(12) PATENT
(19) AUSTRALIAN PATENT OFFICE

(11) Application No. AU 200023731 B2
(10) Patent No. 776828

(54) Title
Streptococcus pneumoniae proteins and immunogenic fragments for vaccines

(51)6 International Patent Classification(s)
A61K 039/09 A61P 031/04
A61K 039/40 C07K 014/315

(21) Application No: 200023731 (22) Application Date: 1999.12.21

(87) WIPO No: WO00/37105

(30) Priority Data

(31) Number	(32) Date	(33) Country
60/113048	1998.12.21	US

(43) Publication Date : 2000.07.12
(43) Publication Journal Date : 2000.09.07
(44) Accepted Journal Date : 2004.09.23

(71) Applicant(s)
MedImmune, Inc.

(72) Inventor(s)
Leslie S. Johnson; Scott Koenig; John E. Adamou

(74) Agent/Attorney
SPRUSON and FERGUSON,GPO Box 3898,SYDNEY NSW 2001

(56) Related Art
SEE REFERENCES OF WO 2000/037105 A3

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
A61K 39/09, 39/40, A61P 31/04 // C07K 14/315

A2

(11) International Publication Number: **WO 00/37105**

(43) International Publication Date: 29 June 2000 (29.06.00)

(21) International Application Number: PCT/US99/30390

(22) International Filing Date: 21 December 1999 (21.12.99)

(30) Priority Data:
60/113,048 21 December 1998 (21.12.98) US

(71) Applicant: MEDIMMUNE, INC. [US/US]; 35 West Watkins Mill Road, Gaithersburg, MD 20878 (US).

(72) Inventors: JOHNSON, Leslie, S.; 13545 Ambassador Drive, Germantown, MD 20874 (US). KOENIG, Scott; 10732 Ralston Road, Rockville, MD 20852 (US). ADAMOU, John, E.; 20822 Shamrock Glen Circle, Germantown, MD 20874 (US).

(74) Agents: GRANT, Alan, J. et al.; Carella, Byrne, Bain, Gilfillan, Cecchi, Stewart & Olstein, 6 Becker Farm Road, Roseland, NJ 07068 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: STREPTOCOCCUS PNEUMONIAE PROTEINS AND IMMUNOGENIC FRAGMENTS FOR VACCINES

(57) Abstract

A vaccine composition is disclosed that comprises polypeptides and fragments of polypeptides containing histidine triad residues or coiled–coil regions, some of which polypeptides or fragments lie between 80 and 680 residues in length. Also disclosed are processes for preventing infection caused by *S. pneumoniae* comprising administering of vaccine compositions.

STREPTOCOCCUS PNEUMONIAE PROTEINS AND IMMUNOGENIC FRAGMENTS FOR VACCINES

FIELD OF THE INVENTION

This invention relates generally to the field of bacterial antigens and their use, for example, as immunogenic agents in humans and animals to stimulate an immune response. More specifically, it relates to the vaccination of mammalian species with a polypeptide comprising at least one conserved histidine triad residue (HxxHxH) and at least one helix-forming polypeptide obtained from *Streptococcus pneumoniae* as a mechanism for stimulating production of antibodies that protect the vaccine recipient against infection by a wide range of serotypes of pathogenic *S. pneumoniae*. Further, the invention relates to antibodies against such polypeptides useful in diagnosis and passive immune therapy with respect to diagnosing and treating such pneumococcal infections.

In a particular aspect, the present invention relates to the prevention and treatment of pneumococcal infections such as infections of the middle ear, nasopharynx, lung and bronchial areas, blood, CSF, and the like, that are caused by pneumococcal bacteria.

Background of the Invention

Streptococcus pneumoniae is a gram positive bacteria which is a major causative agent in invasive infections in animals and humans, such as sepsis, meningitis, otitis media and lobar pneumonia (Tuomanen *et al. New Engl. J. Med.* **322**:1280-1284 (1995)). As part of the infective process, pneumococci readily bind to non-inflamed human epithelial cells of the upper and lower respiratory tract by binding to eukaryotic carbohydrates in a lectin-like manner (Cundell *et al., Micro. Path.* **17**:361-374 (1994)). Conversion to invasive pneumococcal infections for bound bacteria may involve the local generation of inflammatory factors which may activate the epithelial cells to change the number and type of receptors on their surface (Cundell *et al., Nature*, **377**:435-438 (1995)). Apparently, one such receptor, platelet activating factor (PAF) is engaged by the pneumococcal bacteria and within a very short period of time (minutes) from the appearance of PAF, pneumococci exhibit strongly enhanced adherence and invasion of tissue. Certain soluble receptor analogs have been shown to prevent the progression of pneumococcal infections (Idanpaan-Heikkila *et al., J. Inf. Dis.*, **176**:704-712 (1997)). A number of various other proteins have been suggested as being involved in the pathogenicity of *S. pneumoniae*. There remains a need for identifying polypeptides having epitopes in common from various strains of *S. pneumoniae* in order to utilize such polypeptides as vaccines to provide protection against a wide variety of *S. pneumoniae*.

Summary of the Invention

According to a first embodiment of the invention, there is provided a composition comprising a polypeptide at least 95% identical to amino acids 20-838 of SEQ ID NO:4 in a pharmaceutically acceptable carrier in an amount effective to elicit production of an antibody that binds to *S. pneumoniae* when said composition is administered to a mammal.

According to a second embodiment of the invention, there is provided a composition comprising an active fragment of a polypeptide at least 95% identical to amino acids 20-838 of SEQ ID NO:4 wherein said active fragment comprises at least two coiled coil regions and is in a pharmaceutically acceptable carrier in an amount effective to elicit production of an antibody that binds to said polypeptide when said composition is administered to a mammal.

According to a third embodiment of the invention, there is provided a composition comprising a polypeptide comprising the sequence of amino acid residues 139-791 of SEQ ID NO:4.

According to a fourth embodiment of the invention, there is provided a method for preventing infection caused by *S. pneumoniae* comprising administering the vaccine comprising the composition in accordance with any one of the first to third embodiments of the present invention.

According to a fifth embodiment of the invention, there is provided an isolated polypeptide at least 95% identical to amino acids 20-838 of SEQ ID NO:4 wherein said polypeptide binds to an antibody that binds to *S. pneumoniae*.

According to a sixth embodiment of the invention, there is provided a method for producing a polypeptide comprising an amino acid sequence that has at least 95% identity to amino acids 20-838 of SEQ ID NO:4 comprising expressing said polypeptide from a cell that comprises a polynucleotide containing a nucleotide sequence that encodes said polypeptide.

According to a seventh embodiment of the invention, there is provided a method for producing an active fragment of the polypeptide that comprises amino acids 20-838 of SEQ ID NO:4 comprising expressing said active fragment from a cell that contains a polynucleotide containing a nucleotide sequence that encodes said active fragment.

According to an eighth embodiment of the invention, there is provided an isolated polynucleotide comprising a nucleotide sequence having at least 90% identity to the sequence of SEQ ID NO:5.

According to a ninth embodiment of the invention, there is provided a vector comprising the isolated polynucleotide in accordance with the eighth embodiment of the present invention.

According to a tenth embodiment of the invention, there is provided a method for eliciting an immune response to *Streptococcus pneumoniae* in a mammal comprising administering to said mammal a polypeptide comprising an amino acid sequence that has at least 95% identity to amino acids 20-838 of SEQ ID NO:4.

According to an eleventh embodiment of the invention, there is provided a method for eliciting an immune response to *Streptococcus pneumoniae* in a mammal comprising administering to said mammal a polypeptide comprising an amino acid sequence that has at least 95% identity to amino acids 20-838 of SEQ ID NO:4.

According to a twelfth embodiment of the invention, there is provided a method for eliciting an immune response to *Streptococcus pneumoniae* in a mouse comprising administering to said mouse a polypeptide comprising an amino acid sequence that has at least 95% identity to amino acids 20-838 of SEQ ID NO:4.

According to a thirteenth embodiment of the invention, there is provided a method for eliciting an immune response to *Streptococcus pneumoniae* in a mammal comprising administering to said mammal a polypeptide comprising an active fragment of amino acids 20-838 of SEQ ID NO:4.

According to a fourteenth embodiment of the invention, there is provided a method for eliciting an immune response to *Streptococcus pneumoniae* in a mouse comprising administering to said mouse a polypeptide comprising an amino acid sequence that has at least 95% identity to amino acids 20-838 of SEQ ID NO:4.

According to a fifteenth embodiment of the invention, there is provided a composition comprising a polypeptide having at least 90% identity to amino acids 21-480 of SEQ ID NO:6,

wherein said polypeptide is in a pharmaceutically acceptable carrier in an amount effective to elicit production of an antibody that binds to *S. pneumoniae* when said composition is administered to a mammal.

According to a sixteenth embodiment of the invention, there is provided a composition comprising an active fragment of the polypeptide comprising amino acids 21-480 of SEQ ID NO:6,

wherein said active fragment comprises at least about 10% and no more than about 85% of said polypeptide, wherein said active fragment comprises at least one histidine triad and at least one coiled coil region and wherein said active fragment is in a pharmaceutically acceptable carrier in an amount effective to elicit production of an antibody that binds to *S. pneumoniae* when said composition is administered to a mammal.

According to a seventeenth embodiment of the invention, there is provided a vaccine comprising a polypeptide containing the sequence of amino acids 21-480 of SEQ ID NO:6 in a pharmaceutically acceptable carrier in an amount effective to protect against pneumococcal infection when administered to a mammal.

According to an eighteenth embodiment of the invention, there is provided an isolated polypeptide comprising at least 90% identity to amino acids 21-480 of SEQ ID NO:6, wherein said polypeptide binds to an antibody that binds to *S. pneumoniae*.

According to a nineteenth embodiment of the invention, there is provided a composition comprising at least one antibody that binds to a polypeptide comprising an amino acid sequence at least 90% identical to a member selected from the group consisting of:

(i) a sequence having at least 90% identity to amino acids 20-838 of SEQ ID NO:4 and

(ii) a sequence comprising amino acids 21-480 of SEQ ID NO:6,

wherein said antibody is in a pharmaceutically acceptable carrier.

According to a twentieth embodiment of the invention, there is provided a composition comprising at least one antibody that binds to an active fragment of a polypeptide comprising a member selected from the group consisting of:

(i) amino acids 20-838 of SEQ ID NO:4 and

(ii) amino acids 21-480 of SEQ ID NO:6,

wherein said active fragment comprises at least about 10% and no more than about 85% of said polypeptide, wherein said active fragment comprises at least one histidine triad and at least one coiled coil region and wherein said active fragment is in a pharmaceutically acceptable carrier in an amount effective to elicit production of an antibody that binds to *S. pneumoniae* when said composition is administered to a mammal.

According to a twenty-first embodiment of the invention, there is provided a process for treating or preventing infection caused by *S. pneumoniae* comprising administering to a mammal afflicted with said infection, or at risk of said infection, an effective amount of the composition in accordance with the twentieth embodiment of the present invention.

According to a twenty-second embodiment of the invention, there is provided a process for treating or preventing infection caused by *S. pneumoniae* comprising administering to a mammal afflicted with said infection, or at risk of said infection, an effective amount of the composition in accordance with the twenty-first embodiment of the present invention.

According to a twenty-third embodiment of the invention, there is provided the use of a polypeptide comprising an amino acid sequence that has at least 95% identity to amino acids 20-838 of SEQ ID NO:4 for the manufacture of a medicament for eliciting an immune response to *Streptococcus pneumoniae* in a mammal.

According to a twenty-fourth embodiment of the invention, there is provided the use of a polypeptide comprising an active fragment of amino acids 20-838 of SEQ ID

NO:4 for the manufacture of a medicament for eliciting an immune response to *Streptococcus pneumoniae* in a mammal.

According to a twenty-fifth embodiment of the invention, there is provided the use of the polypeptide in accordance with the twenty-fourth embodiment of the present invention wherein said active fragment is a contiguous segment of amino acids 20-838 of SEQ ID NO:4 and at least 42 amino acid residues in length and not more than 356 residues in length.

In accordance with the present invention, there is provided vaccines and

vaccine compositions that include polypeptides obtained from *S. pneumoniae* and/or variants of said polypeptides and/or active fragments of such polypeptides.

The active fragments, as hereinafter defined, include a histidine triad residue(s) and/or coiled coil regions of such polypeptides.

The term "percent identity" or "percent identical," when referring to a sequence, means that a sequence is compared to a claimed or described sequence from an alignment of the sequence to be compared (the "Compared Sequence") with the described or claimed sequence (the "Reference Sequence"). The percent identity is determined as follows:

$$\text{Percent Identity} = [1 - (C/R)]\ 100$$

wherein C is the number of differences between the Reference Sequence and the Compared Sequence over the length of the alignment between the Compared Sequence and the Reference Sequence wherein (i) each base or amino acid in the Reference Sequence that does not have an aligned base or amino acid in the Compared Sequence and (ii) each gap in the Reference Sequence and (iii) each aligned base or amino acid in the Reference Sequence that is different from an aligned base or amino acid in the Compared Sequence, each being a difference; and R is the number of bases or amino acids in the Reference Sequence over the length of the alignment with the Compared Sequence with any gap created in the Reference Sequence also being counted as a base or amino acid.

If an alignment exists between the Compared Sequence and the Reference Sequence in which the Percent Identity as calculated above is about

equal to or greater than a specified minimum Percent Identity then the Compared Sequence has the specified minimum Percent Identity to the Reference Sequence even though alignments may exist in which the hereinabove calculated Percent Identity is less than the specified Percent Identity.

"Isolated" in the context of the present invention with respect to polypeptides and/or polynucleotides means that the material is removed from its original environment (*e.g.*, the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or polypeptide present in a living organism is not isolated, but the same polynucleotide or polypeptide, separated from some or all of the co-existing materials in the natural system, is isolated. Such polynucleotides could be part of a vector and/or such polynucleotides or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment. The polypeptides and polynucleotides of the present invention are preferably provided in an isolated form, and preferably are purified to homogeneity.

BRIEF DESCRIPTION OF DRAWINGS

Figures 1A-1C, respectively, report the results of three experiments using different preparations of SP36. The results demonstrate that active immunization with recombinant SP36 derived from pneumococcal strain Norway serotype 4 is able to protect mice from death in a model of pneumococcal sepsis using a heterologous strain, SJ2 (serotype 6B). In each of the three experiments shown, one hundred percent of the mice immunized

with SP36 survived for the 14-day observation period following challenge with approximately 500 cfu of pneumococci, while eighty to one hundred percent of sham-immunized mice (injected with PBS and adjuvant) died during the same period.

Figures 2A-2B show that passive administration of rabbit antiserum raised against Sp36 derived from Norway type 4 was able to protect mice in the pneumococcal sepsis model using two heterologous strains. Figure 2A shows that one hundred percent of the mice immunized with the SP36 antiserum survived the 21-day observation period after challenge with 172 CFU of strain SJ2 (serotype 6B). Eighty percent of the mice immunized with a control serum (rabbit anti-FimC) died by day 8, and ninety percent died by day 12. Figure 2B shows that 90 percent of the mice immunized with the Sp36 antiserum survived the 8-day observation after challenge with 862 CFU of strain EF6796 (serotype 6A). Ninety percent of the mice immunized with a control serum (collected before immunization) died by day 5.

Figure 3 is a western blot demonstrating the ability of antisera raised against recombinant Sp36 derived from strain Norway type 4 to react with Sp36 of heterologous strains. Total cell lysates were immunoblotted with mouse antisera to Sp36. A band representing Sp36 protein was detected in all 23 *S. pneumoniae* strains tested, which included isolates from each of the 23 pneumococcal serotypes represented in the current polysaccharide vaccine.

Figure 4 is a Southern blot showing that the Sp36 gene from Norway type 4 hybridizes with genomic DNA from 24 other pneumococcal strains, indicating the presence of similar sequences in all these strains.

5

Figure 5 is a western blot showing the reactivity of patient sera with Sp36. Sp36 (either full-length, panel A; N-terminal half, panel B; or C-terminal half, panel C) was electrophoresed by SDS-PAGE and transferred to nitrocellulose. Patient sera collected soon after the onset of illness (acute serum, lanes A) or eight to 30 days later (convalescent serum, lanes C) were used to probe the blots. For patients 2, 3, and 5, convalescent serum reacted more strongly with Sp36 than did the corresponding acute serum.

Figure 6 is an amino acid alignment comparison of four related pneumococcal proteins, namely Sp36A (PhtA; SEQ ID NO:8), Sp36B (PhtB; SEQ ID NO:10), Sp36D (PhtD; SEQ ID NO:4), Sp36E (PhtE; SEQ ID NO:6), respectively. Dashes in a sequence indicate gaps introduced to maximize the sequence similarity. Amino acid residues that match are boxed.

Figure 7 is a nucleotide alignment comparison of four related pneumococcal genes, namely Sp36A (PhtA; SEQ ID NO:9), Sp36B (PhtB; SEQ ID NO:11), Sp36D (PhtD; SEQ ID NO:5), Sp36E (PhtE; SEQ ID NO:7), respectively. Dashes in a sequence indicate gaps introduced to maximize the sequence similarity.

Figure 8 shows the results of immunization of mice with PhtD recombinant protein, which leads to protection from lethal sepsis. C3H/HeJ (Panel A and B) or Balb/cByJ (Panel C) mice were immunized subcutaneously with PhtD protein (15 μg in 50 μl PBS emulsified in 50 μl complete Freund's adjuvant (CFA)). The recombinant PhtD protein used in protection experiments consisted of 819 amino acid residues, starting with the cysteine

(residue 20). A group of 10 sham-immunized mice received PBS with adjuvant. A second immunization of 15 μg protein with incomplete Freund's adjuvant (IFA) was administered 3 weeks later; the sham group received PBS with IFA. Blood was drawn (retro-orbital bleed) at week 7; and sera from each group was pooled for analysis of anti-PhtD antibody by ELISA. Mice were challenged at week 8 by an intraperitonial (i.p.) injection of approximately 550 CFU *S. pneumoniae* strain SJ2, serotype 6B (Panel A), 850 CFU of strain EF6796, serotype 6A (Panel B) or 450 CFU of strain EF5668, serotype 4 (Panel C). In preliminary experiments, the LD_{50} for strain SJ2 and EF6796 were determined to be approximately 10 CFU for both strains. The LD_{50} for strain EF5668 was determined to be < 5 CFU. Survival was determined in all groups over the course of 15 days following challenge. Data are presented as the percent survival for a total of 10 mice per experimental group. Two-sample Log-rank test was used for statistical analysis comparing recombinant Pht immunized mice to sham-immunized mice.

SUMMARY OF THE INVENTION

In accordance with one aspect of the present invention, there is provided a vaccine, generally in the form of a composition, that includes at least one polypeptide that is at least 90% identical to (c) a polypeptide

sequence comprising amino acids 20-838 of SEQ ID NO:4 or (ii) a polypeptide sequence comprising amino acids 21-480 of SEQ ID NO:6 or an active fragment of the foregoing.

In accordance with another aspect of the present invention, there is provided a vaccine, generally in the form of a composition, that includes an active fragment of a polypeptide that is at least 90% identical to (i) a polypeptide comprising amino acids 20-819 of SEQ ID NO:8 or (ii) a polypeptide comprising amino acids 20-819 of SEQ ID NO:10.

The term "active fragment" means a fragment that includes one or more histidine triad residues and/or one or more coiled coil regions. A "histidine triad residue" is the portion of the polypeptide that has the sequence HxxHxH wherein H is histidine and x is an amino acid other than histidine

A coiled coil region is the region predicted by "Coils" algorithm: Lupas, A., Van Dyke, M., and Stock, J. (1991) Predicting Coiled Coils from Protein Sequences, *Science* **252**:1162-1164.

In accordance with one embodiment, the active fragment includes both one or more histidine triad residues and at least one coiled coil region of the applicable polypeptide sequence. In accordance with another embodiment, the active fragment includes at least two histidine triad residues.

In another embodiment, the active fragment that includes at least one histidine triad residue or at least one coiled-coil region of the applicable polypeptide includes at least about ten percent of the applicable polypeptide and no more than about 85% of the applicable polypeptide.

The polypeptide of SEQ ID NO:4 includes five histidine triad residues, as follows: amino acids 83-88, 207-212, 315-320, 560-565 and 644-649.

The polypeptide of SEQ ID NO:6 includes five histidine triad residues, as follows: amino acids 83-88, 205-210, 309-314, 396-401 and 461-466.

In addition, the polypeptide of SEQ ID NO:4 includes two coiled-coil regions (amino acids 139-159 and amino acids 769-791) and the polypeptide of SEQ ID NO:6 includes one coiled-coil region (amino acids 139-172.)

The polypeptide of SEQ ID NO:8 includes the following regions:

HxxHxH: amino acids 82-87, 208-213, 328-333, 569-574 and 653-658.

Coiled-coils: amino acids 137-164, 425-453, 481-512 and 743-770.

In accordance with a further aspect of the invention, a vaccine of the type hereinabove described is administered for the purpose of preventing or treating infection caused by *S. pneumoniae*.

A vaccine, or vaccine composition, in accordance with the present invention may include one or more of the hereinabove described polypeptides or active fragments thereof. When employing more than one polypeptide or active fragment, such two or more polypeptides and/or active fragments may be used as a physical mixture or as a fusion of two or more polypeptides or active fragments. The fusion fragment or fusion polypeptide may be produced,

for example, by recombinant techniques or by the use of appropriate linkers for fusing previously prepared polypeptides or active fragments.

In an embodiment of the invention, there is provided (a) a polypeptide that is at least 95% identical or at least 97% identical or 100% identical to (i) a polypeptide sequence comprising amino acids 20-838 of SEQ ID NO:4 or (ii) a polypeptide sequence comprising amino acids 21-480 of SEQ ID NO:6; or (b) an active fragment of the polypeptide of (a).

In the case where the polypeptide is a variant of the polypeptide comprising the mature polypeptide of SEQ ID NO:4 or SEQ ID NO:6, or any of the active fragments of the invention, the variation in the polypeptide or fragment is generally in a portion thereof other than the histidine triad residues and the coiled-coil region, although variations in one or more of these regions may be made.

In many cases, the variation in the polypeptide or active fragment is a conservative amino acid substitution, although other substitutions are within the scope of the invention.

In accordance with the present invention, a polypeptide variant includes variants in which one or more amino acids are substituted and/or deleted and/or inserted.

In another aspect, the invention relates to passive immunity vaccines formulated from antibodies against a polypeptide or active fragment of a polypeptide of the present invention. Such passive immunity vaccines can be utilized to prevent and/or treat pneumococcal infections in patients. In this manner, according to a further aspect of the invention, a vaccine can be

produced from a synthetic or recombinant polypeptide of the present invention or an antibody against such polypeptide.

In still another aspect the present invention relates to a method of using one or more antibodies (monoclonal, polyclonal or sera) to the polypeptides of the invention as described above for the prophylaxis and/or treatment of diseases that are caused by pneumococcal bacteria. In particular, the invention relates to a method for the prophylaxis and/or treatment of infectious diseases that are caused by *S. pneumoniae*. In a still further preferred aspect, the invention relates to a method for the prophylaxis and/or treatment of otitis media, nasopharyngeal, bronchial infections, and the like in humans by utilizing a vaccine of the present invention.

Generally, vaccines are prepared as injectables, in the form of aqueous solutions or suspensions. Vaccines in an oil base are also well known such as for inhaling. Solid forms which are dissolved or suspended prior to use may also be formulated. Pharmaceutical carriers are generally added that are compatible with the active ingredients and acceptable for pharmaceutical use. Examples of such carriers include, but are not limited to, water, saline solutions, dextrose, or glycerol. Combinations of carriers may also be used.

Vaccine compositions may further incorporate additional substances to stabilize pH, or to function as adjuvants, wetting agents. or emulsifying agents, which can serve to improve the effectiveness of the vaccine.

Vaccines are generally formulated for parental administration and are injected either subcutaneously or intramuscularly. Such vaccines can also be formulated as suppositories or for oral administration, using methods known in the art.

The amount of vaccine sufficient to confer immunity to pathogenic bacteria is determined by methods well known to those skilled in the art. This quantity will be determined based upon the characteristics of the vaccine recipient and the level of immunity required. Typically, the amount of vaccine to be administered will be determined based upon the judgment of a skilled physician. Where vaccines are administered by subcutaneous or intramuscular injection, a range of 50 to 500 μg purified protein may be given.

The present invention is also directed to a vaccine in which a polypeptide or active fragment of the present invention is delivered or administered in the form of a polynucleotide encoding the polypeptide or active fragment, whereby the polypeptide or active fragment is produced *in vivo*. The polynucleotide may be included in a suitable expression vector and combined with a pharmaceutically acceptable carrier.

In addition, the polypeptides of the present invention can be used as immunogens to stimulate the production of antibodies for use in passive immunotherapy, for use as diagnostic reagents, and for use as reagents in other processes such as affinity chromatography.

In another aspect the present invention provides polynucleotides which encode the hereinabove described polypeptides and active fragments of the invention. The polynucleotide of the present invention may be in the form of RNA or in the form of DNA, which DNA includes cDNA, genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand.

In accordance with another aspect of the present invention, there is

provided

(A) an isolated polynucleotide that is at least 90% identical to a polynucleotide sequence encoding (i) a polypeptide comprising amino acids 20-838 of SEQ ID NO:4 or (ii) a polypeptide comprising amino acids 21-480 of SEQ ID NO:6, or

(B) a fragment of the polynucleotide of (A) that encodes an active polypeptide fragment or

(C) a polynucleotide that is at least 90% identical to a polynucleotide sequence encoding an active fragment of (i) a polypeptide comprising amino acids 20-819 of SEQ ID NO:8 or (ii) a polypeptide comprising amino acids 20-819 of SEQ ID NO:10.

In specific embodiments, the polynucleotide is at least 95% identical, preferably at least 97% identical, and even 100% identical to such polynucleotide sequence.

The term "polynucleotide encoding a polypeptide" encompasses a polynucleotide which includes only coding sequence for the polypeptide as well as a polynucleotide which includes additional coding and/or non-coding sequence.

The present invention further relates to variants of polynucleotides. The variants of the polynucleotides may be a naturally occurring allelic variant of the polynucleotides or a non-naturally occurring variant of the polynucleotides. The variants include variants in which one or more bases are substituted, deleted or inserted. Complements to such coding polynucleotides may be utilized to isolate polynucleotides encoding the same or similar polypeptides. In particular, such procedures are useful to obtain native immunogenic portions of polypeptides from different serotypes of *S. pneumoniae*, which is especially

useful in the production of "chain" polypeptide vaccines containing multiple immunogenic segments.

SEQ ID NO:5 is a representative example of a polynucleotide encoding the polypeptide of SEQ ID NO:4 and SEQ ID NO:7 is a representative example of a polynucleotide encoding the polypeptide of SEQ ID NO:6. SEQ ID NO:9 is a representative example of a polynucleotide encoding the polypeptide of SEQ ID NO:8, and SEQ ID NO:11 is a representative example of a polynucleotide encoding the polypeptide of SEQ ID NO:10. As a result of the known degeneracy of the genetic code, other polynucleotides that encode the polypeptides of SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 and SEQ ID NO:10 should be apparent to those skilled in the art from the teachings herein.

The polynucleotides encoding the immunogenic polypeptides described above may also have the coding sequence fused in frame to a marker sequence which allows for purification of the polypeptides of the present invention. The marker sequence may be, for example, a hexa-histidine tag supplied by a pQE-9 vector to provide for purification of the mature polypeptides fused to the marker in the case of a bacterial host, or, for example, the marker sequence may be a hemagglutinin (HA) tag when a mammalian host, e.g. COS-7 cells, is used. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson, I., et al., Cell, 37:767 (1984)).

The present invention also relates to vectors which include polynucleotides encoding one or more of the polypeptides of the invention, host cells which are genetically engineered with vectors of the invention and the production of such immunogenic polypeptides by recombinant techniques in an isolated and substantially immunogenically pure form.

Host cells are genetically engineered (transduced or transformed or transfected) with the vectors comprising a polynucleotide encoding a polypeptide of the invention. The vector may be, for example, in the form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the polynucleotides which encode such polypeptides. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

Vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. However, any other vector may be used as long as it is replicable and viable in the host.

The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Such procedures and others are deemed to be within the scope of those skilled in the art.

The DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. As representative examples of such promoters, there may be mentioned: LTR or SV40 promoter, the E. coli. lac or trp, the phage lambda P_L promoter and other promoters known to control expression of genes in

prokaryotic or eukaryotic cells or their viruses. The expression vector also contains a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression.

In addition, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in E. coli.

The vector containing the appropriate DNA sequence as hereinabove described, as well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the proteins.

As representative examples of appropriate hosts, there may be mentioned: bacterial cells, such as E. coli, Streptomyces, Salmonella typhimurium; fungal cells, such as yeast; insect cells such as Drosophila S2 and Spodoptera Sf9; animal cells such as CHO, COS or Bowes melanoma; adenoviruses; plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein.

More particularly, the present invention also includes recombinant constructs comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter,

operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available. The following vectors are provided by way of example. Bacterial: pQE70, pQE60, pQE-9 (Qiagen, Inc.), pbs, pD10, phagescript, psiX174, pbluescript SK, pbsks, pNH8A, pNH16a, pNH18A, pNH46A (Stratagene); ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia). Eukaryotic: pWLNEO, pSV2CAT, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia). However, any other plasmid or vector may be used as long as they are replicable and viable in the host.

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda P_R, P_L and TRP. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

In a further embodiment, the present invention relates to host cells containing the above-described constructs. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation (Davis, L., Dibner, M., Battey, I., Basic Methods in Molecular Biology, (1986)).

The constructs in host cells can be used in a conventional manner to

produce the gene product encoded by the recombinant sequence. Alternatively, the polypeptides of the invention can be synthetically produced by conventional peptide synthesizers.

Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook, et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), the disclosure of which is hereby incorporated by reference.

Transcription of the DNA encoding the polypeptides of the present invention by higher eukaryotes is increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp that act on a promoter to increase its transcription. Examples including the SV40 enhancer on the late side of the replication origin bp 100 to 270, a cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of E. coli and S. cerevisiae TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), α-factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with

translation initiation and termination sequences. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include E. coli, Bacillus subtilis, Salmonella typhimurium and various species within the genera Pseudomonas, Streptomyces, and Staphylococcus, although others may also be employed as a matter of choice.

As a representative but nonlimiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period.

Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, a french press, mechanical disruption, or use of cell lysing agents, such methods are well know to those skilled in the art. However, preferred are host cells which secrete the polypeptide of the invention and permit recovery of the polypeptide from the culture media.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, Cell, 23:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

The polypeptides can be recovered and/or purified from recombinant cell cultures by well-known protein recovery and purification methods. Such methodology may include ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity

chromatography, hydroxylapatite chromatography and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. In this respect, chaperones may be used in such a refolding procedure. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

The polypeptides that are useful as immunogens in the present invention may be a naturally purified product, or a product of chemical synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant, insect and mammalian cells in culture). Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated.

Procedures for the isolation of the individually expressed polypeptides may be isolated by recombinant expression/isolation methods that are well-known in the art. Typical examples for such isolation may utilize an antibody to a conserved area of the protein or to a His tag or cleavable leader or tail that is expressed as part of the protein structure.

The polypeptides, their fragments or other derivatives, or analogs thereof, or cells expressing them can be used as an immunogen to produce antibodies thereto. These antibodies can be, for example, polyclonal or monoclonal antibodies. The present invention also includes chimeric, single chain, and humanized antibodies, as well as Fab fragments, or the product of an Fab expression library. Various procedures known in the art may be used for the production of such antibodies and fragments.

Antibodies generated against the polypeptides corresponding to a

sequence of the present invention can be obtained by direct injection of the polypeptides into an animal.

For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler and Milstein, 1975, Nature, 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole, et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96).

Techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce single chain antibodies to immunogenic polypeptide products of this invention. Also, transgenic mice may be used to express humanized antibodies to immunogenic polypeptide products of this invention.

The invention will be further described with respect to the following examples; however, the scope of the invention is not limited thereby:

Example 1

Active Protection with Anti-Sp36

A. Cloning, expression, and purification of SP36

The genomic DNA used as target for amplification was isolated from *S. pneumoniae* Norway strain (serotype 4), the same strain used for genomic sequencing. The complete sequence of the Sp36 gene (SEQ ID NO:9), and its predicted amino acid sequence (SEQ ID NO:8), are given in the Sequence Listing appended hereto. It was noted that the predicted amino acid

sequence included a hydrophobic leader sequence followed by a sequence (LSVC) similar to the consensus sequence for Type II signal peptidase (LxxC, in which both x's typically represent small amino acids). Primers (listed as SEQ ID NOS:1-3) were designed that would amplify the Sp36 gene and allow its cloning into pQE10 and expression as a histidine-tagged protein lacking the signal sequence for purification by nickel-affinity chromatography. Cloning of the fragment amplified by SEQ ID Nos 1 and 3 would result in a protein containing amino acids 21 through 819 of Sp36; cloning of the fragment amplified by SEQ ID Nos 2 and 3 would result in a protein containing amino acids 26 through 819 of Sp36 (amino acid numbers refer to SEQ ID NO:8).

B. Active Protection With Sp36 Vaccination

In each of the three experiments shown in Figures 1A-1C, C3H/HeJ mice (10/group) were immunized intraperitoneally (i.p.) with Sp36 protein (15 µg in 50 µl PBS emulsified in 50 µl complete Freund's adjuvant (CFA)). A group of 10 sham-immunized mice received PBS with adjuvant. A second immunization of 15 µg protein with incomplete Freund's adjuvant (IFA) was administered 4 weeks later; the sham group received PBS with IFA. Blood was drawn (retro-orbital bleed) at weeks 3, 6, and 9; and sera from each group were pooled for analysis of anti-Sp36 antibody by ELISA. Mice were challenged at week 10 by an i.p. injection of approximately 500 CFU *S. pneumoniae* strain SJ2 (serotype 6B; provided by P. Flynn, St. Jude Children's Research Hospital, Memphis, TN). In preliminary experiments, the LD_{50} of this strain was determined to be approximately 10 CFU. Mice were monitored for 14 days for survival.

The three experiments shown in Figures 1A-1C used slightly different

preparations of recombinant Sp36. The experiments shown in Figure 1A and 1B both used Sp36 containing amino acids 20-815, but different batches of protein were used in the two experiments. The experiment shown in Figure 1C used Sp36 containing amino acids 25-815.

In the experiment shown in Figure 1A, 9-week sera collected from the ten mice immunized with Sp36 (first batch) had an endpoint ELISA titer of 1:4,096,000. No anti-Sp36 antibody was detected in sera from sham-immunized mice. One hundred percent of the mice immunized with Sp36 protein survived the challenge (520 cfu of pneumococci) for 14 days. Eighty percent of sham-immunized mice were dead by day 4, and the remainder survived.

In the experiment shown in Figure 1B, 9-week sera collected from the ten mice immunized with Sp36 (second batch) had an endpoint ELISA titer of >1:4,096,000. No anti-Sp36 antibody was detected in sera from sham-immunized mice. One hundred percent of the mice immunized with Sp36 protein survived the challenge (510 cfu of pneumococci) for 14 days. Of the sham-immunized mice, eighty percent were dead by day 4, and all died by day 9.

In the experiment shown in Figure 1C, 9-week sera collected from the ten mice immunized with Sp36 (containing amino acids 25- 815) had an endpoint ELISA titer of 1:4,096,000. No anti-Sp36 antibody was detected in sera from sham-immunized mice. One hundred percent of the mice immunized with Sp36 protein survived the challenge (510 cfu of pneumococci) for 14 days. Of the sham-immunized mice, ninety percent died by day 4, and all died by day 12. These data demonstrate that immunization of mice with recombinant Sp36 proteins elicits a response capable of

protecting against systemic pneumococcal infection and death. This protection was not strain-specific: the recombinant pneumococcal protein was cloned from a serotype 4 strain, while the challenge was with a heterologous strain, SJ2 (serotype 6B).

Example 2

Passive Protection with Anti-Sp36 Antisera

A. Generation of Rabbit Immune Sera

Following collection of preimmune serum, a New Zealand White rabbit was immunized with 250 µg of Sp36 (containing amino acids 20-815) in CFA. The rabbit was given two boosts of 125 µg Sp36 in IFA on days 29 and 50 and bled on days 39 and 60. A second rabbit was immunized with a control antigen, *E. coli* FimC.

B. Passive Protection in Mice

C3H/HeJ mice (10 mice/group) were passively immunized by two i.p. injections of 100 µl of rabbit serum. The first injection was administered twenty-four hours before challenge with 172 cfu of *S. pneumoniae* strain SJ2, and the second injection was given four hours after challenge. Figure 2 shows the survival of mice after infection with two different strains of pneumococci.

Figure 2A shows that of mice injected with 172 cfu of strain SJ2 (Figure 2A), one hundred percent of the mice immunized with rabbit immune serum raised against Sp36 protein survived the 21-day observation period. Of the mice immunized with the control serum (anti-FimC), eighty percent

died by day 8, and ninety percent died by day 12. Figure 2B shows that of mice injected with 862 cfu of strain EF6796, ninety percent of the mice immunized with rabbit immune serum raised against Sp36 protein survived the 8-day observation period. Of those given a control serum (collected from a rabbit before immunization), ninety percent died by day 8.

These data indicate that the protection against pneumococcal infection resulting from immunization with Sp36 is antibody-mediated, since mice can be protected by passive transfer of serum from a hyperimmunized rabbit. As seen in the mouse active challenge experiments described above, serum directed against recombinant Sp36 protein cloned from a serotype 4 strain was protective against challenge with heterologous strains.

Example 3

Conservation of Sp36 Among Strains of *S. pneumoniae*

A. Western blotting

The 23 pneumococcal strains used in this experiment were obtained from the American Type Culture Collection (Rockville, MD) and include one isolate each of the 23 serotypes in the multivalent pneumococcal vaccine. For total cell lysates, pneumococci were grown to mid-logarithmic phase (optical density at 620 nm, 0.4 to 0.6) in 2 ml Todd-Hewitt broth with 0.5% yeast extract (Difco, Detroit, ME) at 37°C. Bacteria were harvested by centrifugation and washed twice with water. Pellets were resuspended in 200 µl lysis buffer (0.01% sodium dodecyl sulfate, 0.15 M sodium citrate and 0.1% sodium deoxycholate) and incubated at 37°C for 30 min, then diluted in an equal volume 2x SSC (0.3 M sodium chloride, 0.03 M sodium citrate). Lysates were separated by SDS-PAGE, transferred to nitrocellulose

membranes (Bio-Rad Laboratories, Hercules, CA), and probed with antibody in a standard Western blotting procedure. Sera from ten C3H/HeJ mice immunized with Sp36 (as described in Example 1) were pooled and used at a dilution of 1:3000. Bound antibody was detected with peroxidase-conjugated sheep anti- mouse IgG using the chemiluminescence kit from Amersham, Inc. (Cambridge, MA).

The mouse anti-Sp36 sera detected two major bands with apparent molecular weights of 97 and 100 kDa in all 23 pneumococcal lysates tested (shown in Figure 3). The Sp36 signals obtained from *S. pneumoniae* serotypes 1, 5, 17F and 22F were lower, indicating either that the level of Sp36 expression is reduced in these strains, or that Sp36 in these strains is antigenically different.

These data show that Sp36 is antigenically conserved among strains of the 23 pneumococcal serotypes represented in the current polysaccharide vaccine.

B. Southern blotting

Genomic DNA was prepared from each of the 23 pneumococcal strains listed in the previous section and also from strain SJ2. DNA was digested with *Pvu*II and *Bam*HI, electrophoresed in an agarose gel and transferred to a nylon membrane. A probe was prepared by amplifying the Sp36 gene from Norway type 4 DNA (as in Example 1) and labeling the amplified fragment with fluorescein by the random-priming method, using a kit from Amersham. Hybridization, washing, and exposure of film were carried out as in the protocol supplied by Amersham. Figure 4 shows that

the Sp36 probe hybridized with DNA from each of the 24 strains studied. The lane marked "M" contained DNA from lambda phage, digested with *Hin*dIII and labeled with fluorescein, as molecular weight markers.

Example 4

Immunogenicity of Sp36 in Humans

In order to determine whether Sp36 is immunogenic during human pneumococcal infection, sera from patients with culture-proven pneumococcal bacteremia were used in Western blots containing recombinant Sp36 protein. In the experiment shown in Figure 5, sera from five patients (indicated as 1 through 5) were diluted 1:3000 and used to probe blots containing full-length Sp36, the N-terminal half of Sp36 (preceding the proline-rich region), or the C-terminal half of Sp36 (following the proline-rich region). Lanes labeled A (acute) were probed with serum collected shortly after diagnosis of pneumococcal infection; lanes C (convalescent) were probed with serum collected either one month later (patients 1, 2, and 3) or eight days after the first serum collection (patients 4 and 5). For patients 2, 3 and 5, reactivity of the convalescent serum with Sp36 was stronger that that of the corresponding acute serum. The difference between the acute and convalescent sera was particularly evident for reactivity with the C-terminal half of the protein.

In additional experiments (not shown), convalescent sera from 23 patients with pneumococcal infections were tested individually for reactivity with full-length Sp36: 20 of the 23 sera were found to bind Sp36 on a Western blot.

These experiments indicate that Sp36 is recognized by the human

immune system and suggest that antibodies able to bind the Sp36 protein may be produced during natural *S. pneumoniae* infection in humans. Since the patients were infected with a variety of pneumococcal strains, these data also support the idea that Sp36 is antigenically conserved.

Example 5

Table 1 provides the percent identity between the various sequences.

Alignment of the predicted amino acid sequences of PhtA, PhtB, PhtD, and PhtE using the MEGALIGN program of Lasergene showed strong N-terminal homology with substantial divergence of the C-termini (Figure 6). The alignment of the nucleotide sequences of the same genes is shown in Figure 7. Amino acid and nucleotide sequences were compared using the identity weighting in a Lipman-Pearson pairwise alignment, in which the number of matching residues is divided by the total of matching residues plus the number of mismatched residues plus the number of residues in gaps. In the table below, the percent identity between each pair of sequences is shown at the intersection of the corresponding row and column.

Example 6

Active Protection with PhtD Vaccination.

Mice immunized with recombinant PhtD derived from strain N4 generated potent antibody titers (reciprocal endpoint titers ranging from 2,048,00 to 4,096,000). Mice immunized with PhtD were protected against death following intraperitoneal injection with either of three heterologous strains, SJ2 (serotype 6B; provided by P. Flynn, St. Jude Children's Research

Hospital; Memphis, TN), EF6796 (serotype 6A) or EF5668 (serotype 4; both strains provided by D. Briles, University of Alabama, Birmingham). In the experiment shown in Figure 8 (Panel A), all ten of the sham-immunized mice died within 10-days after challenge with virulent pneumococci (strain SJ2), while eighty percent of the PhtD-immunized mice survived the 15-day observation period. Immunization with PhtD also protected against a serotype 6A strain, EF6796 (Panel B) and a serotype 4 strain, EF5668 (Panel C). In the experiment shown in Figure 8 (Panel B), all ten of the sham-immunized mice died within 7-days after challenge with virulent pneumococci (strain EF6796), while ninety percent of the PhtD-immunized mice survived the 15-day observation period. In the experiment shown in Figure 8 (Panel C), all ten of the sham-immunized mice died within 6-days after challenge with virulent pneumoccoci (strain EF5668), while eight of nine mice immunized with PhtD survived the 15-day observation period.

Table 1. Percent Identities

Percent Identity Between Amino Acid Sequences				
	PhtA	PhtB	PhtD	PhtE
PhtA	---	66.4	63.9	49.5
PhtB		---	87.2	49.5
PhtD			---	49.8
PhtE				---
Percent Identity Between Nucleotide Sequences				
	PhtA	PhtB	PhtD	PhtE
PhtA	---	58.3	59.3	47.9
PhtB		---	86.4	47.4
PhtD			---	47.9
PhtE				---

EDITORIAL NOTE

APPLICATION NUMBER - 2373100

The following Sequence Listing pages 1 **to** 18 **are part of the description. The claims pages follow on pages** 32 **to** 39 **.**

SEQUENCE LISTING

<110> Johnson, Leslie S.
Koenig, Scott
Adamou, John E.

<120> Streptococcus Pneumoniae and Immunogenic Fragments for Vaccines

<130> 469201-444

<140>
<141>

<150> 60/113,048
<151> 1998-12-21

<160> 11

<170> PatentIn Ver. 2.0

<210> 1
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Forward primer used in amplification of the Sp36 gene sequence.

<400> 1
atcggatcct tcttacgagt tgggactgta tcaagc 36

<210> 2
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Forward primer used in amplification of the Sp36 gene sequence.

<400> 2
atcggatcca ctgtatcaag ctagaacggt taagg 35

<210> 3
<211> 40
<212> DNA

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Reverse primer
used in amplification of the Sp36 gene sequence.

<400> 3
agtcaagctt gtttatttttt tccttactta cagatgaagg 40

<210> 4
<211> 838
<212> PRT
<213> Streptococcus pneumoniae

<400> 4
Met Lys Ile Asn Lys Lys Tyr Leu Ala Gly Ser Val Ala Val Leu Ala
1 5 10 15

Leu Ser Val Cys Ser Tyr Glu Leu Gly Arg His Gln Ala Gly Gln Val
20 25 30

Lys Lys Glu Ser Asn Arg Val Ser Tyr Ile Asp Gly Asp Gln Ala Gly
35 40 45

Gln Lys Ala Glu Asn Leu Thr Pro Asp Glu Val Ser Lys Arg Glu Gly
50 55 60

Ile Asn Ala Glu Gln Ile Val Ile Lys Ile Thr Asp Gln Gly Tyr Val
65 70 75 80

Thr Ser His Gly Asp His Tyr His Tyr Tyr Asn Gly Lys Val Pro Tyr
85 90 95

Asp Ala Ile Ile Ser Glu Glu Leu Leu Met Lys Asp Pro Asn Tyr Gln
100 105 110

Leu Lys Asp Ser Asp Ile Val Asn Glu Ile Lys Gly Gly Tyr Val Ile
115 120 125

Lys Val Asp Gly Lys Tyr Tyr Val Tyr Leu Lys Asp Ala Ala His Ala
130 135 140

Asp Asn Ile Arg Thr Lys Glu Glu Ile Lys Arg Gln Lys Gln Glu His
145 150 155 160

Ser His Asn His Gly Gly Gly Ser Asn Asp Gln Ala Val Val Ala Ala
165 170 175

```
Arg Ala Gln Gly Arg Tyr Thr Thr Asp Asp Gly Tyr Ile Phe Asn Ala
            180                 185                 190

Ser Asp Ile Ile Glu Asp Thr Gly Asp Ala Tyr Ile Val Pro His Gly
        195                 200                 205

Asp His Tyr His Tyr Ile Pro Lys Asn Glu Leu Ser Ala Ser Glu Leu
    210                 215                 220

Ala Ala Ala Glu Ala Tyr Trp Asn Gly Lys Gln Gly Ser Arg Pro Ser
225                 230                 235                 240

Ser Ser Ser Ser Tyr Asn Ala Asn Pro Ala Gln Pro Arg Leu Ser Glu
                245                 250                 255

Asn His Asn Leu Thr Val Thr Pro Thr Tyr His Gln Asn Gln Gly Glu
            260                 265                 270

Asn Ile Ser Ser Leu Leu Arg Glu Leu Tyr Ala Lys Pro Leu Ser Glu
        275                 280                 285

Arg His Val Glu Ser Asp Gly Leu Ile Phe Asp Pro Ala Gln Ile Thr
    290                 295                 300

Ser Arg Thr Ala Arg Gly Val Ala Val Pro His Gly Asn His Tyr His
305                 310                 315                 320

Phe Ile Pro Tyr Glu Gln Met Ser Glu Leu Glu Lys Arg Ile Ala Arg
                325                 330                 335

Ile Ile Pro Leu Arg Tyr Arg Ser Asn His Trp Val Pro Asp Ser Arg
            340                 345                 350

Pro Glu Gln Pro Ser Pro Gln Ser Thr Pro Glu Pro Ser Pro Ser Pro
        355                 360                 365

Gln Pro Ala Pro Asn Pro Gln Pro Ala Pro Ser Asn Pro Ile Asp Glu
    370                 375                 380

Lys Leu Val Lys Glu Ala Val Arg Lys Val Gly Asp Gly Tyr Val Phe
385                 390                 395                 400

Glu Glu Asn Gly Val Ser Arg Tyr Ile Pro Ala Lys Asp Leu Ser Ala
                405                 410                 415

Glu Thr Ala Ala Gly Ile Asp Ser Lys Leu Ala Lys Gln Glu Ser Leu
            420                 425                 430
```

3

```
Ser His Lys Leu Gly Ala Lys Lys Thr Asp Leu Pro Ser Ser Asp Arg
        435                 440                 445

Glu Phe Tyr Asn Lys Ala Tyr Asp Leu Leu Ala Arg Ile His Gln Asp
    450                 455                 460

Leu Leu Asp Asn Lys Gly Arg Gln Val Asp Phe Glu Ala Leu Asp Asn
465                 470                 475                 480

Leu Leu Glu Arg Leu Lys Asp Val Pro Ser Asp Lys Val Lys Leu Val
                485                 490                 495

Asp Asp Ile Leu Ala Phe Leu Ala Pro Ile Arg His Pro Glu Arg Leu
            500                 505                 510

Gly Lys Pro Asn Ala Gln Ile Thr Tyr Thr Asp Asp Glu Ile Gln Val
        515                 520                 525

Ala Lys Leu Ala Gly Lys Tyr Thr Thr Glu Asp Gly Tyr Ile Phe Asp
    530                 535                 540

Pro Arg Asp Ile Thr Ser Asp Glu Gly Asp Ala Tyr Val Thr Pro His
545                 550                 555                 560

Met Thr His Ser His Trp Ile Lys Lys Asp Ser Leu Ser Glu Ala Glu
                565                 570                 575

Arg Ala Ala Ala Gln Ala Tyr Ala Lys Glu Lys Gly Leu Thr Pro Pro
            580                 585                 590

Ser Thr Asp His Gln Asp Ser Gly Asn Thr Glu Ala Lys Gly Ala Glu
        595                 600                 605

Ala Ile Tyr Asn Arg Val Lys Ala Ala Lys Lys Val Pro Leu Asp Arg
    610                 615                 620

Met Pro Tyr Asn Leu Gln Tyr Thr Val Glu Val Lys Asn Gly Ser Leu
625                 630                 635                 640

Ile Ile Pro His Tyr Asp His Tyr His Asn Ile Lys Phe Glu Trp Phe
                645                 650                 655

Asp Glu Gly Leu Tyr Glu Ala Pro Lys Gly Tyr Thr Leu Glu Asp Leu
            660                 665                 670

Leu Ala Thr Val Lys Tyr Tyr Val Glu His Pro Asn Glu Arg Pro His
        675                 680                 685
```

```
Ser Asp Asn Gly Phe Gly Asn Ala Ser Asp His Val Arg Lys Asn Lys
    690                 695                 700

Val Asp Gln Asp Ser Lys Pro Asp Glu Asp Lys Glu His Asp Glu Val
705                 710                 715                 720

Ser Glu Pro Thr His Pro Glu Ser Asp Glu Lys Glu Asn His Ala Gly
                725                 730                 735

Leu Asn Pro Ser Ala Asp Asn Leu Tyr Lys Pro Ser Thr Asp Thr Glu
            740                 745                 750

Glu Thr Glu Glu Glu Ala Glu Asp Thr Thr Asp Glu Ala Glu Ile Pro
        755                 760                 765

Gln Val Glu Asn Ser Val Ile Asn Ala Lys Ile Ala Asp Ala Glu Ala
    770                 775                 780

Leu Leu Glu Lys Val Thr Asp Pro Ser Ile Arg Gln Asn Ala Met Glu
785                 790                 795                 800

Thr Leu Thr Gly Leu Lys Ser Ser Leu Leu Leu Gly Thr Lys Asp Asn
                805                 810                 815

Asn Thr Ile Ser Ala Glu Val Asp Ser Leu Leu Ala Leu Leu Lys Glu
            820                 825                 830

Ser Gln Pro Ala Pro Ile
        835
```

<210> 5
<211> 2531
<212> DNA
<213> Streptococcus pneumoniae

<400> 5
atgaaaatta ataaaaaata tctagcaggt tcagtggcag tccttgccct aagtgtttgt 60
tcctatgaac ttggtcgtca ccaagctggt caggttaaga aagagtctaa tcgagtttct 120
tatatagatg gtgatcaggc tggtcaaaag gcagaaaact tgacaccaga tgaagtcagt 180
aagagggagg ggatcaacgc cgaacaaatc gtcatcaaga ttacggatca aggttatgtg 240
acctctcatg gagaccatta tcattactat aatggcaagg tcccttatga tgccatcatc 300
agtgaagagc tcctcatgaa agatccgaat tatcagttga aggattcaga cattgtcaat 360
gaaatcaagg gtggttatgt tatcaaggta gatggaaaat actatgttta ccttaaggat 420
gcagctcatg cggataatat tcggacaaaa gaagagatta aacgtcagaa gcaggaacac 480
agtcataatc acgggggtgg ttctaacgat caagcagtag ttgcagccag agcccaagga 540
cgctatacaa cggatgatgg ttatatcttc aatgcatctg atatcattga ggacacgggt 600
gatgcttata tcgttcctca cggcgaccat taccattaca ttcctaagaa tgagttatca 660

gctagcgagt tagctgctgc agaagcctat tggaatggga agcagggatc tcgtccttct 720
tcaagttcta gttataatgc aaatccagct caaccaagat tgtcagagaa ccacaatctg 780
actgtcactc caacttatca tcaaaatcaa ggggaaaaca tttcaagcct tttacgtgaa 840
ttgtatgcta aacccttatc agaacgccat gtggaatctg atggccttat tttcgaccca 900
gcgcaaatca caagtcgaac cgccagaggt gtagctgtcc ctcatggtaa ccattaccac 960
tttatccctt atgaacaaat gtctgaattg gaaaaacgaa ttgctcgtat tattcccctt 1020
cgttatcgtt caaaccattg ggtaccagat tcaagaccag aacaaccaag tccacaatcg 1080
actccggaac ctagtccaag tccgcaacct gcaccaaatc ctcaaccagc tccaagcaat 1140
ccaattgatg agaaattggt caaagaagct gttcgaaaag taggcgatgg ttatgtcttt 1200
gaggagaatg gagtttctcg ttatatccca gccaaggatc tttcagcaga aacagcagca 1260
ggcattgata gcaaactggc caagcaggaa agtttatctc ataagctagg agctaagaaa 1320
actgacctcc catctagtga tcgagaattt tacaataagg cttatgactt actagcaaga 1380
attcaccaag atttacttga taataaaggt cgacaagttg attttgaggc tttggataac 1440
ctgttggaac gactcaagga tgtcccaagt gataaagtca agttagtgga tgatattctt 1500
gccttcttag ctccgattcg tcatccagaa cgtttaggaa aaccaaatgc gcaaattacc 1560
tacactgatg atgagattca agtagccaag ttggcaggca agtacacaac agaagacggt 1620
tatatctttg atcctcgtga tataaccagt gatgaggggg atgcctatgt aactccacat 1680
atgacccata gccactggat taaaaaagat agtttgtctg aagctgagag agcggcagcc 1740
caggcttatg ctaaagagaa aggtttgacc cctccttcga cagaccatca ggattcagga 1800
aatactgagg caaaaggagc agaagctatc tacaaccgcg tgaaagcagc taagaaggtg 1860
ccacttgatc gtatgcctta caatcttcaa tatactgtag aagtcaaaaa cggtagttta 1920
atcatacctc attatgacca ttaccataac atcaaatttg agtggtttga cgaaggcctt 1980
tatgaggcac ctaaggggta tactcttgag gatcttttgg cgactgtcaa gtactatgtc 2040
gaacatccaa acgaacgtcc gcattcagat aatggttttg gtaacgctag cgaccatgtt 2100
cgtaaaaata aggtagacca agacagtaaa cctgatgaag ataaggaaca tgatgaagta 2160
agtgagccaa ctcaccctga atctgatgaa aaagagaatc acgctggttt aaatccttca 2220
gcagataatc tttataaacc aagcactgat acggaagaga cagaggaaga agctgaagat 2280
accacagatg aggctgaaat tcctcaagta gagaattctg ttattaacgc taagatagca 2340
gatgcggagg ccttgctaga aaaagtaaca gatcctagta ttagacaaaa tgctatggag 2400
acattgactg gtctaaaaag tagtcttctt ctcggaacga aagataataa cactatttca 2460
gcagaagtag atagtctctt ggctttgtta aaagaaagtc aaccggctcc tatatagtaa 2520
aagcttaagc c 2531

<210> 6
<211> 484
<212> PRT
<213> Streptococcus pneumoniae

<400> 6
Met Lys Phe Ser Lys Lys Tyr Ile Ala Ala Gly Ser Ala Val Ile Val
1 5 10 15

Ser Leu Ser Leu Cys Ala Tyr Ala Leu Asn Gln His Arg Ser Gln Glu
20 25 30

Asn Lys Asp Asn Asn Arg Val Ser Tyr Val Asp Gly Ser Gln Ser Ser
35 40 45

6

```
Gln Lys Ser Glu Asn Leu Thr Pro Asp Gln Val Ser Gln Lys Glu Gly
 50                  55                  60

Ile Gln Ala Glu Gln Ile Val Ile Lys Ile Thr Asp Gln Gly Tyr Val
65                  70                  75                  80

Thr Ser His Gly Asp His Tyr His Tyr Tyr Asn Gly Lys Val Pro Tyr
                85                  90                  95

Asp Ala Leu Phe Ser Glu Glu Leu Leu Met Lys Asp Pro Asn Tyr Gln
            100                 105                 110

Leu Lys Asp Ala Asp Ile Val Asn Glu Val Lys Gly Gly Tyr Ile Ile
        115                 120                 125

Lys Val Asp Gly Lys Tyr Tyr Val Tyr Leu Lys Asp Ala Ala His Ala
    130                 135                 140

Asp Asn Val Arg Thr Lys Asp Glu Ile Asn Arg Gln Lys Gln Glu His
145                 150                 155                 160

Val Lys Asp Asn Glu Lys Val Asn Ser Asn Val Ala Val Ala Arg Ser
                165                 170                 175

Gln Gly Arg Tyr Thr Thr Asn Asp Gly Tyr Val Phe Asn Pro Ala Asp
            180                 185                 190

Ile Ile Glu Asp Thr Gly Asn Ala Tyr Ile Val Pro His Gly Gly His
        195                 200                 205

Tyr His Tyr Ile Pro Lys Ser Asp Leu Ser Ala Ser Glu Leu Ala Ala
    210                 215                 220

Ala Lys Ala His Leu Ala Gly Lys Asn Met Gln Pro Ser Gln Leu Ser
225                 230                 235                 240

Tyr Ser Ser Thr Ala Ser Asp Asn Asn Thr Gln Ser Val Ala Lys Gly
                245                 250                 255

Ser Thr Ser Lys Pro Ala Asn Lys Ser Glu Asn Leu Gln Ser Leu Leu
            260                 265                 270

Lys Glu Leu Tyr Asp Ser Pro Ser Ala Gln Arg Tyr Ser Glu Ser Asp
        275                 280                 285

Gly Leu Val Phe Asp Pro Ala Lys Ile Ile Ser Arg Thr Pro Asn Gly
    290                 295                 300
```

Val Ala Ile Pro His Gly Asp His Tyr His Phe Ile Pro Tyr Ser Lys
305 310 315 320

Leu Ser Ala Leu Glu Glu Lys Ile Ala Arg Met Val Pro Ile Ser Gly
325 330 335

Thr Gly Ser Thr Val Ser Thr Asn Ala Lys Pro Asn Glu Val Val Ser
340 345 350

Ser Leu Gly Ser Leu Ser Ser Asn Pro Ser Ser Leu Thr Thr Ser Lys
355 360 365

Glu Leu Ser Ser Ala Ser Asp Gly Tyr Ile Phe Asn Pro Lys Asp Ile
370 375 380

Val Glu Glu Thr Ala Thr Ala Tyr Ile Val Arg His Gly Asp His Phe
385 390 395 400

His Tyr Ile Pro Lys Ser Asn Gln Ile Gly Gln Pro Thr Leu Pro Asn
405 410 415

Asn Ser Leu Ala Thr Pro Ser Pro Ser Leu Pro Ile Asn Pro Gly Thr
420 425 430

Ser His Glu Lys His Glu Glu Asp Gly Tyr Gly Phe Asp Ala Asn Arg
435 440 445

Ile Ile Ala Glu Asp Glu Ser Gly Phe Val Met Ser His Gly Asp His
450 455 460

Asn His Tyr Phe Phe Lys Lys Asp Leu Thr Glu Glu Gln Ile Lys Val
465 470 475 480

Arg Lys Asn Ile

<210> 7
<211> 1455
<212> DNA
<213> Streptococcus pneumoniae

<400> 7
atgaaattta gtaaaaaata tatagcagct ggatcagctg ttatcgtatc cttgagtcta 60
tgtgcctatg cactaaacca gcatcgttcg caggaaaata aggacaataa tcgtgtctct 120
tatgtggatg gcagccagtc aagtcagaaa agtgaaaact tgacaccaga ccaggttagc 180
cagaaagaag gaattcaggc tgagcaaatt gtaatcaaaa ttacagatca gggctatgta 240
acgtcacacg gtgaccacta tcattactat aatgggaaag ttccttatga tgccctcttt 300

agtgaagaac tcttgatgaa ggatccaaac tatcaactta aagacgctga tattgtcaat 360
gaagtcaagg gtggttatat catcaaggtc gatggaaaat attatgtcta cctgaaagat 420
gcagctcatg ctgataatgt tcgaactaaa gatgaaatca atcgtcaaaa acaagaacat 480
gtcaaagata atgagaaggt taactctaat gttgctgtag caaggtctca gggacgatat 540
acgacaaatg atggttatgt ctttaatcca gctgatatta tcgaagatac gggtaatgct 600
tatatcgttc ctcatggagg tcactatcac tacattccca aaagcgattt atctgctagt 660
gaattagcag cagctaaagc acatctggct ggaaaaaata tgcaaccgag tcagttaagc 720
tattcttcaa cagctagtga caataacacg caatctgtag caaaaggatc aactagcaag 780
ccagcaaata aatctgaaaa tctccagagt cttttgaagg aactctatga ttcacctagc 840
gcccaacgtt acagtgaatc agatggcctg gtctttgacc ctgctaagat tatcagtcgt 900
acaccaaatg gagttgcgat tccgcatggc gaccattacc actttattcc ttacagcaag 960
ctttctgcct tagaagaaaa gattgccaga atggtgccta tcagtggaac tggttctaca 1020
gtttctacaa atgcaaaacc taatgaagta gtgtctagtc taggcagtct ttcaagcaat 1080
ccttcttctt taacgacaag taaggagctc tcttcagcat ctgatggtta tatttttaat 1140
ccaaaagata tcgttgaaga aacggctaca gcttatattg taagacatgg tgatcatttc 1200
cattacattc caaaatcaaa tcaaattggg caaccgactc ttccaaacaa tagtctagca 1260
acaccttctc catctcttcc aatcaatcca ggaacttcac atgagaaaca tgaagaagat 1320
ggatacggat ttgatgctaa tcgtattatc gctgaagatg aatcaggttt tgtcatgagt 1380
cacggagacc acaatcatta tttcttcaag aaggacttga cagaagagca aattaaggtg 1440
cgcaaaaaca tttag 1455

<210> 8
<211> 819
<212> PRT
<213> Streptococcus pneumoniae

<400> 8
Met Lys Ile Asn Lys Lys Tyr Leu Val Gly Ser Ala Ala Ala Leu Ile
1 5 10 15

Leu Ser Val Cys Ser Tyr Glu Leu Gly Leu Tyr Gln Ala Arg Thr Val
20 25 30

Lys Glu Asn Asn Arg Val Ser Tyr Ile Asp Gly Lys Gln Ala Thr Gln
35 40 45

Lys Thr Glu Asn Leu Thr Pro Asp Glu Val Ser Lys Arg Glu Gly Ile
50 55 60

Asn Ala Glu Gln Ile Val Ile Lys Ile Thr Asp Gln Gly Tyr Val Thr
65 70 75 80

Ser His Gly Asp His Tyr His Tyr Tyr Asn Gly Lys Val Pro Tyr Asp
85 90 95

Ala Ile Ile Ser Glu Glu Leu Leu Met Lys Asp Pro Asn Tyr Lys Leu
100 105 110

```
Lys Asp Glu Asp Ile Val Asn Glu Val Lys Gly Gly Tyr Val Ile Lys
        115                 120                 125

Val Asp Gly Lys Tyr Tyr Val Tyr Leu Lys Asp Ala Ala His Ala Asp
    130                 135                 140

Asn Val Arg Thr Lys Glu Glu Ile Asn Arg Gln Lys Gln Glu His Ser
145                 150                 155                 160

Gln His Arg Glu Gly Gly Thr Pro Arg Asn Asp Gly Ala Val Ala Leu
                165                 170                 175

Ala Arg Ser Gln Gly Arg Tyr Thr Thr Asp Asp Gly Tyr Ile Phe Asn
            180                 185                 190

Ala Ser Asp Ile Ile Glu Asp Thr Gly Asp Ala Tyr Ile Val Pro His
        195                 200                 205

Gly Asp His Tyr His Tyr Ile Pro Lys Asn Glu Leu Ser Ala Ser Glu
    210                 215                 220

Leu Ala Ala Ala Glu Ala Phe Leu Ser Gly Arg Gly Asn Leu Ser Asn
225                 230                 235                 240

Ser Arg Thr Tyr Arg Arg Gln Asn Ser Asp Asn Thr Ser Arg Thr Asn
                245                 250                 255

Trp Val Pro Ser Val Ser Asn Pro Gly Thr Thr Asn Thr Asn Thr Ser
            260                 265                 270

Asn Asn Ser Asn Thr Asn Ser Gln Ala Ser Gln Ser Asn Asp Ile Asp
        275                 280                 285

Ser Leu Leu Lys Gln Leu Tyr Lys Leu Pro Leu Ser Gln Arg His Val
    290                 295                 300

Glu Ser Asp Gly Leu Val Phe Asp Pro Ala Gln Ile Thr Ser Arg Thr
305                 310                 315                 320

Ala Arg Gly Val Ala Val Pro His Gly Asp His Tyr His Phe Ile Pro
                325                 330                 335

Tyr Ser Gln Met Ser Glu Leu Glu Glu Arg Ile Ala Arg Ile Ile Pro
            340                 345                 350

Leu Arg Tyr Arg Ser Asn His Trp Val Pro Asp Ser Arg Pro Glu Gln
        355                 360                 365
```

10

```
Pro Ser Pro Gln Pro Thr Pro Glu Pro Ser Pro Gly Pro Gln Pro Ala
    370                 375                 380

Pro Asn Leu Lys Ile Asp Ser Asn Ser Ser Leu Val Ser Gln Leu Val
385                 390                 395                 400

Arg Lys Val Gly Glu Gly Tyr Val Phe Glu Glu Lys Gly Ile Ser Arg
                405                 410                 415

Tyr Val Phe Ala Lys Asp Leu Pro Ser Glu Thr Val Lys Asn Leu Glu
            420                 425                 430

Ser Lys Leu Ser Lys Gln Glu Ser Val Ser His Thr Leu Thr Ala Lys
        435                 440                 445

Lys Glu Asn Val Ala Pro Arg Asp Gln Glu Phe Tyr Asp Lys Ala Tyr
    450                 455                 460

Asn Leu Leu Thr Glu Ala His Lys Ala Leu Phe Glu Asn Lys Gly Arg
465                 470                 475                 480

Asn Ser Asp Phe Gln Ala Leu Asp Lys Leu Leu Glu Arg Leu Asn Asp
                485                 490                 495

Glu Ser Thr Asn Lys Glu Lys Leu Val Asp Asp Leu Leu Ala Phe Leu
            500                 505                 510

Ala Pro Ile Thr His Pro Glu Arg Leu Gly Lys Pro Asn Ser Gln Ile
        515                 520                 525

Glu Tyr Thr Glu Asp Glu Val Arg Ile Ala Gln Leu Ala Asp Lys Tyr
    530                 535                 540

Thr Thr Ser Asp Gly Tyr Ile Phe Asp Glu His Asp Ile Ile Ser Asp
545                 550                 555                 560

Glu Gly Asp Ala Tyr Val Thr Pro His Met Gly His Ser His Trp Ile
                565                 570                 575

Gly Lys Asp Ser Leu Ser Asp Lys Glu Lys Val Ala Ala Gln Ala Tyr
            580                 585                 590

Thr Lys Glu Lys Gly Ile Leu Pro Pro Ser Pro Asp Ala Asp Val Lys
        595                 600                 605

Ala Asn Pro Thr Gly Asp Ser Ala Ala Ala Ile Tyr Asn Arg Val Lys
    610                 615                 620
```

```
Gly Glu Lys Arg Ile Pro Leu Val Arg Leu Pro Tyr Met Val Glu His
625                 630                 635                 640

Thr Val Glu Val Lys Asn Gly Asn Leu Ile Ile Pro His Lys Asp His
                645                 650                 655

Tyr His Asn Ile Lys Phe Ala Trp Phe Asp Asp His Thr Tyr Lys Ala
            660                 665                 670

Pro Asn Gly Tyr Thr Leu Glu Asp Leu Phe Ala Thr Ile Lys Tyr Tyr
        675                 680                 685

Val Glu His Pro Asp Glu Arg Pro His Ser Asn Asp Gly Trp Gly Asn
    690                 695                 700

Ala Ser Glu His Val Leu Gly Lys Lys Asp His Ser Glu Asp Pro Asn
705                 710                 715                 720

Lys Asn Phe Lys Ala Asp Glu Glu Pro Val Glu Glu Thr Pro Ala Glu
                725                 730                 735

Pro Glu Val Pro Gln Val Glu Thr Glu Lys Val Glu Ala Gln Leu Lys
            740                 745                 750

Glu Ala Glu Val Leu Leu Ala Lys Val Thr Asp Ser Ser Leu Lys Ala
        755                 760                 765

Asn Ala Thr Glu Thr Leu Ala Gly Leu Arg Asn Asn Leu Thr Leu Gln
    770                 775                 780

Ile Met Asp Asn Asn Ser Ile Met Ala Glu Ala Glu Lys Leu Leu Ala
785                 790                 795                 800

Leu Leu Lys Gly Ser Asn Pro Ser Ser Val Ser Lys Glu Lys Ile Asn
                805                 810                 815

Lys Leu Asn
```

<210> 9
<211> 2451
<212> DNA
<213> Streptococcus pneumoniae

<400> 9
atgaaaatta ataagaaata ccttgttggt tctgcggcag ctttgatttt aagtgtttgt 60
tcttacgagt tgggactgta tcaagctaga acggttaagg aaaataatcg tgtttcctat 120

atagatggaa aacaagcgac gcaaaaaacg gagaatttga ctcctgatga ggttagcaag 180
cgtgaaggaa tcaatgctga gcaaatcgtc atcaagataa cagaccaagg ctatgtcact 240
tcacatggcg accactatca ttattacaat ggtaaggttc cttatgacgc tatcatcagt 300
gaagaattac tcatgaaaga tccaaactat aagctaaaag atgaggatat tgttaatgag 360
gtcaagggtg gatatgttat caaggtagat ggaaaatact atgtttacct taaggatgct 420
gcccacgcgg ataacgtccg tacaaaagag gaaatcaatc gacaaaaaca agagcatagt 480
caacatcgtg aaggtggaac tccaagaaac gatggtgctg ttgccttggc acgttcgcaa 540
ggacgctata ctacagatga tggttatatc tttaatgctt ctgatatcat agaggatact 600
ggtgatgctt atatcgttcc tcatggagat cattaccatt acattcctaa gaatgagtta 660
tcagctagcg agttggctgc tgcagaagcc ttcctatctg gtcgaggaaa tctgtcaaat 720
tcaagaacct atcgccgaca aaatagcgat aacacttcaa gaacaaactg ggtaccttct 780
gtaagcaatc caggaactac aaatactaac acaagcaaca acagcaacac taacagtcaa 840
gcaagtcaaa gtaatgacat tgatagtctc ttgaaacagc tctacaaact gcctttgagt 900
caacgacatg tagaatctga tggccttgtc tttgatccag cacaaatcac aagtcgaaca 960
gctagaggtg ttgcagtgcc acacggagat cattaccact tcatccctta ctctcaaatg 1020
tctgaattgg aagaacgaat cgctcgtatt attccccttc gttatcgttc aaaccattgg 1080
gtaccagatt caaggccaga acaaccaagt ccacaaccga ctccggaacc tagtccaggc 1140
ccgcaacctg caccaaatct taaaatagac tcaaattctt ctttggttag tcagctggta 1200
cgaaaagttg gggaaggata tgtattcgaa gaaaagggca tctctcgtta tgtctttgcg 1260
aaagatttac catctgaaac tgttaaaaat cttgaaagca agttatcaaa acaagagagt 1320
gtttcacaca ctttaactgc taaaaaagaa aatgttgctc ctcgtgacca agaattttat 1380
gataaagcat ataatctgtt aactgaggct cataaagcct tgtttgnaaa taagggtcgt 1440
aattctgatt tccaagcctt agacaaatta ttagaacgct tgaatgatga atcgactaat 1500
aaagaaaaat tggtagatga tttattggca ttcctagcac caattaccca tccagagcga 1560
cttggcaaac caaattctca aattgagtat actgaagacg aagttcgtat tgctcaatta 1620
gctgataagt atacaacgtc agatggttac atttttgatg aacatgatat aatcagtgat 1680
gaaggagatg catatgtaac gcctcatatg ggccatagtc actggattgg aaaagatagc 1740
ctttctgata aggaaaaagt tgcagctcaa gcctatacta aagaaaaagg tatcctacct 1800
ccatctccag acgcagatgt taaagcaaat ccaactggag atagtgcagc agctatttac 1860
aatcgtgtga aaggggaaaa acgaattcca ctcgttcgac ttccatatat ggttgagcat 1920
acagttgagg ttaaaaacgg taatttgatt attcctcata aggatcatta ccataatatt 1980
aaatttgctt ggtttgatga tcacacatac aaagctccaa atggctatac cttggaagat 2040
ttgtttgcga cgattaagta ctacgtagaa caccctgacg aacgtccaca ttctaatgat 2100
ggatggggca atgccagtga gcatgtgtta ggcaagaaag accacagtga agatccaaat 2160
aagaacttca aagcggatga agagccagta gaggaaacac ctgctgagcc agaagtccct 2220
caagtagaga ctgaaaaagt agaagcccaa ctcaaagaag cagaagtttt gcttgcgaaa 2280
gtaacggatt ctagtctgaa agccaatgca acagaaactc tagctggttt acgaaataat 2340
ttgactcttc aaattatgga taacaatagt atcatggcag aagcagaaaa attacttgcg 2400
ttgttaaaag gaagtaatcc ttcatctgta agtaaggaaa aaataaacta a 2451

<210> 10
<211> 819
<212> PRT
<213> Streptococcus pneumoniae

<400> 10
Met Lys Ile Asn Lys Lys Tyr Leu Ala Gly Ser Val Ala Val Leu Ala
1 5 10 15

```
Leu Ser Val Cys Ser Tyr Glu Leu Gly Arg Tyr Gln Ala Gly Gln Asp
             20                  25                  30

Lys Lys Glu Ser Asn Arg Val Ala Tyr Ile Asp Gly Asp Gln Ala Gly
         35                  40                  45

Gln Lys Ala Glu Asn Leu Thr Pro Asp Glu Val Ser Lys Arg Glu Gly
     50                  55                  60

Ile Asn Ala Glu Gln Ile Val Ile Lys Ile Thr Asp Gln Gly Tyr Val
 65                  70                  75                  80

Thr Ser His Gly Asp His Tyr His Tyr Tyr Asn Gly Lys Val Pro Tyr
                 85                  90                  95

Asp Ala Ile Ile Ser Glu Glu Leu Leu Met Lys Asp Pro Asn Tyr Gln
            100                 105                 110

Leu Lys Asp Ser Asp Ile Val Asn Glu Ile Lys Gly Gly Tyr Val Ile
        115                 120                 125

Lys Val Asn Gly Lys Tyr Tyr Val Tyr Leu Lys Asp Ala Ala His Ala
    130                 135                 140

Asp Asn Ile Arg Thr Lys Glu Glu Ile Lys Arg Gln Lys Gln Glu Arg
145                 150                 155                 160

Ser His Asn His Asn Ser Arg Ala Asp Asn Ala Val Ala Ala Ala Arg
                165                 170                 175

Ala Gln Gly Arg Tyr Thr Thr Asp Asp Gly Tyr Ile Phe Asn Ala Ser
            180                 185                 190

Asp Ile Ile Glu Asp Thr Gly Asp Ala Tyr Ile Val Pro His Gly Asp
        195                 200                 205

His Tyr His Tyr Ile Pro Lys Asn Glu Leu Ser Ala Ser Glu Leu Ala
    210                 215                 220

Ala Ala Glu Ala Tyr Trp Asn Gly Lys Gln Gly Ser Arg Pro Ser Ser
225                 230                 235                 240

Ser Ser Ser Tyr Asn Ala Asn Pro Ala Gln Pro Arg Leu Ser Glu Asn
                245                 250                 255

His Asn Leu Thr Val Thr Pro Thr Tyr His Gln Asn Gln Gly Glu Asn
            260                 265                 270
```

```
Ile Ser Ser Leu Leu Arg Glu Leu Tyr Ala Lys Pro Leu Ser Glu Arg
        275                 280                 285

His Val Glu Ser Asp Gly Leu Ile Phe Asp Pro Ala Gln Ile Thr Ser
    290                 295                 300

Arg Thr Ala Arg Gly Val Ala Val Pro His Gly Asn His Tyr His Phe
305                 310                 315                 320

Ile Pro Tyr Glu Gln Met Ser Glu Leu Glu Lys Arg Ile Ala Arg Ile
                325                 330                 335

Ile Pro Leu Arg Tyr Arg Ser Asn His Trp Val Pro Asp Ser Arg Pro
            340                 345                 350

Glu Glu Pro Ser Pro Gln Pro Thr Pro Glu Pro Ser Pro Ser Pro Gln
        355                 360                 365

Pro Ala Pro Ser Asn Pro Ile Asp Gly Lys Leu Val Lys Glu Ala Val
    370                 375                 380

Arg Lys Val Gly Asp Gly Tyr Val Phe Glu Glu Asn Gly Val Ser Arg
385                 390                 395                 400

Tyr Ile Pro Ala Lys Asp Leu Ser Ala Glu Thr Ala Ala Gly Ile Asp
                405                 410                 415

Ser Lys Leu Ala Lys Gln Glu Ser Leu Ser His Lys Leu Gly Thr Lys
            420                 425                 430

Lys Thr Asp Leu Pro Ser Ser Asp Arg Glu Phe Tyr Asn Lys Ala Tyr
        435                 440                 445

Asp Leu Leu Ala Arg Ile His Gln Asp Leu Leu Asp Asn Lys Gly Arg
    450                 455                 460

Gln Val Asp Phe Glu Ala Leu Asp Asn Leu Leu Glu Arg Leu Lys Asp
465                 470                 475                 480

Val Ser Ser Asp Lys Val Lys Leu Val Glu Asp Ile Leu Ala Phe Leu
                485                 490                 495

Ala Pro Ile Arg His Pro Glu Arg Leu Gly Lys Pro Asn Ala Gln Ile
            500                 505                 510

Thr Tyr Thr Asp Asp Glu Ile Gln Val Ala Lys Leu Ala Gly Lys Tyr
        515                 520                 525
```

```
Thr Ala Glu Asp Gly Tyr Ile Phe Asp Pro Arg Asp Ile Thr Ser Asp
530                 535                 540

Glu Gly Asp Ala Tyr Val Thr Pro His Met Thr His Ser His Trp Ile
545                 550                 555                 560

Lys Lys Asp Ser Leu Ser Glu Ala Glu Arg Ala Ala Ala Gln Ala Tyr
                565                 570                 575

Ala Glu Glu Lys Gly Leu Thr Pro Pro Ser Thr Asp His Gln Asp Ser
            580                 585                 590

Gly Asn Thr Glu Ala Lys Gly Ala Glu Ala Ile Tyr Asn Arg Val Lys
        595                 600                 605

Ala Ala Lys Lys Val Pro Leu Asp Arg Met Pro Tyr Asn Leu Gln Tyr
    610                 615                 620

Thr Val Glu Val Lys Asn Gly Ser Leu Ile Ile Pro His Tyr Asp His
625                 630                 635                 640

Tyr His Asn Ile Lys Phe Glu Trp Phe Asp Glu Gly Leu Tyr Glu Ala
                645                 650                 655

Pro Lys Gly Tyr Thr Leu Glu Asp Leu Leu Ala Thr Val Lys Tyr Tyr
            660                 665                 670

Val Glu His Pro Asn Glu Arg Pro His Ser Asp Asn Gly Phe Gly Asn
        675                 680                 685

Ala Ser Asp His Val Gln Arg Asn Lys Asn Gly Gln Ala Asp Thr Asn
    690                 695                 700

Gln Thr Glu Lys Pro Ser Glu Glu Lys Pro Gln Thr Glu Lys Pro Glu
705                 710                 715                 720

Glu Glu Thr Pro Arg Glu Glu Lys Pro Gln Ser Glu Lys Pro Glu Ser
                725                 730                 735

Pro Lys Pro Thr Glu Glu Pro Glu Glu Ser Pro Glu Glu Ser Glu Glu
            740                 745                 750

Pro Gln Val Glu Thr Glu Lys Val Glu Glu Lys Leu Arg Glu Ala Glu
        755                 760                 765

Asp Leu Leu Gly Lys Ile Gln Asp Pro Ile Ile Lys Ser Asn Ala Lys
    770                 775                 780
```

Glu Thr Leu Thr Gly Leu Lys Asn Asn Leu Leu Phe Gly Thr Gln Asp
785 790 795 800

Asn Asn Thr Ile Met Ala Glu Ala Glu Lys Leu Leu Ala Leu Leu Lys
805 810 815

Glu Ser Lys

<210> 11
<211> 2531
<212> DNA
<213> Streptococcus pneumoniae

<400> 11
atgaaaatta ataaaaaata tctagcaggt tcagtggcag tccttgccct aagtgtttgt 60
tcctatgagc ttggacgtta ccaagctggt caggataaga aagagtctaa tcgagttgct 120
tatatagatg gtgatcaggc tggtcaaaag gcagaaaact tgacaccaga tgaagtcagt 180
aagagggagg ggatcaacgc cgaacaaatt gttatcaaga ttacggatca aggttatgtg 240
acctctcatg gagaccatta tcattactat aatggcaagg ttccttatga tgccatcatc 300
agtgaagagc tcctcatgaa agatccgaat tatcagttga aggattcaga cattgtcaat 360
gaaatcaagg gtggttatgt cattaaggta aacggtaaat actatgttta ccttaaggat 420
gcrgctcatg cggataatat tcggacaaaa gaagagatta aacgtcagaa gcaggaacgc 480
agtcataatc ataactcaag agcagataat gctgttgctg cagccagagc ccaaggacgt 540
tatacaacgg atgatgggta tatcttcaat gcatctgata tcattgagga cacgggtgat 600
gcttatatcg ttcctcacgg cgaccattac cattacattc ctaagaatga gttatcagct 660
agcgagttag ctgctgcaga agcctattgg aatgggaagc agggatctcg tccttcttca 720
agttctagtt ataatgcaaa tccagctcaa ccaagattgt cagagaacca caatctgact 780
gtcactccaa cttatcatca aaatcaaggg gaaaacattt caagcctttt acgtgaattg 840
tatgctaaac ccttatcaga acgccatgtg gaatctgatg gccttatttt cgacccagcg 900
caaatcacaa gtcgaaccgc cagaggtgta gctgtccctc atggtaacca ttaccacttt 960
atcccttatg aacaaatgtc tgaattggaa aaacgaattg ctcgtattat tcccccttcgt 1020
tatcgttcaa accattgggt accagattca agaccagaag aaccaagtcc acaaccgact 1080
ccagaaccta gtccaagtcc gcaaccagct ccaagcaatc caattgatgg gaaattggtc 1140
aaagaagctg ttcgaaaagt aggcgatggt tatgtctttg aggagaatgg agtttctcgt 1200
tatatcccag ccaaggatct ttcagcagaa acagcagcag gcattgatag caaactggcc 1260
aagcaggaaa gtttatctca taagctagga actaagaaaa ctgacctccc atctagtgat 1320
cgagaatttt acaataaggc ttatgactta ctagcaagaa ttcaccaaga tttacttgat 1380
aataaaggtc gacaagttga ttttgaggct ttggataacc tgttggaacg actcaaggat 1440
gtctcaagtg ataaagtcaa gttagtggaa gatattcttg ccttcttagc tccgattcgt 1500
catccagaac gtttaggaaa accaaatgcg caaattacct acactgatga tgagattcaa 1560
gtagccaagt tggcaggcaa gtacacagca gaagacggtt atatctttga tcctcgtgat 1620
ataaccagtg atgaggggga tgcctatgta actccacata tgacccatag ccactggatt 1680
aaaaaagata gtttgtctga agctgagaga gcggcagccc aggcttatgc traagagaaa 1740
ggtttgaccc ctccttcgac agaccatcag gattcaggaa atactgaggc aaaaggagca 1800
gaagctatct acaaccgmgt gaaagcagct aagaaggtgc cacttgatcg tatgccttac 1860

```
aatcttcaat atactgtaga agtcaaaaac ggtagtttaa tcatacctca ttatgaccat 1920
taccataaca tcaaatttga gtggtttgac gaaggccttt atgaggcacc taaggggtat 1980
actcttgagg atcttttggc gactgtcaag tactatgtcg aacatccaaa cgaacgtccg 2040
cattcagata atggttttgg taacgctagc gaccatgttc aaagaaacaa aaatggtcaa 2100
gctgatacca atcaaacgga aaaaccaagc gaggagaaac ctcagacaga aaaacctgag 2160
gaagaaaccc ctcgagaaga gaaaccgcaa agcgagaaac cagagtctcc aaaaccaaca 2220
gaggaaccag aagaatcacc agaggaatca gaagaacctc aggtcgagac tgaaaaggtt 2280
gaagaaaaac tgagagaggc tgaagattta cttggaaaaa tccaggatcc aattatcaag 2340
tccaatgcca aagagactct cacaggatta aaaaataatt tactatttgg cacccaggac 2400
aacaatacta ttatggcaga agctgaaaaa ctattggctt tattaaagga gagtaagtaa 2460
aggtagaagc ttaagggcga atttggcacc caggacaaca atactattat ggcagaagct 2520
gaaaaactat t                                                      2531
```

The claims defining the invention are as follows:

1. A composition comprising a polypeptide at least 95% identical to amino acids 20-838 of SEQ ID NO:4 in a pharmaceutically acceptable carrier in an amount effective to elicit production of an antibody that binds to *S. pneumoniae* when said composition is administered to a mammal.

2. The composition of claim 1 wherein said percent identity is at least 97%.

3. The composition of claim 1 wherein said polypeptide has the sequence of amino acids 20-838 of SEQ ID NO:4.

4. A composition comprising an active fragment of a polypeptide at least 95% identical to amino acids 20-838 of SEQ ID NO:4 wherein said active fragment comprises at least two coiled coil regions and is in a pharmaceutically acceptable carrier in an amount effective to elicit production of an antibody that binds to said polypeptide when said composition is administered to a mammal.

5. The composition of claim 4 wherein said active fragment further comprises at least 5 histidine triad regions.

6. A composition comprising a polypeptide comprising the sequence of amino acid residues 139-791 of SEQ ID NO:4.

7. A vaccine comprising the composition of claim 1, 2 or 3 wherein said polypeptide is present in an amount effective to protect against pneumococcal infection when administered to a mammal.

8. A method for preventing infection caused by *S. pneumoniae* comprising administering the vaccine of claim 7.

9. An isolated polypeptide at least 95% identical to amino acids 20-838 of SEQ ID NO:4 wherein said polypeptide binds to an antibody that binds to *S. pneumoniae.*

10. The isolated polypeptide of claim 9 wherein said percent identity is at least 97%.

11. The isolated polypeptide of claim 9 wherein said isolated polypeptide has the sequence of amino acids 20-838 of SEQ ID NO:4.

12. A method for producing a polypeptide comprising an amino acid sequence that has at least 95% identity to amino acids 20-838 of SEQ ID NO:4 comprising expressing said polypeptide from a cell that comprises a polynucleotide containing a nucleotide sequence that encodes said polypeptide.

13. The method of claim 12 wherein said percent identity is at least 97%.

14. The method of claim 12 wherein said polypeptide comprises the amino acid sequence of SEQ ID NO:4.

15. The method of claim 12 wherein said polypeptide binds to an antibody specific for *Streptococcus pneumoniae*.

16. The method of claim 12 wherein the nucleotide sequence contained in said polynucleotide has at least 90% identity to the sequence of SEQ ID NO:5.

17. The method of claim 12 wherein said nucleotide sequence contained in said polynucleotide has at least 95% identity to the sequence of SEQ ID NO:5.

18. The method of claim 12 wherein said nucleotide sequence contained in said polynucleotide has at least 97% identity to the sequence of SEQ ID NO:5.

19. The method of claim 12 wherein said nucleotide sequence contained in said polynucleotide has the sequence of SEQ ID NO:5.

20. A method for producing an active fragment of the polypeptide that comprises amino acids 20-838 of SEQ ID NO:4 comprising expressing said active fragment from a cell that contains a polynucleotide containing a nucleotide sequence that encodes said active fragment.

21. The method of claim 20 wherein said active fragment is a contiguous segment of amino acids 20-838 of SEQ ID NO:4 and at least 42 amino acid residues in length and not more than 356 residues in length.

22. The method of claim 20 wherein said active fragment comprises at least one coiled coil region.

23. The method of claim 20 wherein said active fragment comprises at least two coiled coil regions.

24. The method of claim 20 wherein said active fragment comprises at least one histidine triad region.

25. The method of claim 20 wherein said active fragment comprises at least two histidine triad regions.

26. The method of claim 20 wherein said active fragment comprises at least three histidine triad regions.

27. An isolated polynucleotide comprising a nucleotide sequence having at least 90% identity to the sequence of SEQ ID NO:5.

28. The isolated polynucleotide of claim 27 wherein said nucleotide sequence has at least 95% identity to the sequence of SEQ ID NO:5.

29. The isolated polynucleotide of claim 27 wherein said nucleotide sequence has at least 97% identity to the sequence of SEQ ID NO:5.

30. The isolated polynucleotide of claim 27 wherein said nucleotide sequence comprises the sequence of SEQ ID NO:5.

31. A vector comprising the isolated polynucleotide of claim 27, 28, 29, or 30.

32. A vector comprising the polynucleotide used in 12, 16, 17, 18, 19 or 20.

33. A recombinant cell genetically engineered with the vector of claim 31.

34. A recombinant cell genetically engineered with the vector of claim 32.

35. A method for eliciting an immune response to *Streptococcus pneumoniae* in a mammal comprising administering to said mammal a polypeptide comprising an amino acid sequence that has at least 95% identity to amino acids 20-838 of SEQ ID NO:4.

36. The method of claim 35 wherein said percent identity is at least 97%.

37. The method of claim 35 wherein said polypeptide comprises the amino acid sequence of SEQ ID NO:4.

38. A method for eliciting an immune response to *Streptococcus pneumoniae* in a mammal comprising administering to said mammal a polypeptide comprising an active fragment of amino acids 20-838 of SEQ ID NO:4.

39. The method of claim 38 wherein said active fragment is a contiguous segment of amino acids 20-838 of SEQ ID NO:4 and at least 42 amino acid residues in length and not more than 356 residues in length.

40. The method of claim 39 wherein said active fragment comprises at least one coiled coil region.

41. The method of claim 40 wherein said active fragment comprises at least two coiled coil regions.

42. The method of claim 39 wherein said active fragment comprises at least one histidine triad region.

43. The method of claim 39 wherein said active fragment comprises at least two histidine triad regions.

44. The method of claim 39 wherein said active fragment comprises at least three histidine triad regions.

45. The method of claim 35 wherein said polypeptide is administered in a pharmaceutically acceptable carrier.

46. The method of claim 39 wherein said active fragment is administered in a pharmaceutically acceptable carrier.

47. A method for eliciting an immune response to *Streptococcus pneumoniae* in a mouse comprising administering to said mouse a polypeptide comprising an amino acid sequence that has at least 95% identity to amino acids 20-838 of SEQ ID NO:4.

48. A composition comprising a polypeptide having at least 90% identity to amino acids 21-480 of SEQ ID NO:6,

wherein said polypeptide is in a pharmaceutically acceptable carrier in an amount effective to elicit production of an antibody that binds to *S. pneumoniae* when said composition is administered to a mammal.

49. The composition of claim 48 wherein said percent identity is at least 95%.

50. The composition of claim 48 wherein said percent identity is at least 97%.

51. The composition of claim 48 wherein said polypeptide has the sequence of amino acids 21-480 of SEQ ID NO:6.

52. A composition comprising an active fragment of the polypeptide comprising amino acids 21-480 of SEQ ID NO:6,

wherein said active fragment comprises at least about 10% and no more than about 85% of said polypeptide, wherein said active fragment comprises at least one histidine triad and at least one coiled coil region and wherein said active fragment is in a pharmaceutically acceptable carrier in an amount effective to elicit production of an antibody that binds to *S. pneumoniae* when said composition is administered to a mammal.

53. The composition of claim 52 wherein said active fragment further comprises at least 2 histidine triad regions.

54. The composition of claim 52 wherein said active fragment further comprises at least 2 coiled coil regions.

55. A vaccine comprising a polypeptide containing the sequence of amino acids 21-480 of SEQ ID NO:6 in a pharmaceutically acceptable carrier in an amount effective to protect against pneumococcal infection when administered to a mammal.

56. A process for preventing infection caused by *S. pneumoniae* comprising administering the vaccine of claim 55.

57. A process for preventing infection caused by *S. pneumoniae* comprising administering the composition of claim 52.

58. An isolated polypeptide comprising at least 90% identity to amino acids 21-480 of SEQ ID NO:6, wherein said polypeptide binds to an antibody that binds to *S. pneumoniae*.

59. The isolated polypeptide of claim 58 wherein said percent identity is at least 95%.

60. The isolated polypeptide of claim 58 wherein said percent identity is at least 97%.

61. The isolated polypeptide of claim 58 wherein said isolated polypeptide has the amino acid sequence of amino acids 21-480 of SEQ ID NO:6.

62. A composition comprising at least one antibody that binds to a polypeptide comprising an amino acid sequence at least 90% identical to a member selected from the group consisting of:

(i) a sequence having at least 90% identity to amino acids 20-838 of SEQ ID NO:4 and

(ii) a sequence comprising amino acids 21-480 of SEQ ID NO:6,

wherein said antibody is in a pharmaceutically acceptable carrier.

63. The composition of claim 62 wherein the antibody is the antibody against the polypeptide in (i).

64. The composition of claim 62 wherein the antibody is the antibody against the fragment in (ii).

65. A composition comprising at least one antibody that binds to an active fragment of a polypeptide comprising a member selected from the group consisting of:

(i) amino acids 20-838 of SEQ ID NO:4 and

(ii) amino acids 21-480 of SEQ ID NO:6,

wherein said active fragment comprises at least about 10% and no more than about 85% of said polypeptide, wherein said active fragment comprises at least one histidine triad and at least one coiled coil region and wherein said active fragment is in a pharmaceutically acceptable carrier in an amount effective to elicit production of an antibody that binds to *S. pneumoniae* when said composition is administered to a mammal.

66. A process for treating or preventing infection caused by *S. pneumoniae* comprising administering to a mammal afflicted with said infection, or at risk of said infection, an effective amount of the composition of claim 62.

67. A process for treating or preventing infection caused by *S. pneumoniae* comprising administering to a mammal afflicted with said infection, or at risk of said infection, an effective amount of the composition of claim 65.

68. A composition comprising a polypeptide at least 95% identical to amino acids 20-838 of SEQ ID NO:4 in a pharmaceutically acceptable carrier in an amount effective to elicit production of an antibody that binds to *S. pneumoniae*, substantially as hereinbefore described with reference to any one of the examples.

69. A composition comprising an active fragment of a polypeptide at least 95% identical to amino acids 20-838 of SEQ ID NO:4 wherein said active fragment comprises

at least two coiled coil regions and is in a pharmaceutically acceptable carrier in an amount effective to elicit production of an antibody that binds to said polypeptide, substantially as hereinbefore described with reference to any one of the examples.

70. A vaccine comprising the composition of claim 68 or 69 wherein said polypeptide is present in an amount effective to protect against pneumococcal infection when administered to a mammal.

71. A method for preventing infection caused by *S. pneumoniae* comprising administering the vaccine of claim 70.

72. A method for producing a polypeptide comprising an amino acid sequence that has at least 95% identity to amino acids 20-838 of SEQ ID NO:4, substantially as hereinbefore described with reference to any one of the examples.

73. An isolated polynucleotide comprising a nucleotide sequence having at least 90% identity to the sequence of SEQ ID NO:5, substantially as hereinbefore described with reference to any one of the examples.

74. A method for eliciting an immune response to *Streptococcus pneumoniae* in a mammal comprising administering to said mammal a polypeptide comprising an amino acid sequence that has at least 95% identity to amino acids 20-838 of SEQ ID NO:4, substantially as hereinbefore described with reference to any one of the examples.

75. A vaccine comprising a polypeptide containing the sequence of amino acids 21-480 of SEQ ID NO:6 in a pharmaceutically acceptable carrier, substantially as hereinbefore described with reference to any one of the examples.

76. A method for preventing infection caused by *S. pneumoniae*, comprising administering the vaccine of claim 75.

77. Use of a polypeptide comprising an amino acid sequence that has at least 95% identity to amino acids 20-838 of SEQ ID NO:4 for the manufacture of a medicament for eliciting an immune response to *Streptococcus pneumoniae* in a mammal.

78. Use of a polypeptide of claim 77 wherein said percent identity is at least 97%.

79. Use of a polypeptide of claim 77 wherein said polypeptide comprises the amino acid sequence of residues 20-838 of SEQ ID NO:4.

80. Use of a polypeptide comprising an active fragment of amino acids 20-838 of SEQ ID NO:4 for the manufacture of a medicament for eliciting an immune response to *Streptococcus pneumoniae* in a mammal.

81. Use of the polypeptide of claim 80 wherein said active fragment is a contiguous segment of amino acids 20-838 of SEQ ID NO:4 and at least 42 amino acid residues in length and not more than 356 residues in length.

82. Use of the polypeptide of claim 81 wherein said active fragment comprises at least one coiled coil region.

83. Use of the polypeptide of claim 82 wherein said active fragment comprises at least two coiled coil regions.

84. Use of the polypeptide of claim 81 wherein said active fragment comprises at least one histidine triad region.

85. Use of the polypeptide of claim 81 wherein said active fragment comprises at least two histidine triad regions.

86. Use of the polypeptide of claim 81 wherein said active fragment comprises at least three histidine triad regions.

87. Use of the polypeptide of claim 77 wherein said polypeptide is administered in a pharmaceutically acceptable carrier.

88. Use of the polypeptide of claim 81 wherein said active fragment is administered in a pharmaceutically acceptable carrier.

89. Use of a polypeptide comprising an amino acid sequence that has at least 95% identity to amino acids 20-838 of SEQ ID NO:4 for the manufacture of a medicament for eliciting an immune response to *Streptococcus pneumoniae* in a mouse.

90. Use of an effective amount of a composition comprising a polypeptide having at least 90% identity to amino acids 21-480 of SEQ ID NO:6,

wherein said polypeptide is in a pharmaceutically acceptable carrier in an amount effective to elicit production of an antibody that binds to *S. pneumoniae* when said composition is administered to a mammal.

91. Use of a polypeptide containing the sequence of amino acids 21-480 of SEQ ID NO:6 in a pharmaceutically acceptable carrier in an amount effective to protect against pneumococcal infection when administered to a mammal, for the manufacture of a medicament for preventing infection caused by *S. pneumoniae*.

92. Use of the composition of claim 52 for the manufacture of a medicament for preventing infection caused by *S. pneumoniae*.

93. Use of the composition of claim 62 for the manufacture of a medicament for treating or preventing infection caused by *S. pneumoniae* of a mammal afflicted with said infection or at risk of said infection.

94. Use of the composition of claim 65 for the manufacture of a medicament for treating or preventing infection caused by *S. pneumoniae* of a mammal afflicted with said infection or at risk of said infection.

95. An isolated polynucleotide comprising a nucleotide sequence that encodes the polypeptide of SEQ ID NO:4.

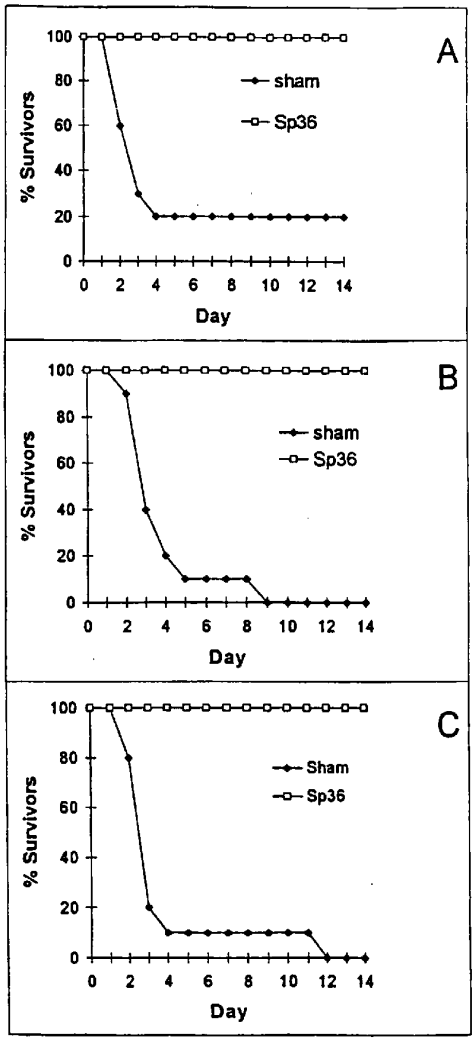
96. An isolated polynucleotide comprising a nucleotide sequence that encodes the polypeptide of SEQ ID NO:6.

97. The isolated polynucleotide of claim 96 wherein said nucleotide sequence is the sequence of SEQ ID NO:7.

Dated 19 July, 2004
Med Immune, Inc.

Patent Attorneys for the Applicant/Nominated Person
SPRUSON & FERGUSON

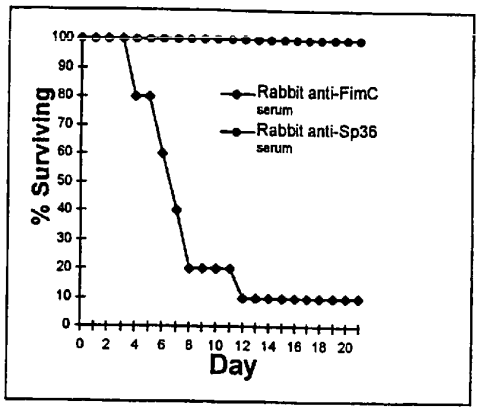
Figure 1


A
% Survivors
100
80
60
40
20
0
0 2 4 6 8 10 12 14
Day
sham
Sp36
B
% Survivors
100
80
60
40
20
0
0 2 4 6 8 10 12 14
Day
sham
Sp36
C
% Survivors
100
80
60
40
20
0
0 2 4 6 8 10 12 14
Day
Sham
Sp36

1/17

Figure 2

A. Strain SJ2 (serotype 6B)



B. Strain EF6796 (serotype 6A)

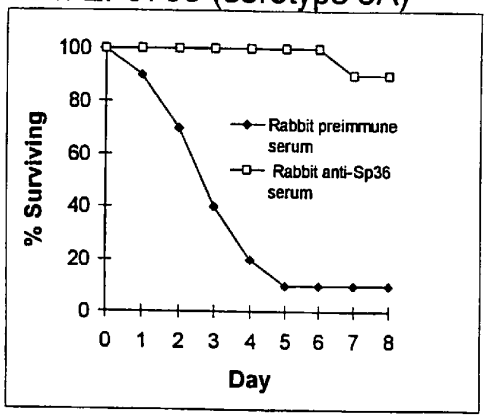


FIG. 3A

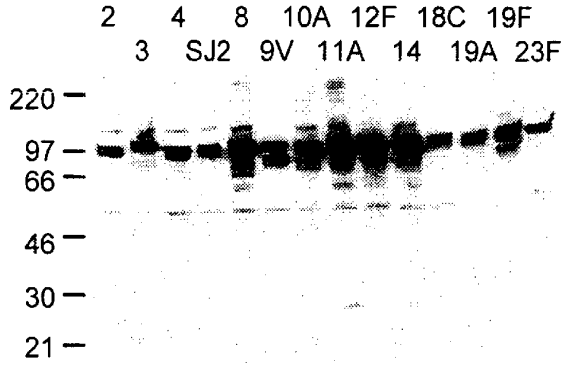


FIG. 3B

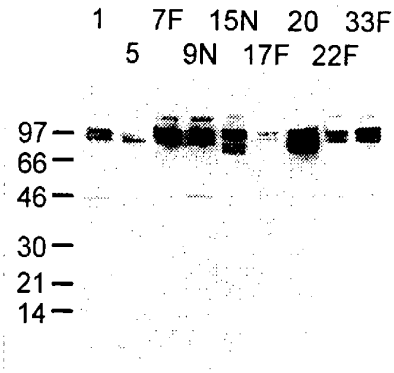
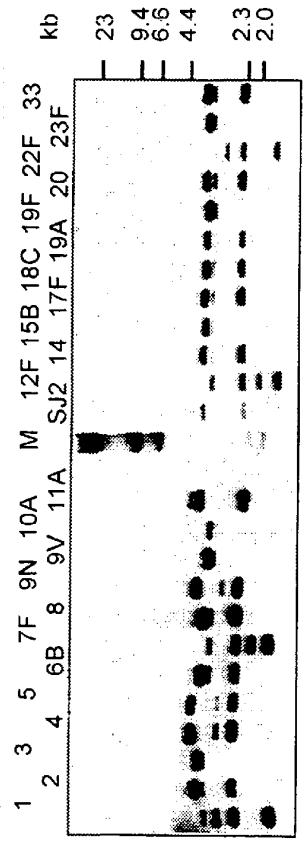
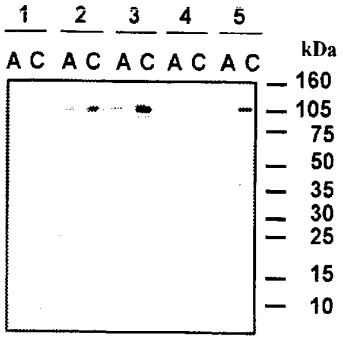


FIG. 4



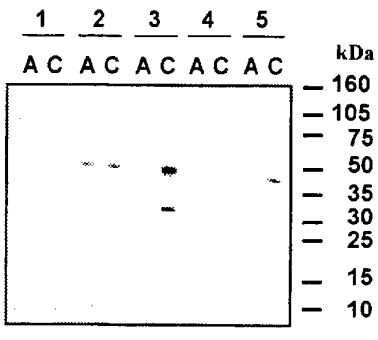
SUBSTITUTE SHEET (RULE 26)

FIG. 5A



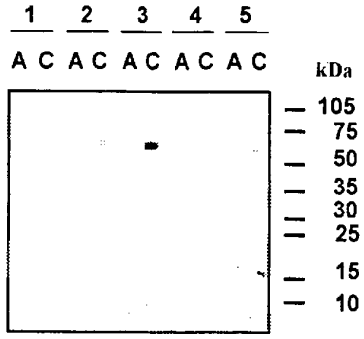
PhtA

FIG. 5B



PhtA N-terminal

FIG. 5C



PhtA C-terminal

Figure 6(a)

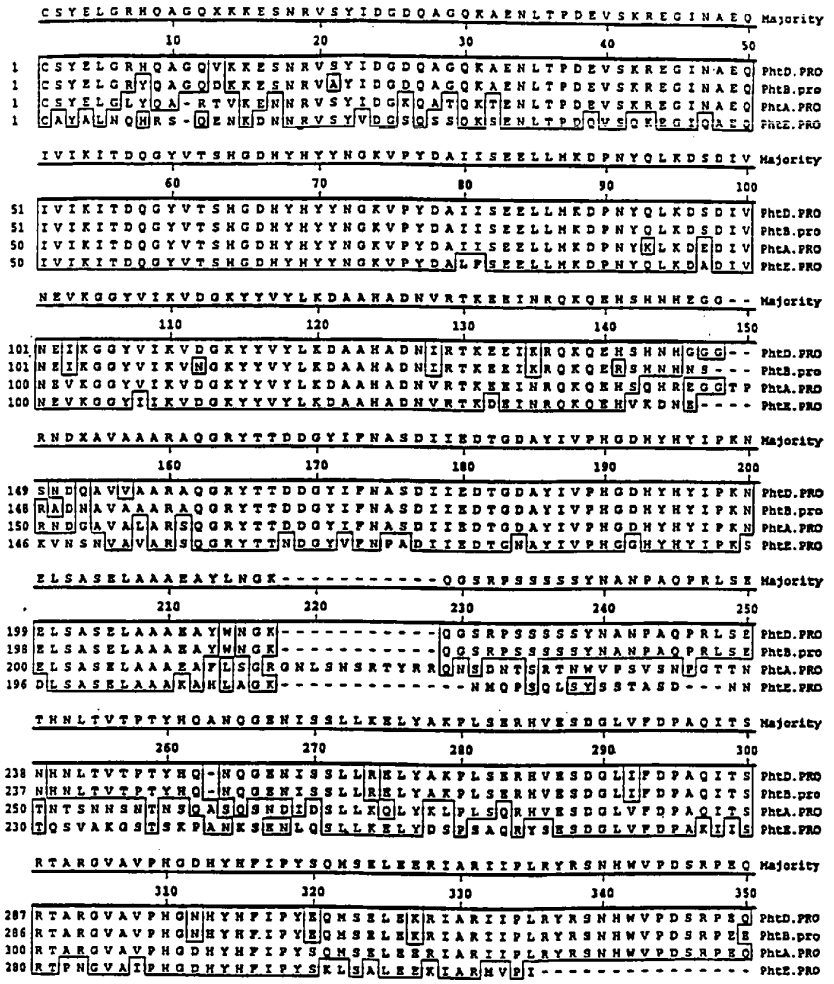


Figure 6(b)

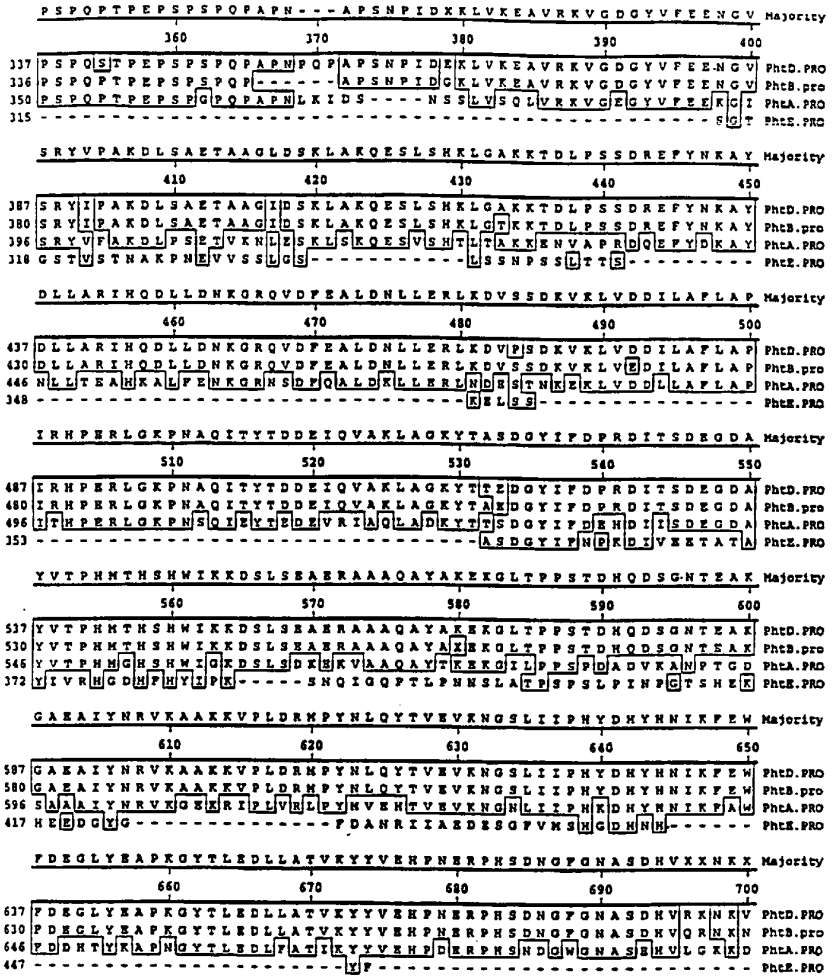
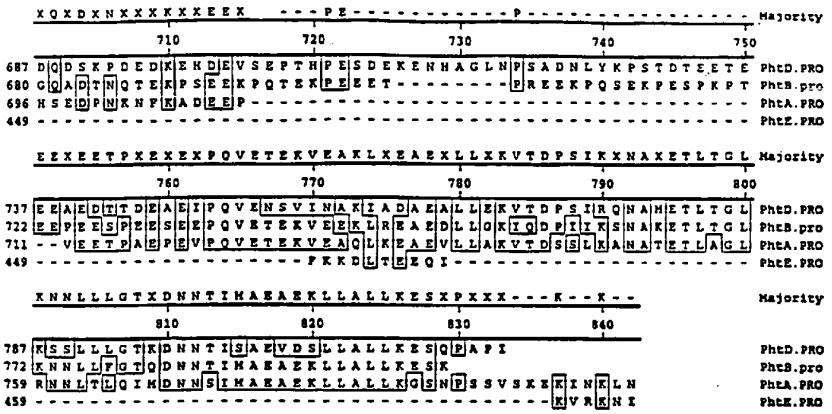


Figure 6(c)



Decoration 'Decoration #2': Box residues that match the Consensus exactly.

8/17

Figure 7(a)

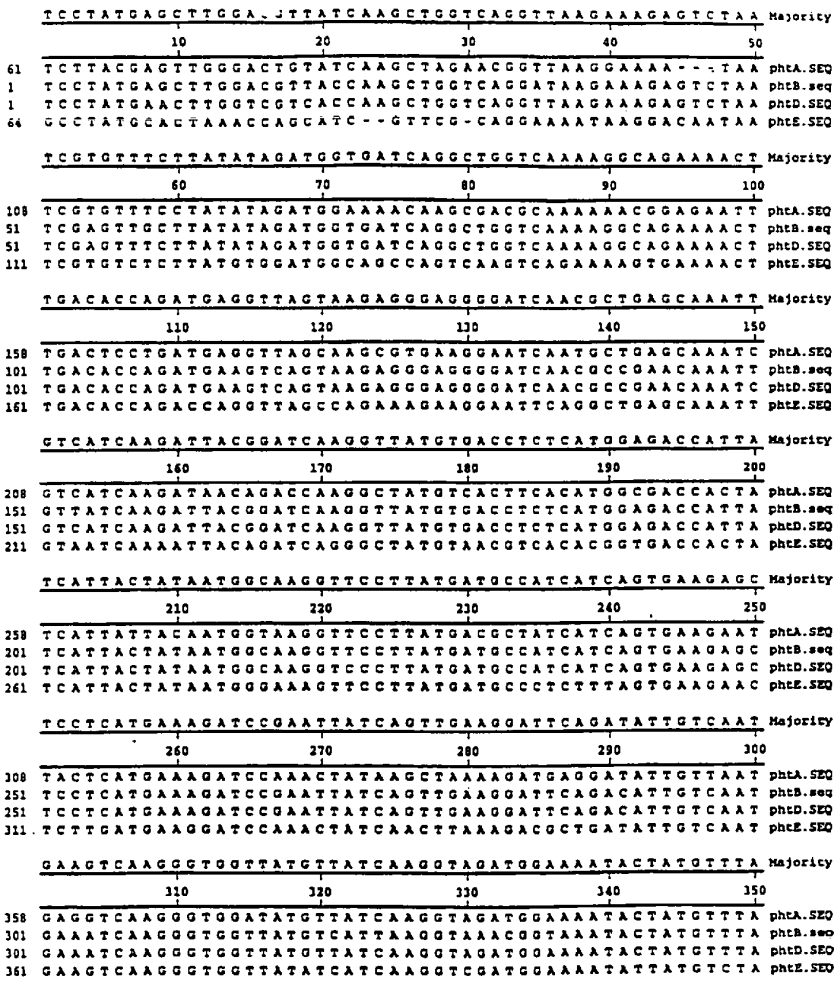


Figure 7(b)

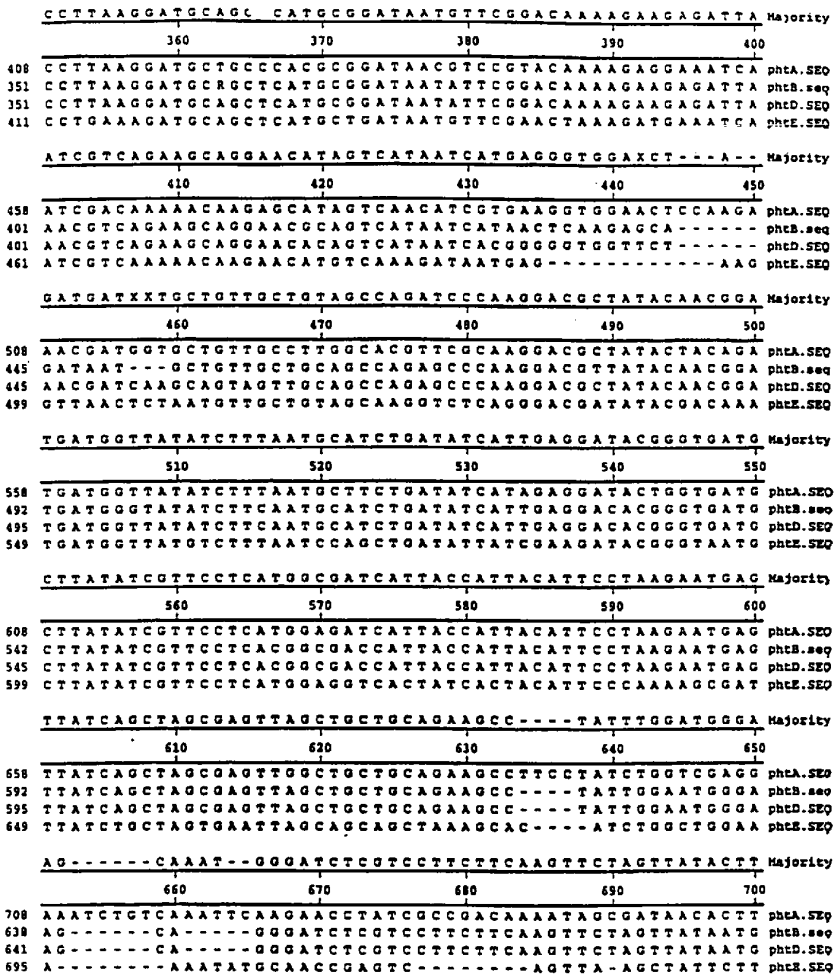


Figure 7(c)

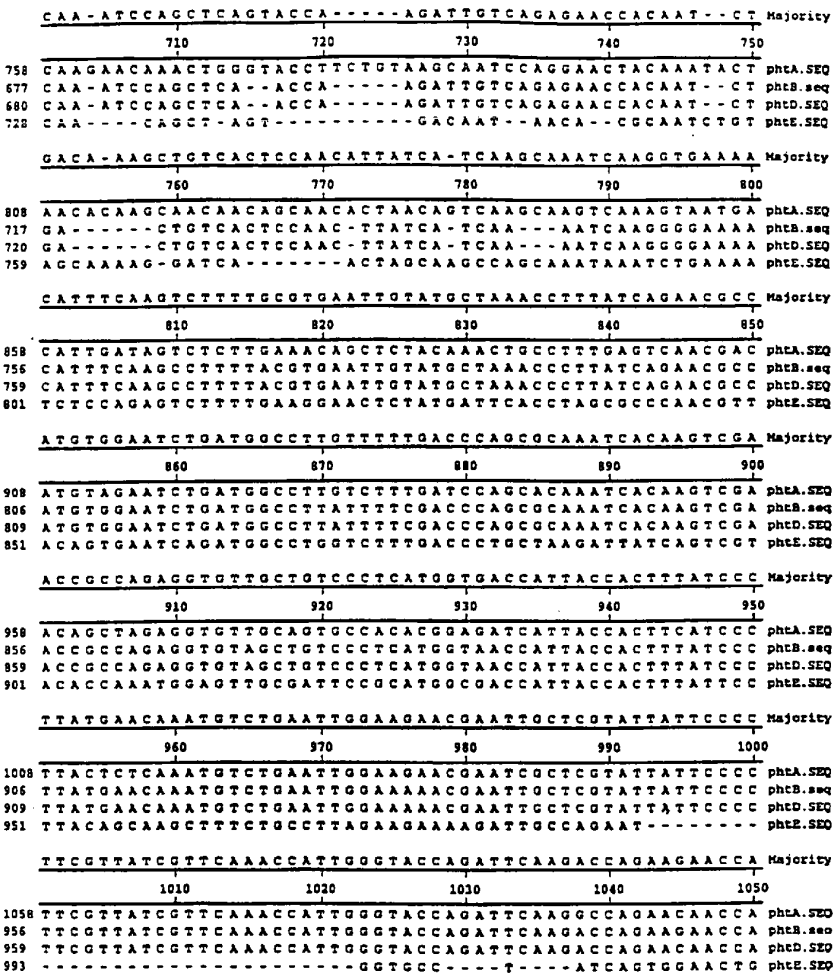


Figure 7(d)

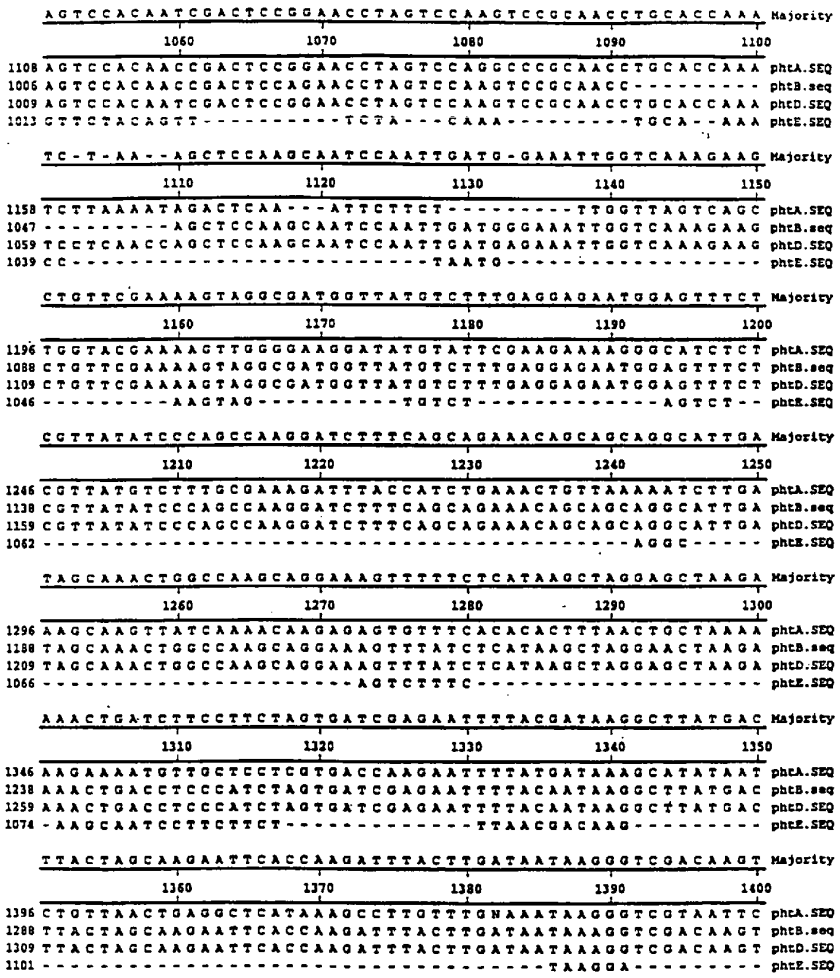


Figure 7(e)

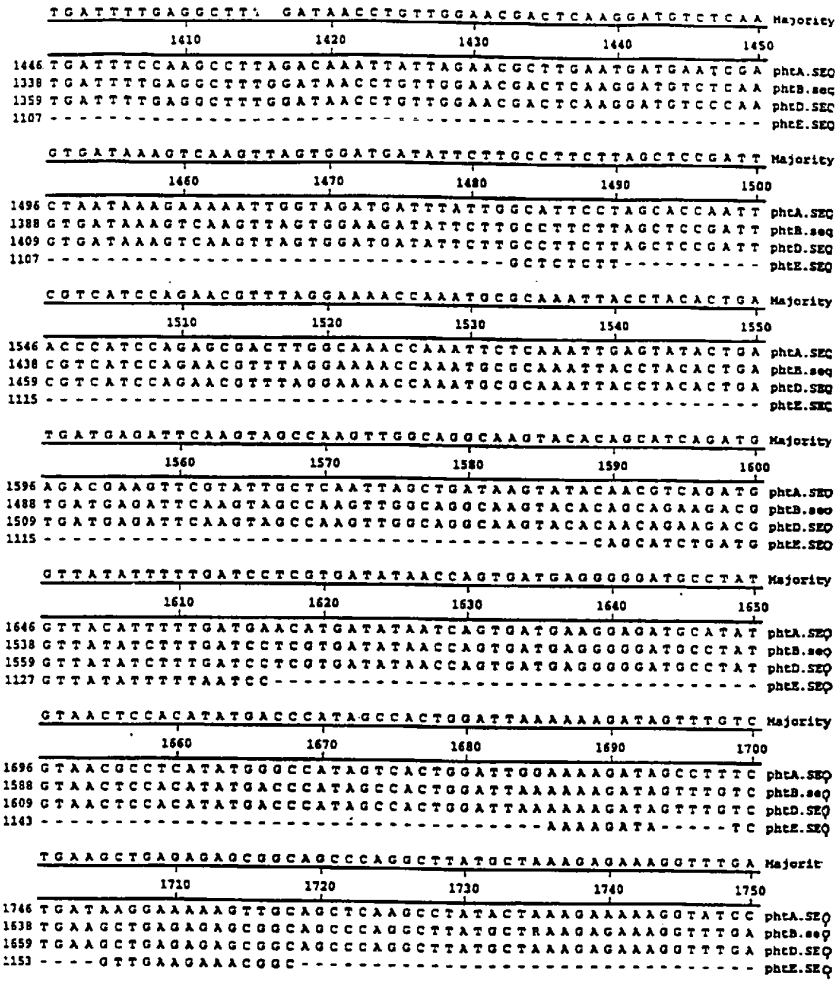


Figure 7(f)

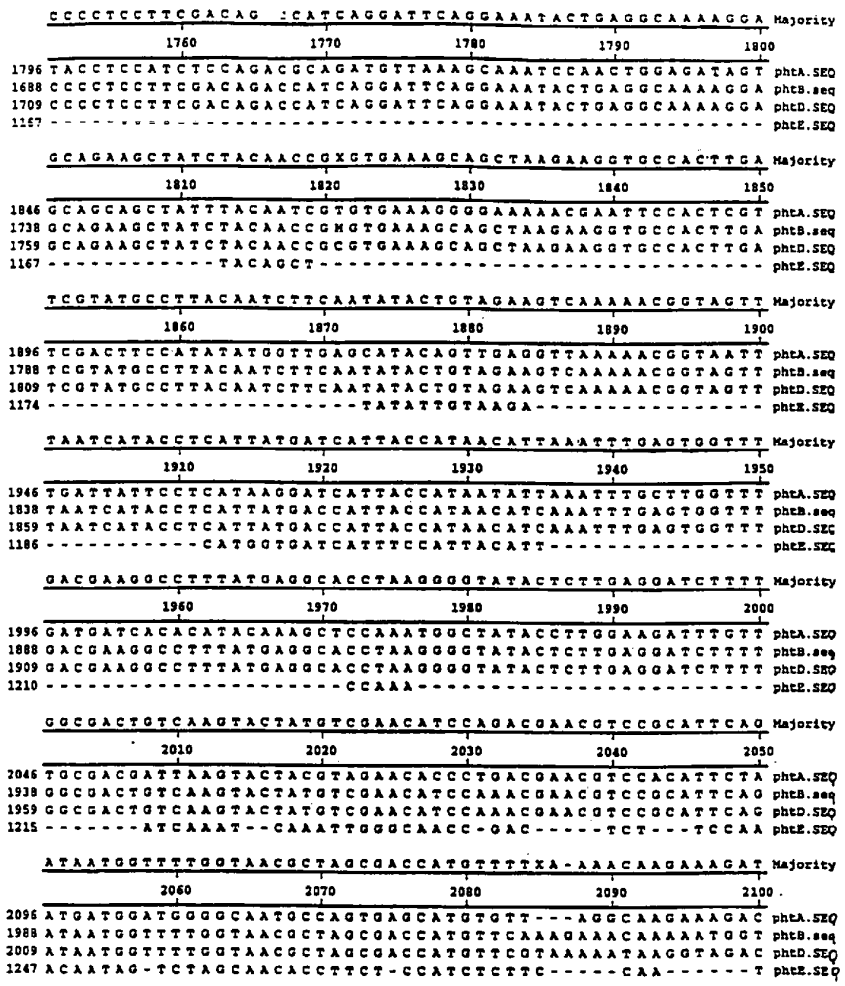


Figure 7(g)

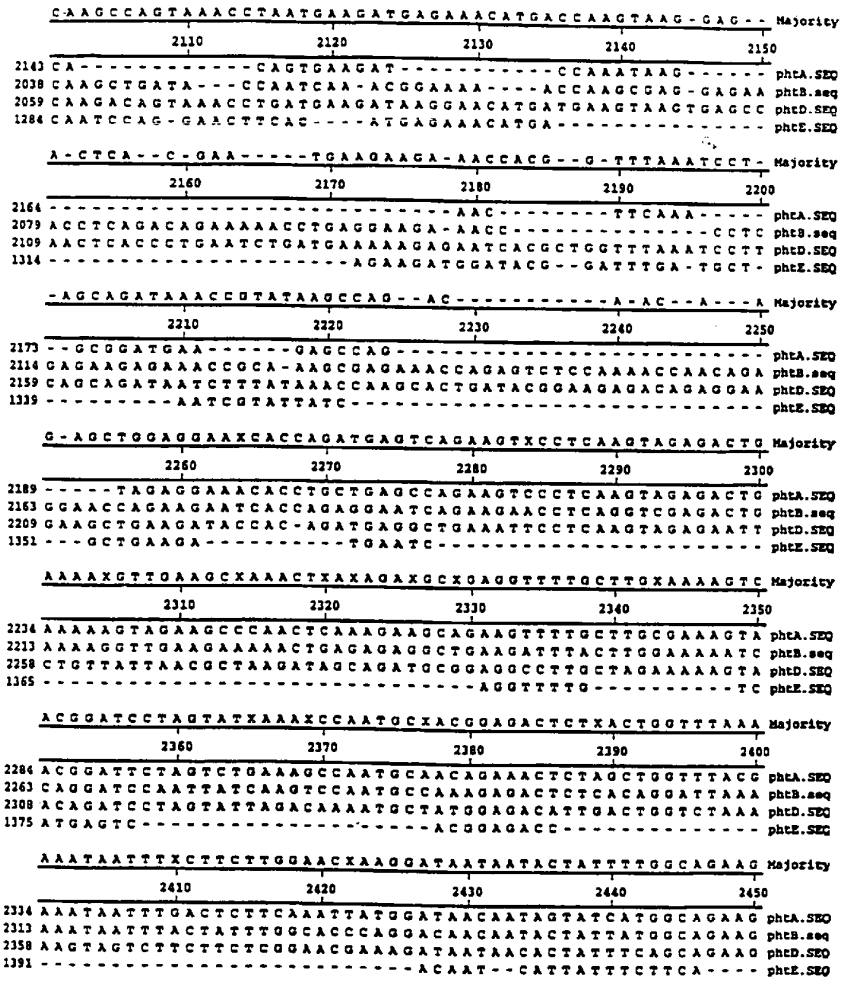


Figure 7(h)

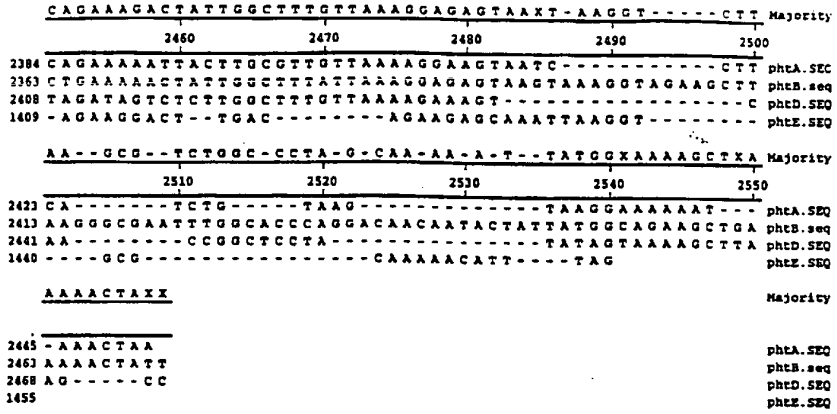
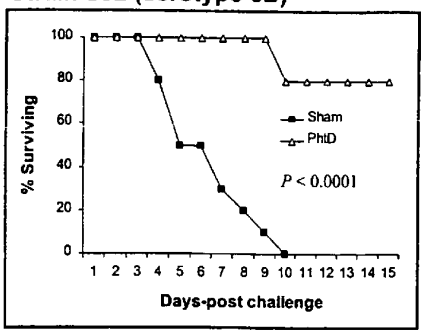
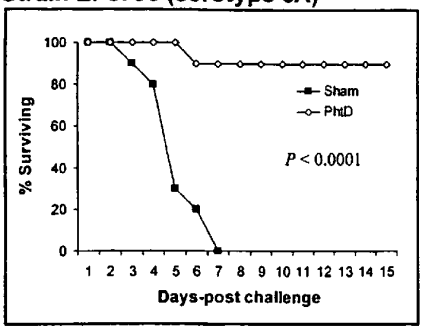


Figure 8

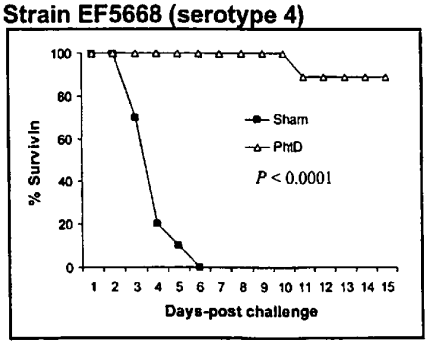
A. Strain SJ2 (serotype 6B)



B. Strain EF6796 (serotype 6A)



C. Strain EF5668 (serotype 4)



17/17