Bearing Heterologous PspA," J Infect Dis., 182:1694-701 (2000); Crain et al., "Pneumococcal Surface Protein A (PspA) is Serologically Highly Variable and is Expressed by All Clinically Important Capsular Serotypes of *Streptococcus pneumoniae*," Infect. Immun., 58:3293-3299 (1990); Nabors et al., "Immunization of Healthy Adults with a Single Recombinant Pneumococcal Surface Protein A (PspA) Variant Stimulates Broadly Cross-Reactive Antiboides," Vaccine, 18:1743-1754 (2000)). Immune sera elicited to the alpha helical region of a single family 1 PspA cross react with virtually all PspAs of family 1 or family 2 (Crain et al., "Pneumococcal Surface Protein A (PspA) is Serologically Highly Variable and is Expressed by All Clinically Important Capsular Serotypes of *Streptococcus pneumoniae*," Infect. Immun., 58:3293-3299 (1990); Nabors et al., "Immunization of Healthy Adults with a Single Recombinant Pneumococcal Surface Protein A (PspA) Variant Stimulates Broadly Cross-Reactive Antibodies," Vaccine, 18:1743-1754 (2000)). Moreover, immune responses to a PspA of one family can protect against strains bearing PspAs of either family Briles et al., "The Potential for Using Protein Vaccines to Protect Against Otitis Media Caused by *Streptococcus pneumoniae*," Vaccine, 19:S87-S95 (2001); Briles et al., "Immunization of Humans with rPspA Elicits Antibodies, which Passively Protect Mice from Fatal Infection with *Streptococcus pneumoniae* Bearing Heterologous PspA," J Infect Dis., 182:1694-701 (2000)). These findings make it very likely that broadly protective PspA-containing vaccines can be developed by including only one or two PspAs. In the mouse, antibody to PspA can also protect against carriage and pneumonia. In the case of carriage, the best protection is elicited by immunization with a mixture of PsaA and PspA. In the case of pneumonia and sepsis, a combination of PspA and pneumolysin is often better than either immunogen alone (Briles et al., "Intranasal Immunization of Mice with a Mixture of the Pneumococcal Proteins PsaA and PspA is Highly Protective Against Nasopharyngeal Carriage of *Streptococcus pneumoniae*," Infect Immun., 68:796-800 (2000); Ogunniyi et al., "Immunization of Mice with Combinations of Pneumococcal Virulence Proteins Elicits Enhanced Protection Against Challenge with *Streptococcus pneumoniae*," Infect. Immun., 68:3028-3033 (2000)).

[0020] The best way to prevent the deleterious effects of pneumococcal infections in SCDPs would be the development of an effective, affordable pneumococcal vaccine that could compensate for the high disease susceptibility of SCDP. Although the 7-valent polysaccharide-protein conjugate vaccine offers type-specific protection against bacteremia, it has several limitations: (1) it offers protection against only 7 of the 90 different capsular types; (2) the use of the vaccine is increasing the incidence of infections with the non-vaccine capsular types; and (3) it is far too expensive for use in the developing world. It is not yet known how protective the conjugate vaccine will be in patients without splenic function. Moreover, the need for an effective vaccine is heightened by the rise of antibiotic resistance among pneumococci, which compromises prophylactic antibiotic therapy of SCDP.

[0021] The present invention is directed to achieving these objectives.

SUMMARY OF THE INVENTION

[0022] The present invention relates to a method of treating *Streptococcus pneumoniae* infection in a subject lacking a functional spleen. The method involves administering to the subject lacking a functional spleen an antibody that recognizes pneumococcal surface protein A (PspA) or a binding portion thereof under conditions effective to treat *Streptococcus pneumoniae* infection in the subject.

[0023] The present invention also relates to a method of immunization against *Streptococcus pneumoniae* infection in a subject lacking a functional spleen. The method involves administering to the subject lacking a functional spleen pneumococcal surface protein A (PspA) or a fragment thereof under conditions effective to elicit immunity that can prevent *Streptococcus pneumoniae* infection in the subject.

[0024] The present invention provides evidence that passive antibody to PspA can protect subjects without spleens from fatal infection with *Streptococcus pneumoniae* and that immunizing splenectomized subjects with PspA can give protection against infection with *Streptococcus pneumoniae*. This suggests that PspA can be used as an efficacious immunogen in immuno-compromised individuals such

as those with sickle anemia or spleen dysfunctions, which may be important in developing countries.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] Figure 1 shows modular diagrams of representative PspA and PspA fragments that can be used for the method of the present invention.

[0026] Figure 2 shows titers of serum antibody to PspA in splenectomized and mock splenectomized mice following systemic immunization with purified UAB103.

[0027] Figure 3 shows survival time of immunized, splenectomized and mock splenectomized infected mice following intravenous infection with *S. pneumoniae*.

DETAILED DESCRIPTION OF THE INVENTION

[0028] The present invention relates to a method of treating *Streptococcus pneumoniae* infection in a subject lacking a functional spleen. The method involves administering to the subject lacking a functional spleen an antibody that recognizes pneumococcal surface protein A (PspA) or a binding portion thereof under conditions effective to treat *Streptococcus pneumoniae* infection in the subject.

[0029] In one embodiment of the present invention, the subject is a human. The subject may lack a functional spleen due to trauma, such as automobile accidents or falls, where hemorrhage of the spleen caused by the trauma requires the spleen to be surgically removed to prevent internal bleeding. Alternatively, the subject may lack a functional spleen due to a genetic defect. The subject may also lack a functional spleen due to disease-associated injury. The disease associated injury can be derived from a hemolytic anemia disease such as sickle cell anemia disease. The disease associated injury can also be derived from leukemia or lymphoma such as Hodgkins disease, where the spleen is removed as a part of a diagnostic procedure.

[0030] The antibodies used in the present invention may be monoclonal or polyclonal. Monoclonal antibody production may be effected by techniques which are well-known in the art. Basically, the process involves first obtaining immune cells (lymphocytes) from the spleen of a mammal (e.g., mouse) which has been previously immunized with the antigen of interest either *in vivo* or *in vitro*. The

antibody-secreting lymphocytes are then fused with (mouse) myeloma cells or transformed cells, which are capable of replicating indefinitely in cell culture, thereby producing an immortal, immunoglobulin-secreting cell line. The resulting fused cells, or hybridomas, are cultured, and the resulting colonies screened for the production of the desired monoclonal antibodies. Colonies producing such antibodies are cloned, and grown either *in vivo* or *in vitro* to produce large quantities of antibody. A description of the theoretical basis and practical methodology of fusing such cells is set forth in Kohler and Milstein, "Continuous Culture of Fused Cells Secreting Antibody of Predefined Specificity," Nature, 256:495-7 (1975), which is hereby incorporated by reference in its entirety.

[0031] Mammalian lymphocytes are immunized by *in vivo* immunization of the animal (e.g., a mouse) with the protein or polypeptide used in the present invention. Such immunizations are repeated as necessary at intervals of up to several weeks to obtain a sufficient titer of antibodies. Following the last antigen boost, the animals are sacrificed and spleen cells removed.

[0032] Fusion with mammalian myeloma cells or other fusion partners capable of replicating indefinitely in cell culture is effected by standard and well-known techniques, for example, by using polyethylene glycol ("PEG") or other fusing agents (Milstein et al., "Derivation of Specific Antibody-Producing Tissue Culture and Tumor Lines by Cell Fusion," Eur. J. Immunol., 6:511-19 (1976), which is hereby incorporated by reference in its entirety). This immortal cell line, which may be derived from cells of any mammalian species, including, but not limited to, mouse, rat, and human, is selected to be deficient in enzymes necessary for the utilization of certain nutrients, to be capable of rapid growth, and to have good fusion capability. Many such cell lines are known to those skilled in the art, and others are regularly described.

[0033] Procedures for raising polyclonal antibodies are also well known. Typically, such antibodies can be raised by administering the protein or polypeptide of the present invention subcutaneously to New Zealand white rabbits which have first been bled to obtain pre-immune serum. The antigens can be injected at a total volume of 100 l per site at six different sites. Each injected material will contain synthetic surfactant adjuvant pluronic polyols, or pulverized acrylamide gel containing the protein or polypeptide after SDS-polyacrylamide gel electrophoresis.

The rabbits are then bled two weeks after the first injection and periodically boosted with the same antigen three times every six weeks. A sample of serum is then collected 10 days after each boost. Polyclonal antibodies are then recovered from the serum by affinity chromatography using the corresponding antigen to capture the antibody. Ultimately, the rabbits are euthenized with pentobarbital 150 mg/Kg IV. This and other procedures for raising polyclonal antibodies are disclosed in E. Harlow, et. al., Editors, Antibodies: a Laboratory Manual (1988), which is hereby incorporated by reference in its entirety.

[0034] The present invention also relates to a method of immunization against *Streptococcus pneumoniae* infection in a subject lacking a functional spleen. The method involves administering to the subject lacking a functional spleen pneumococcal surface protein A (PspA) or a fragment thereof under conditions effective to elicit immunity that can prevent *Streptococcus pneumoniae* infection in the subject.

[0035] A suitable PspA molecule in accordance with the present invention is from the Rx1 strain and has an amino acid sequence of SEQ ID NO: 1 (GenBank Accession No. AAC62252; Hollingshead et al., "Diversity of PspA: Mosaic Genes and Evidence for Past Recombination in *Streptococcus pneumoniae*," Infect. Immun., 68(10):5889-5900 (2000), which is hereby incorporated by reference in its entirety), as follows:

MNKKKMILTSLASVAILGAGFVASSPTFVRAEEAPVANQSKAEKDYDAAVKKSEAAKKDY
ETAKKKAEDAQKKYDEDQKKTEAKAEKERKASEKIAEATKEVQQAYLAYLQASNESQRKE
ADKKIKEATQRKDEAEAAFATIRTTIVVPEPSELAETKKKAEEATKEAEVAKKKSEEAAK
EVEVEKNKILEQDAENEKKIDVLQNKVADLEKGIAPYQNEVAELNKEIARLQSDLKDAEE
NNVEDYIKEGLEQAITNKKAELATTQQNIDKTQKDLEDAELELEKVLATLDPEGKTQDEL
DKEAAEAELNEKVEALQNQVAELEEELSKLEDNLKDAETNNVEDYIKEGLEEAIATKKAE
LEKTQKELDAALNELGPDGDEEETPAPAPQPEKPAEEPENPAPAPKPEKSADQQAEEDYA
RRSEEEYNRLTQQQPPKAEKPAPAPQPEQPAPAPKIGWKQENGMWYFYNTDGSMATGWLQ
NNGSWYYLNSNGAMATGWLQYNGSWYYLNANGAMATGWLQYNGSWYYLNANGAMATGWLQ
YNGSWYYLNANGDMATGWLQYNGSWYYLNANGDMATGWAKVHGSWYYLNANGSMATGWVK
DGETWYYLEASGSMKANQWFQVSDKWYYVNGLGSLSVNTTVDGYKVNANGEWV

[0036] Another suitable PspA molecule in accordance with the present invention is from the EF3296 strain and has an amino acid sequence of SEQ ID NO: 3 (GenBank Accession No. AF071816; Hollingshead et al., "Diversity of PspA: Mosaic Genes and Evidence for Past Recombination in *Streptococcus pneumoniae*," Infect.

Immun., 68(10):5889-5900 (2000), which is hereby incorporated by reference in its entirety), as follows:

MNKKKMILTSLASVAILGAGLVTSQPTFVRAEESPQVVEKSSLEKKYEEAKAKADTAKKD
YETAKKKAEDAQKKYEDDQKRTEEKARKEAEASQKLNDVALVVQNAYKEYREVQNQRSKY
KSDAEYQKKLTEVDSKIEKARKEQQDLQNKFNEVRAVVVPEPNALAETKKKAEEAKAEEK
VAKRKYDYATLKVALAKKEVEAKELEIEKLQYEISTLEQEVATAQHQVDNLKKLLAGADP
DDGTEVIEAKLKKGEAELNAKQAELAKKQTELEKLLDSLDPEGKTQDELDKEAEEAELDK
KADELQNKVADLEKEISNLEILLGGADPEDDTAALQNKLAAKKAELAKKQTELEKLLDSL
DPEGKTQDELDKEAEEAELDKKADELQNKVADLEKEISNLEILLGGADSEDDTAALQNKL
ATKKAELEKTQKELDAALNELGPDGDEEETPAPAPQPEQPAPAPKPEQPAPAPKPEQPAP
APKPEQPAPAPKPEQPAPAPKPEQPAKPEKPAEEPTQPEKPATPKT

The corresponding nucleotide sequence for SEQ ID NO: 3 is also disclosed in http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=nucleotide&list_uids=6752402&dopt=GenBank, as GenBank Accession No. AF071816, which is hereby incorporated by reference in its entirety.

[0037] The PspA protein or PspA fragment can be recombinantly produced by methods well known in the art. Typically, the proteins or polypeptides used in the present invention are secreted into the growth medium of recombinant *E. coli*. To isolate the desired protein, the *E. coli* host cell carrying a recombinant plasmid is propagated, homogenized, and the homogenate is centrifuged to remove bacterial debris. The supernatant is then subjected to sequential ammonium sulfate precipitation. The fraction containing the desired protein of the present invention is subjected to gel filtration in an appropriately sized dextran or polyacrylamide column to separate the proteins. If necessary, the protein fraction may be further purified by HPLC. Alternative methods may be used as suitable.

[0038] Mutations or variants of the above polypeptides or proteins are encompassed by the present invention.

[0039] Variants may be modified by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure, and hydropathic nature of the desired polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide.

[0040] Fragments of the above proteins are also encompassed by the present invention. Suitable fragments can be produced by several means. In the first, subclones of the gene encoding the desired protein of the present invention are produced by conventional molecular genetic manipulation by subcloning gene fragments. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or peptide.

[0041] In another approach, based on knowledge of the primary structure of the proteins of the present invention, fragments of the genes of the present invention may be synthesized by using the polymerase chain reaction ("PCR") technique together with specific sets of primers chosen to represent particular portions of the protein. These then would be cloned into an appropriate vector for increased expression of an accessory peptide or protein.

[0042] Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences for the proteins of the present invention. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE) and used in the methods of the present invention.

[0043] Thus, in another embodiment of the present invention, a fragment of PspA can be administered. The fragment can contain α-helical domains and proline rich regions of PspA. A suitable PspA fragment molecule in accordance with the present invention, identified herein as UAB103 (Brooks-Walter et al., "The *pspC* Gene of *Streptococcus pneumoniae* Encodes a Polymorphic Protein, PspA, Which Elicits Cross-Reactive Antibodies to PspA and Provides Immunity to Pneumococcal Bactermia," Infect. Immun., 67(12): 6533-6542 (1999), which is hereby incorporated by reference in its entirety), contains the α-helical region as well as the entire proline-rich region of PspA from Rx1 strain (amino acid 1-370 of the mature PspA protein) and has an amino acid sequence of SEQ ID NO: 2, as follows:

EEAPVANQSKAEKDYDAAVKKSEAAKKDYETAKKKAEDAQKKYDEDQKKTEAKAEKERK
ASEKIAEATKEVQQAYLAYLQASNESQRKEADKKIKEATQRKDEAEAAFATIRTTIVVPE
PSELAETKKKAEEATKEAEVAKKKSEEAAKEVEVEKNKILEQDAENEKKIDVLQNKVADL
EKGIAPYQNEVAELNKEIARLQSDLKDAEENNVEDYIKEGLEQAITNKKAELATTQQNID
KTQKDLEDAELELEKVLATLDPEGKTQDELDKEAAEAELNEKVEALQNQVAELEEELSKL
EDNLKDAETNNVEDYIKEGLEEAIATKKAELEKTQKELDAALNELGPDGDEEETPAPAPQ
PEKPAEEPEN

[0044] Another suitable PspA fragment molecule in accordance with the present invention contains part of the α-helical region as well as the entire proline-rich region of PspA from the EF3296 strain (amino acid 75-490 of the mature PspA protein) and has an amino acid sequence of SEQ ID NO: 4, as follows:

AYKEYREVQNQRSKYKSDAEYQKKLTEVDSKIEKARKEQQDLQNKFNEVRAVVVPEPNAL
AETKKKAEEAKAEEKVAKRKYDYATLKVALAKKEVEAKELEIEKLQYEISTLEQEVATAQ
HQVDNLKKLLAGADPDDGTEVIEAKLKKGEAELNAKQAELAKKQTELEKLLDSLDPEGKT
QDELDKEAEEAELDKKADELQNKVADLEKEISNLEILLGGADPEDDTAALQNKLAAKKAE
LAKKQTELEKLLDSLDPEGKTQDELDKEAEEAELDKKADELQNKVADLEKEISNLEILLG
GADSEDDTAALQNKLATKKAELEKTQKELDAALNELGPDGDEEETPAPAPQPEQPAPAPK
PEQPAPAPKPEQPAPAPKPEQPAPAPKPEQPAPAPKPEQPAKPEKPAEEPTQPEKP

[0045] In other embodiments of the present invention, the administering of PspA protein or PspA antibody is carried out orally, parenterally, subcutaneously, intravenously, intramuscularly, intraperitoneally, by intravesical instillation, by intracavitary, intravesical instillation, intraocularly, intraarterially, intralesionally, or by application to mucous membrane.

[0046] The PspA- or PspA antibody-containing compositions can be in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose, or the like. The compositions can also be lyophilized. The compositions can contain auxiliary substances such as wetting or emulsifying agents, pH buffering agents, gelling or viscosity enhancing additives, preservatives, flavoring agents, colors, and the like, depending upon the route of administration and the preparation desired. Standard texts, such as " REMINGTON'S PHARMACEUTICAL SCIENCE", 17th edition (1985), which is hereby incorporated by reference in its entirety, may be consulted to prepare suitable preparations without undue experimentation. The compositions can be conveniently provided as liquid preparations, e.g., isotonic aqueous solutions, suspensions, emulsions, or viscous compositions which may be buffered to a selected pH. If digestive tract absorption is preferred, the compositions can be in the "solid" form of pills, tablets, capsules, caplets, and the like, including "solid" preparations which are time-released or which have a liquid filling, e.g., gelatin covered liquid whereby the gelatin is dissolved in the stomach for delivery to the gut. If nasal or respiratory (mucosal) administration is desired, compositions may be in a form dispensed by a squeeze spray dispenser, pump

dispenser, or aerosol dispenser. Aerosols are usually under pressure by means of a hydrocarbon. Pump dispensers can preferably dispense a metered dose or a dose having a particular particle size. The compositions can contain pharmaceutically acceptable flavors and/or colors for rendering them more appealing, especially if they are administered orally. The viscous compositions may be in the form of gels, lotions, ointments, creams, and the like and will typically contain a sufficient amount of a thickening agent so that the viscosity is from about 2500 to 6500 cps, although more viscous compositions even up to 10,000 cps may be employed. Viscous compositions have a viscosity preferably of 2500 to 5000 cps, since they become more difficult to administer above that range. However, above that range, the compositions can approach solid or gelatin forms which are then easily administered as a swallowed pill for oral ingestion. Liquid preparations are normally easier to prepare than gels, other viscous compositions, and solid compositions. Additionally, liquid compositions are somewhat more convenient to administer, especially by injection or orally, to animals, children, particularly small children, and others who may have difficulty swallowing a pill, tablet, capsule or the like, or in multi-dose situations. Viscous compositions, on the other hand, can be formulated within the appropriate viscosity range to provide longer contact periods with mucosa, such as the lining of the stomach or nasal mucosa. The choice of suitable carriers and other additives will obviously depend on the exact route of administration and the nature of the particular dosage form, e.g., liquid dosage form (e.g., whether the composition is to be formulated into a solution, a suspension, gel, or another liquid form), or solid dosage form (e.g., whether the composition is to be formulated into a pill, tablet, capsule, caplet, time release form, or liquid-filled form). Those skilled in the art will recognize that the components of the compositions must be selected to be chemically inert with respect to PspA immunogens or PspA antibodies.

[0047] The amount of PspA protein or PspA antibody to be administered to a mammal infected with *Streptococcus pneumoniae* can be determined in accordance with standard techniques well known to those of ordinary skill in the arts, taking into consideration such factors such as the particular antigen, the age, sex, weight, species and condition of the particular animal or patient, and the route of administration. Specifically, the dosage can be between 0.1 mg to 3 mg per kg of body weight. The optimal dosage will depend on the post-infection time of administration. For

example, an efficacious dosage will require less antibody if the post-infection time elapsed is less since there is less time for the bacteria to proliferate. In like manner, an efficacious dosage will depend on the bacterial load at the time of diagnosis. Multiple injections administered over a period of days could be considered for therapeutic usage.

EXAMPLES

[0048] The following examples are provided to illustrate embodiments of the present invention but are by no means intended to limit its scope.

Example 1 – Effect on Splenectomy on Pneumococcal Infections and the Protective Effect of Antibody to PspA in Surgically Asplenic Mice

[0049] Although passive antibody to PspA provides protection against intravenous challenge with *S. pneumoniae,* it does not mediate opsonophagocytosis *in vitro* and killing *in vitro* in the presence of phagocytes (Briles et al., "Antipneumococcal Effects of C-Reactive Protein and Monoclonal Antibodies to Pneumococcal Cell Wall and Capsular Antigens," Infect. Immun., 57:1457-1464 (1989), which is hereby incorporated by reference in its entirety). This result suggests that part of the protective mechanism of anti-PspA relies on *in vivo* events. Because of the known importance of splenic clearance for the removal of pneumococci from the blood, it is possible that antibody to PspA might not be effective in the absence of a spleen. If this is the case, a PspA vaccine might not be effective in SCDP. To investigate this, passive protection in surgically asplenic (SAS) mice was examined. Four to six week old BALB/cByJ mice were splenectomized or mock splenectomized. In the SAS mice, the spleen was surgically exposed and a hot wire was used to remove the spleen by severing the blood and lymph vessels coming from it. The incisions were sealed with surgical staples and the mice were allowed 3 weeks to recover from the surgery before infection. *S. pneumoniae* strain WU2 (at 3 x 10^4 CFU i.v.) was significantly less virulent in BALB/cByJ mice with spleens (mock splenectomy) than in BALB/cByJ mice lacking spleens (Table 1). These results were consistent with earlier studies with mice (Shinefield et al., "Effect of Splenectomy on the Susceptibility of Mice Inoculated with *Diplococcus pneumoniae*," J Exp Med., 123:777-94 (1966), which is hereby incorporated by reference in its entirety) and

observations that humans lacking spleens are at increased risk of pneumococcal infection (Buchanan, "Infection," In Embury, eds., Sickle Cell Disease: Basic Principles and Clinical Practice, New York, New York:Raven Press, p. 567-587 (1994), which is hereby incorporated by reference in its entirety).

Table 1. Passive Protection[1] vs. i.v. Challenge with Capsular Type 3 *S. pneumoniae* in Mice With and Without Spleens

Challenge	Spleen[2]	Number	Days to Death			% alive		
		of mice[3]	a-PspA	no Ab	*P*[4]	a-PspA	no Ab	*P*[4]
3 x 10^4 WU2	no	14, 14	>21	4	**0.004**	100	36	**0.006**
"	yes (m)	17, 18	>21	>21[5]	0.4	100	83	0.23
3 x 10^6 WU2	no	6, 6	>21	1	**0.02**	67	0	0.06
"	yes (m)	12, 5	>21	2	**0.007**	67	0	**0.03**
1 x 10^6 A66.1	no	13 , 5	6[6]	1	**0.01**	42[6]	0	0.2
"	yes (m)	12, 5	>21	2	**0.01**	91	20	**0.01**
"	yes (ns)	13, 5	>21	3	**0.003**	82	0	**0.048**

[1] protection by 0.1 ml of immune rabbit serum to a clade 2 family 1 PspA (Rx1);
[2] m = mock splenectomy, ns = no surgery;
[3] 1st number, number of mice given immune rabbit serum; 2nd number, mice given pre-immune serum;
[4] two tailed *P*-value for days to death, by Wilcoxon; % alive, by Fisher's exact test;
[5] days to death of asplenic vs. splenic mice infected at 3 x 10^4 WU2, $P = 0.012$;
[6] days to death of anti-PspA treated asplenic vs. splenic mice infected at 1 x 10^6 A66.1, $P=0.030$; for % alive the *P* value was 0.015.

[0050] To examine the effect of splenectomy on passive protection, mice were injected intraperitoneally with immune sera containing 8 μg of antibody to PspA or a comparable volume of pre-immune sera followed by intravenous challenge with pneumococci 1 hr later. Blood was collected from infected animals by retro-orbital puncture. Mice were monitored for survival for 21 days. Passive antibody to PspA protected SAS mice. At the highest challenge dose of WU2 used (3 x 10^6 CFU), equal protection against pneumococcal infection in SAS and normal mice was observed. In addition, mice were challenged with 1 x 10^6 CFU of strain A66.1, the most virulent capsular type 3 strain in the collection. Although anti-PspA mediated a significant delay in time to death and increase in % survival, the percentage survival in anti-PspA treated SAS mice was not as high as with normal mice treated with

antibody to PspA ($P = 0.015$). Thus, although antibody to PspA can protect asplenic mice, it did not necessarily bring them to the same level of protection as does the same amount of antibody in normal mice. Even with the A66.1 infection, however, the asplenic mice given antibody to PspA were more resistant than the un-protected mice with intact spleens.

Example 2 –Protective Effects of Active Immunity to PspA in Surgically Asplenic Mice

[0051] Four to six week old BALB/cByJ mice were splenectomized or mock splenectomized. In the SAS mice, the spleen was surgically exposed and a hot wire was used to remove the spleen by severing the blood and lymph vessels coming from it. The incisions were sealed with surgical staples and the mice were allowed to recover from the surgery before infection. Mice were immunized subcutaneously with 1 μg of purified UAB103 (Brooks-Walter et al., "The pspC Gene of *Streptococcus pneumoniae* Encodes a Polymorphic Protein, PspC, Which Elicits Cross-Reactive Antibodies to PspA and Provides Immunity to Pneumococcal Bacteremia," Infect. Immun., 67:6533-6542 (1999), which is hereby incorporated by reference in its entirety), using Alum (50 μg) as an adjuvant. Animals were given a first boost 2 weeks after a primary immunization and a second boost 2 weeks following the first boost. Blood was collected by retro-orbital puncture 12 days following the last boost. Mice were infected intravenously with $2.2x10^6$ CFUs via the tail vein, 48 hours following blood collection. Animals were monitored daily and their times of death were recorded.

[0052] Following immunization with PspA, splenectomized mice mounted immune response similar to that of mock splenectomized mice (Figure 2). Antibody titers, which were determined using ELISA, in splenectomized mice immunized with PspA were significantly higher than splenectomized mice receiving only Alum (Figure 2). Antibodies produced to PspA by splenectomized mice were capable of protecting mice against death following infection with $2.2x10^6$ CFUs of WU2 (Figure 3). Survival time in splenectomized mice immunized with PspA was significantly longer than in splenectomized mice receiving adjuvant alone (Figure 3).

[0053] Although the invention has been described in detail, for the purpose of illustration, it is understood that such detail is for that purpose and variations can be

made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.

WHAT IS CLAIMED:

1. A method of treating *Streptococcus pneumoniae* infection in a subject lacking a functional spleen, said method comprising:

administering to the subject lacking a functional spleen an antibody that recognizes pneumococcal surface protein A (PspA) or a binding portion thereof under conditions effective to treat *Streptococcus pneumoniae* infection in the subject.

2. The method according to claim 1, wherein the subject lacks a functional spleen due to trauma, genetic defect, or disease-associated injury.

3. The method according to claim 2, wherein the disease associated injury is derived from a disease selected from the group consisting of hemolytic anemia disease, leukemia, and lymphoma.

4. The method according to claim 3, wherein the disease is sickle cell anemia disease.

5. The method according to claim 3, wherein the disease is Hodgkins disease.

6. The method according to claim 1, wherein the subject is a human.

7. The method according to claim 1, wherein said administering is carried out orally, parenterally, subcutaneously, intravenously, intramuscularly, intraperitoneally, by intravesical instillation, by intracavitary, intravesical instillation, intraocularly, intraarterially, intralesionally, or by application to mucous membrane.

8. A method of immunization against *Streptococcus pneumoniae* infection in a subject lacking a functional spleen comprising:

administering to the subject lacking a functional spleen pneumococcal surface protein A (PspA) or a fragment thereof under conditions effective to elicit immunity that can prevent *Streptococcus pneumoniae* infection in the subject.

9. The method according to claim 8, wherein the subject lacks a functional spleen due to trauma, genetic defect, or disease associated injury.

10. The method according to claim 9, wherein the disease associated injury is derived from a disease selected from the group consisting of hemolytic anemia disease, leukemia, and lymphoma.

11. The method according to claim 10, wherein the disease is sickle cell anemia disease.

12. The method according to claim 10, wherein the disease is Hodgkins disease.

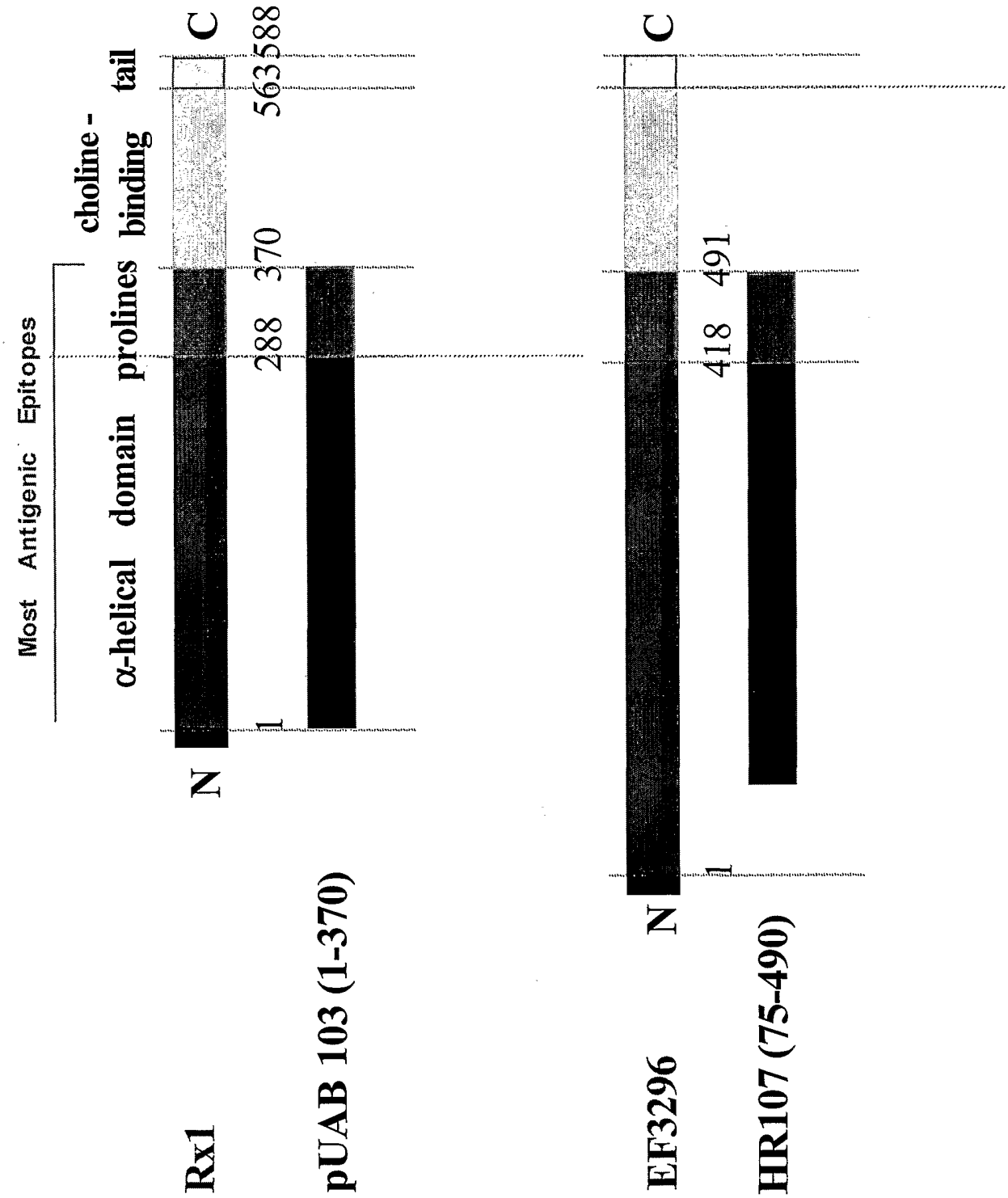
13. The method according to claim 8, wherein said administering is carried out orally, parenterally, subcutaneously, intravenously, intramuscularly, intraperitoneally, by intravesical instillation, by intracavitary, intravesical instillation, intraocularly, intraarterially, intralesionally, or by application to mucous membrane.

14. The method according to claim 8, wherein the pneumococcal surface protein A or fragment is recombinantly produced.

15. The method according to claim 8, wherein a fragment of pneumococcal surface protein A is administered.

16. The method according to claim 15, wherein the fragment contains α-helical domains and proline rich epitopic regions of pneumococcal surface protein A.

17. A method according to claim 8, wherein the pneumococcal surface protein A or fragment thereof is selected from the group consisting of a protein having an amino acid sequence of SEQ. ID. No. 1, a protein having an amino acid of SEQ. ID. No. 2, a protein having an amino acid sequence of SEQ. ID. No. 3, and a protein having an amino acid sequence of SEQ. ID. No. 4.


Most Antigenic Epitopes
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choline - binding
tail
C
N
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288
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C
N
1
418
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EF3296
HR107 (75-490)

Figure 1

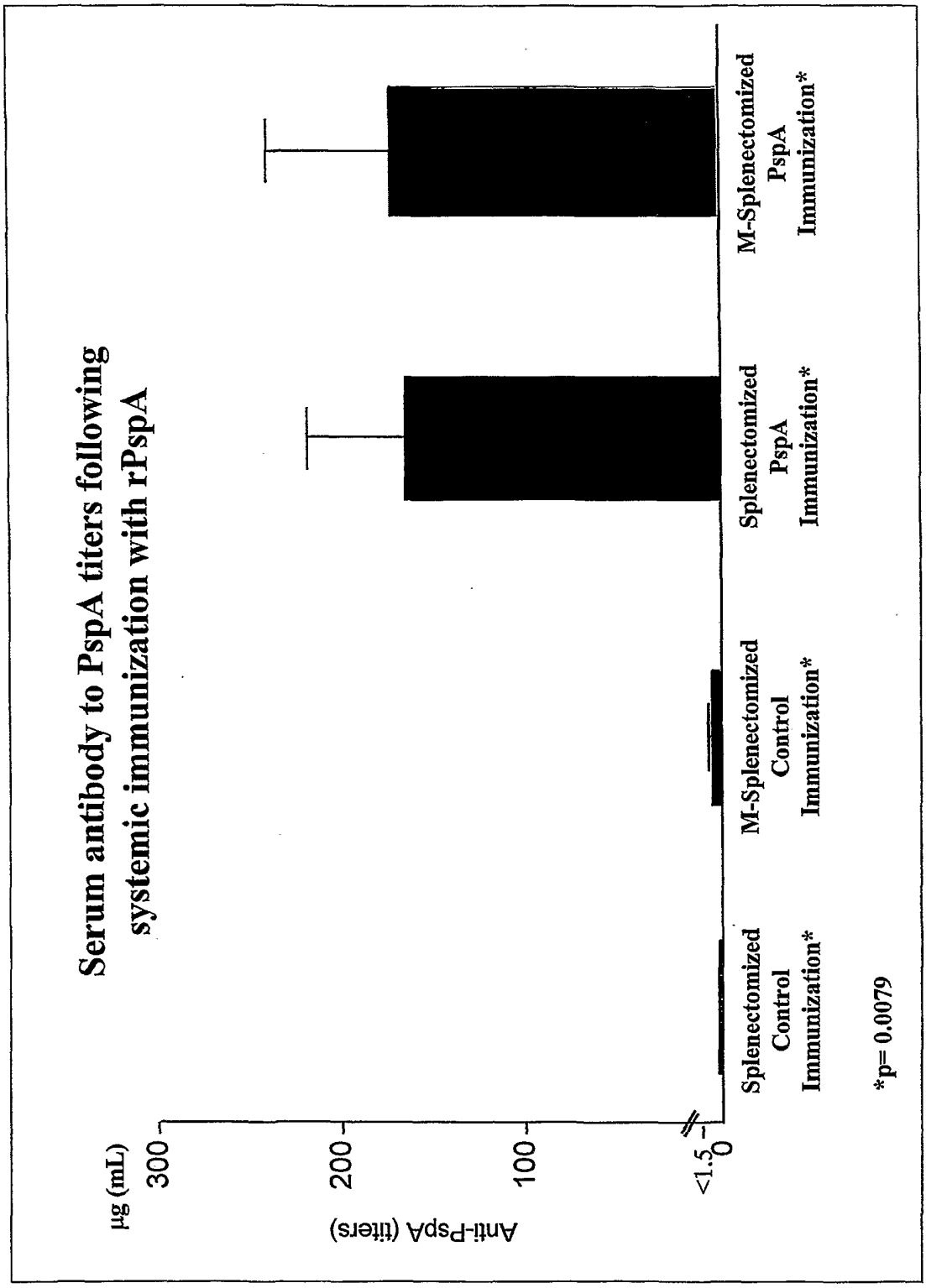
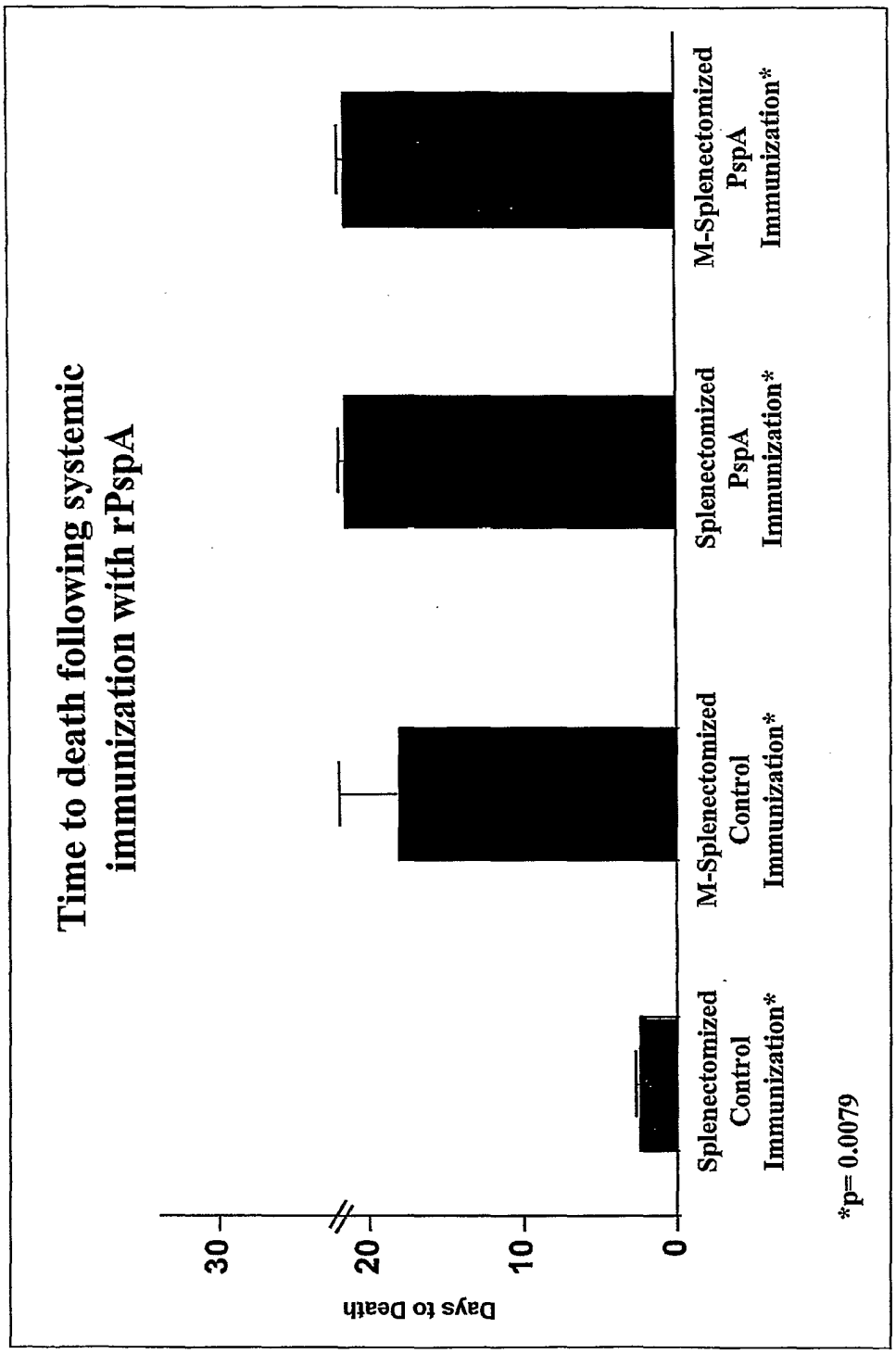

Serum antibody to PspA titers following systemic immunization with rPspA
Anti-PspA (titers)
μg (mL)
300
200
100
<1.5
0
Splenectomized Control Immunization*
M-Splenectomized Control Immunization*
Splenectomized PspA Immunization*
M-Splenectomized PspA Immunization*
*p= 0.0079

Figure 2


Time to death following systemic immunization with rPspA
Days to Death
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10
20
30
Splenectomized Control Immunization*
M-Splenectomized Control Immunization*
Splenectomized PspA Immunization*
M-Splenectomized PspA Immunization*
*p= 0.0079

Figure 3

SEQUENCE LISTING

<110> University of Alabama at Birmingham

<120> PROTECTION OF PATIENTS LACKING A FUNCTIONAL SPLEEN AGAINST S. PNEUMONIAE WITH PNEUMOCOCCAL SURFACE PROTEIN A PspA

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<151> 2002-03-15

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                 85                  90                  95

Glu Ala Thr Lys Glu Val Gln Gln Ala Tyr Leu Ala Tyr Leu Gln Ala
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Ser Asn Glu Ser Gln Arg Lys Glu Ala Asp Lys Lys Ile Lys Glu Ala
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Thr Leu Asp Pro Glu Gly Lys Thr Gln Asp Glu Leu Asp Lys Glu Ala
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325 330 335

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Lys Thr Glu Ala Lys Ala Glu Lys Glu Arg Lys Ala Ser Glu Lys Ile
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