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(54) Titre : VACCIN A COMBINAISON DE PROTEINES DE SURFACE PNEUMOCOCCIQUES
(54) Title: PNEUMOCOCCAL SURFACE PROTEIN COMBINATION VACCINE

(57) Abrégé/Abstract:
The present invention relates to synergistic immunogenic combinations containing two or more pneumococcal surface proteins, including PspA and/or PspC and/or PsaA, advantageously, PspA and PsaA. Also provided are methods of intranasal administration of such immunogenic combinations to reduce nasopharyngeal carriage of pneumococci and methods of use of such immunogenic combinations in the prevention and treatment of S.pneumoniae infection.

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(54) Title: PNEUMOCOCCAL SURFACE PROTEIN COMBINATION VACCINE

(57) Abstract: The present invention relates to synergistic immunogenic combinations containing two or more pneumococcal surface proteins, including PspA and/or PspC and/or PsaA, advantageously, PspA and PsaA. Also provided are methods of intranasal administration of such immunogenic combinations to reduce nasopharyngeal carriage of pneumococci and methods of use of such immunogenic combinations in the prevention and treatment of *S.pneumoniae infection.*

TITLE OF THE INVENTION

PNEUMOCOCCAL SURFACE PROTEIN COMBINATION VACCINE

STATEMENT OF GOVERNMENT SUPPORT

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RELATED APPLICATIONS/PATENTS

This application is based upon and claims priority from U.S. Provisional application Serial No. 60/138,422, filed June 10, 1999 and from the utility application filed June 6, 2000.

Reference is made to: Briles et al., "Pneumococcal Surface Protein C (PspC)...," PCT application No. PCT/US99/08895, publication No. WO 99/53940, claiming priority to U.S. provisional application Serial No. 60/082,728, filed April 23, 1998, and U.S. application Serial No. 09/298,523, filed April 23, 1999; Briles et al. "Compositions and Methods for Administering...," PCT application No. PCT/US97/22847, filed December 4, 1997, publication No. WO 98/24927, published July 11, 1998, claiming priority to U.S. application No. 08/759,505, filed December 4, 1996; Briles et al., "Strain Selection of Pneumococcal Surface Proteins," U.S. Patent No. 5,955,089 (from U.S. application Serial No. 08/710,749, filed September 20, 1996), and PCT application No. PCT/US97/16761, filed September 20, 1997, publication No. WO 98/11915, published March 26, 1998; Briles et al., "Pneumoccocal Genes, Portions Thereof, Expression Products Therefrom, And Uses of Such Genes, Portions and Products," U.S. applications Serial Nos. 08/714,741, filed September 16, 1996, and 08/529,055, filed September 15, 1995, and PCT application No. PCT/US96/14819, filed September 16, 1996, publication No. WO 97/09994, published March 20, 1997; Briles et al. "Oral Administration ...," U.S. application Serial Nos. 08/482,981, filed June 7, 1995 (allowed); U.S. application Serial No. 08/458,399, filed June 2, 1995, and U.S. Patent No. 6,004,802 (from U.S. application Serial No. 08/657,751, filed May 30, 1996) and PCT application No. PCT/96IB/01051, filed May 31, 1996, publication No. WO 96/39113, published December 12, 1996; "Mucosal Administration ...," Briles et al., U.S. Patent No. 6,042,838 (from U.S. application Serial No. 08/446,201, filed May 19, 1995 as a CIP of U.S. Serial No. 08/246,636, filed May 20, 1994 (now U.S. Patent No. 5,965,141), and Briles et al., U.S. Patent No. 6,027,734 (from U.S. application Serial No.

08/312,949, filed September 30, 1994); Briles et al., U.S. Patent No. 5,980,909 (from U.S. application Serial No. 08/319,795, filed May, 20, 1994); Briles et al., "Epitopic Regions of Pneumococcal Surface Protein A," U.S. Patent No. 5,679,768 (from U.S. application Serial No. 08/456,746, filed June 6, 1995 as a cont. U.S. application Serial No. 08/048,896, filed April 20, 1993, now abandoned, which was as a CIP of USSN 07/835,698, filed February 12, 1992, now abandoned, which was as a CIP of U.S. application Serial No. 07/656,773, now abandoned); Briles et al.,"Structural Gene of Pneumococcal Protein," U.S. Patent No. 5,856,170 (from U.S. application Serial No. 08/467,852, filed June 6, 1995 as a cont. of U.S. application Serial No. 08/247,491, filed May 23, 1994), U.S. Patent No. 5,476,929 (from U.S. application Serial No. 08/072,070, filed June 3, 1993) and U.S. Patent Nos. 5,753,463 and 5,728,387 (from U.S. applications Serial Nos. 08/469,434, filed June 6, 1995 and U.S. application Serial No. 214,164, filed March 14, 1994, respectively); Briles et al., "Truncated PspA ...," U.S. application Serial No. 08/214,222, filed March 17, 1994 (now U.S. Patent No. 5,804,193); Briles et al. U.S. Patent No. 5,997,882 (from U.S. application Serial No. 08/468,985); Briles et al., "Immunoassay Comprising a Truncated Pneumococcal Surface Protein A (PspA)," U.S. Patent No. 5,871,943 (from U.S. application Serial No. 08/468,718, filed June 6, 1995); U.S. applications Serial Nos. 08/226,844, filed May 29, 1992, 08/093,907, filed July 5, 1994 and 07/889,918, filed July 5, 1994; PCT/US93/05191; and Briles et al., PCT application No. PCT/US92/00857, filed February 12, 1992, publication No. WO 92/1448, published September 3, 1992, claiming priority to U.S. Serial No. 656,773, filed February 15, 1991; Briles et al. "DNA Encoding ...," U.S. Patent No. 5,965,400 (from U.S. application Serial No. 247,491, filed as a Cont. of U.S. application Serial No. 835,698, filed February 12, 1992, now abandoned, which was filed as a CIP of U.S. application Serial No. 656,773, filed February 15, 1991, now abandoned) and European Patent No. 096119668, filed February 12, 1992, publication No. EP786521.

Reference is also made to U.S. Patent Nos. 4,496,538, 4,673,574, 5,948,900, and 5,474,905; and PCT application Nos. PCT/US98/19740, filed September 18, 1998, publication No. WO 99/14333, published March 25, 1999, claiming priority to U.S. application Serial No. 08/932,982, filed September 18, 1997, PCT/US99/00379, filed January 14, 1999, publication No. WO 99/40200, published August 12, 1999, claiming priority to U.S. application Serial No. 09/017,782, filed February 3, 1998,

PCT/US99/04326, filed February 26, 1999, publication No. WO 99/45121, published September 10, 1999, claiming priority to U.S. provisional application Serial No. 60/076,565, filed March 2, 1998, and PCT/US92/09522, filed November 16, 1992, publication No. WO 93/10238, published May 27, 1993, claiming priority to U.S. Patent No. 5,854,416, filed as a CIP of U.S. application Serial No. 222,179, filed Apr. 4, 1994, now abandoned, which was a CIP of Serial. No. 791,377, filed Sept. 17, 1991, now U.S. Patent No. 5,422,427.

Each of these applications and patents, as well as each document or reference cited in each of these applications and patents (including during the prosecution of each issued patent; "appln cited documents"), and each of the PCT and foreign applications or patents corresponding to and/or claiming priority from any of the foregoing applications and patents, and each of the documents cited or referenced in each of the appln cited documents, are hereby expressly incorporated herein by reference. Documents or references are also cited in this text, either in a Reference List before the claims, or in the text itself; and, each of these documents or references ("herein-cited documents or references"), as well as each document or reference cited in each of the herein-cited documents or references, is hereby expressly incorporated herein by reference.

FIELD OF THE INVENTION

The present invention relates to epitopic regions of Pneumococcal Surface Protein C, "PspC", and/or Pneumococcal Surface Protein A "PspA", and/or Pneumococcal Surface Adhesin Protein A, "PsaA" and different clades of PspC and/or PspA and/or PsaA; isolated and/or purified nucleic acid molecules such as DNA encoding a fragment or portion of PspC and/or PspA and/or PsaA such as an epitopic region of PspC and/or PspA and/or PsaA; vectors or plasmids which contain and/or express such nucleic acid molecles, e.g., *in vitro* or *in vivo*; and immunological, immunogenic or vaccine compositions comprising combinations of at least two of PspC and/or PspA and/or PsaA, and/or a portion thereof (such as epitopic region(s), and/or polypeptide(s) and/or fragment(s) thereof, of at least two of PspC and/or PspA and/or PsaA) e.g. PsaA (or a fragment thereof) and PspA (or a fragment thereof) and/or PspC (or a fragment thereof) and/or vector or vectors expressing such combinations. The composition can contain an immunogen or immunogens or epitope or epitopes of at least two of PspC and/or PspA and/or PsaA, and/or a vector

that so expresses such immunogen(s) or epitope(s). The invention further relates to methods of vaccination; and administration, as well as to methods of producing and of formulating these compositions.

PspC and/or PspA and/or PsaA, or a fragment and/or epitope thereof, and thus an immunological, immunogenic or vaccine composition comprising PspC and/or PspA and/or PsaA, or a fragment and/or epitope thereof, or a vector that so expresses such immunogen(s) or epitope(s), can be administered by the same routes, and in approximately the same amounts, as for PspA or PspC, which have been described. Thus, the invention further provides methods for administering combinations of at least two of PspC and/or PspA and/or PsaA, or a fragment and/or epitope thereof; immunological, immunogenic or vaccine compositions comprising at least two of PspC and/or PspA and/or PsaA, or a fragment and/or epitope thereof, or a vector that so expresses such immunogen(s) or epitope(s); as well as uses of PspC and/or PspA and/or PsaA, or a fragment or/and epitope thereof, or a vector that so expresses such immunogen(s) or epitope(s) to formulate such compositions.

Other aspects of the invention are described in or are obvious from (and within the ambit of the invention) the following disclosure.

BACKGROUND OF THE INVENTION

S. pneumoniae is an important cause of otitis media, meningitis, bacteremia and pneumonia, and a leading cause of fatal infections in the elderly and persons with underlying medical conditions, such as pulmonary disease, liver disease, alcoholism, sickle cell, cerebrospinal fluid leaks, acquired immune deficiency syndrome (AIDS), and patients undergoing immunosuppressive therapy. It is also a leading cause of morbidity in young children. Pneumococcal infections cause approximately 40,000 deaths in the U.S. yearly. The most severe pneumococcal infections involve invasive meningitis and bacteremia infections, of which there are 3,000 and 50,000 cases annually, respectively.

Despite the use of antibiotics and vaccines, the prevalence of pneumococcal infections has declined little over the last twenty-five years; the case-fatality rate for bacteremia is reported to be 15-20% in the general population, 30-40% in the elderly, and 36% in inner-city African Americans. Less severe forms of pneumococcal disease are pneumonia, of which there are 500,000 cases annually in the U.S. , and otitis media in children, of which there are an estimated 7,000,000 cases annually in

the U.S. caused by Pneumococcus. Strains of drug-resistant *S. pneumoniae* are becoming ever more common in the U.S. and worldwide. In some areas, as many as 30% of pneumococcal isolates are resistant to penicillin. The increase in antimicrobial resistant pneumococcus further emphasizes the need for preventing pneumococcal infections.

Humans acquire pneumococci through aerosols or by direct contact. Pneumococci first colonize the upper airways and can remain in nasal mucosa for weeks or months. As many as 50% or more of young children and the elderly are colonized. In most cases, this colonization results in no apparent infection (10-12). Studies of outbreak strains have suggested that even highly virulent strains can colonize without causing disease (13-16). In some individuals, however, the organism carried in the nasopharynx can give rise to symptomatic sinusitis or middle ear infections. If pneumococci are aspirated into the lung, especially with food particles or mucus, they can cause pneumonia. Infections at these sites generally shed some pneumococci into the blood, where they can lead to sepsis, especially if they continue to be shed in large numbers from the original focus of infection. Pneumococci in the blood can reach the brain where they can cause meningitis. Although pneumococcal meningitis is less common than other infections caused by these bacteria it is particularly devastating; some 10% of such patients die and greater than 50% of the remainder have life-long neurological sequelae (17-18).

Pneumococcus asymptomatically colonizes the upper respiratory tract of normal individuals; disease often results from the spread of organisms from the nasopharynx to other tissues during opportunistic events. The incidence of carriage in humans varies with age and circumstances. Carrier rates in children are typically higher than are those of adults. Studies have demonstrated that 38 to 60% of preschool children, 29 to 35% of grammar school children and 9 to 25% of junior high school children are carriers of pneumococcus. Among adults, the rate of carriage drops to 6% for those without children at home and to 18 to 29% for those with children at home. It is not surprising that the higher rate of carnage in children than in adults parallels the incidence of pneumococcal disease in these populations.

An attractive goal for streptococcal vaccination is to reduce carriage in the vaccinated populations and subsequently reduce the incidence of pneumococcal disease. There is speculation that a reduction in pneumococcal carriage rates by

vaccination could reduce the incidence of the disease in non-vaccinated individuals as well as vaccinated individuals. This "herd immunity" induced by vaccination against upper respiratory bacterial pathogens has been observed using the *Haemophilus influenzae* type b conjugate vaccines (20, 21, 22, 23 and 24).

If a vaccine could prevent colonization by pneumococci such vaccine would be expected to prevent virtually all pneumococcal infections in the immunized patents. Since even immunized patients might acquire pneumococci from others, a vaccine that reduced carriage should reduce infections in immuno-compromised as well as uncompromised patients.

It is generally accepted that immunity to S. pneumoniae can be mediated by specific antibodies against the polysaccharide capsule of the pneumococcus. However, neonates and young children fail to make adequate immune response against most capsular polysaccharide immunogens and can have repeated infections involving the same capsular serotype. One approach to immunizing infants against a number of encapsulated bacteria is to conjugate the capsular polysaccharide immunogens to protein to make them immunogenic. This approach has been successful, for example, with *Haemophilus influenzae* B (see U.S. Patent No. 4,496,538 to Gordon and U.S. Patent No. 4,673,574 to Anderson).

However, there are over ninety known capsular serotypes of S. pneumoniae, of which twenty-three account for about 95% of the disease. For a pneumococcal polysaccharide-protein conjugate to be successful, the capsular types responsible for most pneumococcal infections would have to be made adequately immunogenic. This approach may be difficult, because the twenty-three polysaccharides included in the presently available vaccine are not all optimally immunogenic, even in adults.

Protection mediated by anti-capsular polysaccharide antibody responses is restricted to the polysaccharide type. Different polysaccharide types differentially facilitate virulence in humans and other species. Pneumococcal vaccines have been developed by combining the 23 different capsular polysaccharides which are representative of the prevalent types of human pneumococcal disease. These 23 polysaccharide types have been used in a licensed pneumococcal vaccine since 1983 (19). The licensed 23-valent polysaccharide vaccine has a reported efficacy of approximately 60% in preventing bacteremia caused vaccine type pneumococci in healthy adults.

However, the efficacy of the vaccine has been controversial, and at times, the justification for the recommended use of the vaccine questioned. It has been speculated that the efficacy of this vaccine is negatively affected by having to combine 23 different immunogens. Having a large number of immunogens combined in a single formulation may negatively affect the antibody responses to individual types within this mixture because of immunogenic competition. The efficacy is also affected by the fact that the 23 serotypes encompass all serological types associated with human infections and carriage. Also, it is not effective in children less than 2 years of age because of their inability to make adequate responses to most polysaccharides (21,22).

An alternative approach for protecting children, and also the elderly, from pneumococcal infection involves the use of protein immunogens capable of eliciting protective immune responses. Examples of such pneumococcal protein immunogens are Pneumococcal Surface Protein C (PspC), Pneumococcal Surface Protein A (PspA) and Pneumococcal Surface Adhesin Protein A (PsaA). Such proteins may serve as vaccines by themselves, or advantageously, as described herein, may be used in combination to produce an enhanced immune response.

PspA has been identified as a virulence factor and protective immunogen. PspA is a cell surface molecule that is found on all clinical isolates, and the expression of PspA is required for the full virulence of pneumococci in mouse (34). The biological function of PspA has not been well defined, although a preliminary report suggests that it may inhibit complement activation (27).

The PspA protein type is independent of capsular type. It would seem that genetic mutation or exchange in the environment has allowed for the development of a large pool of strains which are highly diverse with respect to capsule, PspA, and possibly other molecules with variable structures. Variability of PspA's from different strains also is evident in their molecular weights, which range from 67 to 99 kD. The observed differences are stably inherited and are not the result of protein degradation.

A number of U.S. Patents and patent applications, including U.S. application Serial No. 08/529,055, filed September 15, 1995; U.S. application Serial No. 08/470,626, filed June 6, 1995; U.S. Patent No. 5,856,170; U.S. Patent No. 5,753,463; U.S. Patent No. 5,871,943; U.S. Patent No. 5,965,400; U.S. Patent No. 5,728,387; U.S. Patent No. 5,728,387; U.S. Patent No. 5,965,141; U.S. Patent No. 5,980,909; and

U.S. Patent No. 5,476,929, relate to vaccines comprising PspA and fragments thereof, methods for expressing DNA encoding PspA and fragments thereof, DNA encoding PspA and fragments thereof, the amino acid sequences of PspA and fragments thereof, compositions containing PspA and fragments thereof and methods of using such compositions. The teachings of these applications are relevant to the present invention and these applications, together with any and all of the references cited therein, are incorporated herein by reference.

Although PspA is a highly variable surface protein, sufficient homologies have been identified to allow for the grouping of pneumococcal isolates into discrete sets of families or clades. Based on this information, the teachings of which are found in U.S. Patent No. 5,955,089, a combination of 4 to 6 different PspA molecules can be used to engender an immunological response to any given pneumococcal strain.

Studies on PspA led to the discovery of a PspA-like protein and a *pspA*-like gene, now termed PspC and *pspC*. Indeed, early patent literature termed PspC as "PspA-like". Epitopic regions of PspC, DNA encoding epitopic regions of PspC, and immunological, immunogenic or vaccine compositions comprising at least one PspC are provided in PCT application No. PCT/US99/08895, publication No. WO 99/53940, claiming priority to U.S. provisional application Serial No. 60/082,728, filed April 23, 1998, and U.S. application Serial No. 09/298,523, filed April 23, 1999. The teachings of these applications are relevant to the present invention and these applications, together with any and all of the references cited therein, are incorporated herein by reference.

Another pneumococcal surface protein of interest is PsaA. Russell *et al.* described an immunogenic, species-common protein from *S. pnueumoniae* designated pneumococcal fimbrial protein A (41). This 37 kDa protein immunogen is also described in U.S. Patent No. 5,422,427, the teachings of which are hereby incorporated in their entirety herein by reference. The 37 kDa protein, which was previously referred to as pneumococcal fimbral protein A, has more recently been designated pneumococcal surface adhesin protein A (PsaA). For the purposes of the present application, references made to PsaA, pneumococcal surface adhesin protein A, pnuemococcal fimbral protein A, or the 37 kDa immunogen, shall all be understood to refer to that certain protein immunogen from *S. pneumoniae* characterized by Russell *et al.* (1990) and described in U.S. Patent No. 5,422,427.

PsaA is common to all 23 pneumococcal vaccine serotypes (41). The gene encoding PsaA has been cloned and sequenced (46). More recently, the *PsaA* gene was cloned from encapsulated strain 6B, and is the subject of pending U.S. application Serial No. 08/222,179. This gene is more representative of clinically relevant strains. A recombinant lipidated PsaA protein suitable for use in development of PsaA-based vaccines is described in pending U. S. application Serial No. 09/017,782.

In order to establish an infection, *S. pneumoniae* must first gain entry to the host through mucosal surfaces. The principal determinant of specific immunity at mucosal surfaces is secretory IgA (S-IgA) which is physiologically and functionally separate from the components of the circulatory immune system. Mucosal S-IgA responses are predominantly generated by the common mucosal immune system (CMIS) (61), in which immunogens are taken up by specialized lymphoepithelial structures collectively referred to as mucosa associated lymphoid tissue (MALT). The term common mucosal immune system refers to the fact that immunization at any mucosal site can elicit an immune response at all other mucosal sites. Thus, immunization in the gut can elicit mucosal immunity in the upper airways and vice versa.

Further, it is important to note that oral immunization can induce an immunogen-specific IgG response in the systemic compartment in addition to mucosal IgA antibodies (62).

The reservoir of *S. pneumoniae* in the world is maintained by nasopharyngeal carriage within human populations. Acquisition of pneumococci is invariably from carriers. Most people carry pneumococci many times during their lives and in the vast majority of cases carriage does not lead to disease. In a minority of cases pneumococci invade from the nasopharynx into the deeper tissue leading to pneumonia, bacteremia, sepsis, and meningitis. Even the frequency of invasion is low per carriage event, the high prevalence of carriage (between 5 and 40% of individuals) means that the morbidity and mortality attributed to *S. pneumoniae* is very high. In this country, there are over 40,000 deaths due to pneumococcal pneumonia each year.

Mucosal immunity to PspA has been shown in published studies to be able to reduce and sometimes eliminate carriage of *S. pneumoniae* in the nasopharynx. More

recently it has been demonstrated that immunity to PsaA can also elicit protection against carriage. However, neither protein alone is capable of reproducibly eliciting complete protection against pneumococcal carriage. Thus, a need exists for immunogenic compositions that are capable of reducing pneumococcal carriage.

Native protein immunogens such as PspC, PspA, and PsaA, or immunogenic fragments or epitopes thereof, stimulate an immune response when administered to a host. Recombinant proteins are promising vaccine or immunogenic composition candidates because they can be produced at high yield and purity and manipulated to maximize desirable activities and minimize undesirable ones. However, because they can be poorly immunogenic, methods to enhance the immune response to recombinant proteins are important in the development of vaccines or immunogenic compositions. Such immunogens, especially when recombinantly produced, may elicit a stronger response when administered in conjunction with an adjuvant. An adjuvant is a substance that enhances the immunogenicity of an immunogen. Adjuvants may act by retaining the immunogen locally near the site of administration to produce a depot effect, facilitating a slow, sustained release of immunogen to cells of the immune system. Adjuvants can also attract cells of the immune system, and may attract immune cells to an immunogen depot and stimulate such cells to elicit an immune response.

Immunostimulating agents or adjuvants have been used for many years to improve the host immune response to, for example, vaccines. Intrinsic adjuvants, such as lipopolysaccharides, normally are components of the killed or attenuated bacteria used as vaccines. Extrinsic adjuvants are immunomodulators that are typically non-covalently linked to immunogens and are formulated to enhance the host immune response. Aluminum hydroxide and aluminum phosphate (collectively commonly referred to as alum) are routinely used as adjuvants in human and veterinary vaccines. Currently, alum is the only adjuvant licensed for human use, although hundreds of experimental adjuvants such as cholera toxin B are being tested.

Alternative vaccination strategies are desirable as such provide alternative immunological, immunogenic or vaccine compositions, as well as alternative routes to administration or alternative routes to responses. It would be advantageous to provide an immunological composition or vaccination regimen which elicits protection against various diversified pneumococcal strains, without having to combine a large

number of possibly competitive immunogens within the same formulation. And, it is advantageous to provide additional immunogens and epitopes for use in immunological, immunogenic and/or vaccine compositions, e.g., to provide alternative compositions containing or comprising such immunogens or epitopes either alone or in combination with different immunogens.

Furthermore it is advantageous to provide a better understanding of the pathogenic mechanisms of pneumococci, as this can lead to the development of improved vaccines, diagnosis and treatments.

OBJECTS AND SUMMARY OF THE INVENTION

An object of the present invention can include providing one or more of: epitopic regions of Pneumococcal Surface Protein C, "PspC", and/or Pneumococcal Surface Protein A "PspA", and/or Pneumococcal Surface Adhesin Protein A, "PsaA" and different clades of PspC and/or PspA and/or PsaA; isolated and/or purified nucleic acid molecules such as DNA encoding a fragment or portion of PspC and/or PspA and/or PsaA such as an epitopic region of PspC and/or PspA and/or PsaA; vectors or plasmids which contain and/or express such nucleic acid molecules, e.g., *in vitro* or *in vivo*; and immunological, immunogenic or vaccine compositions comprising combinations of at least two of PspC and/or PspA and/or PsaA, and/or a portion thereof (such as epitopic region(s), and/or polypeptide(s) and/or fragment(s) thereof, of at least two of PspC and/or PspA and/or PsaA) e.g. PsaA (or a fragment thereof) and PspA (or a fragment thereof) and/or PspC (or a fragment thereof) and/or vector or vectors expressing such combinations. The composition can contain an immunogen or immunogens or epitope or epitopes of at least two of PspC and/or PspA and/or PsaA, and/or a vector that so expresses such immunogen(s) or epitope(s). The invention further relates to methods of vaccination; and administration, as well as to methods of producing and of formulating these compositions.

Thus, the invention provides a composition comprising: (i) PsaA or an epitope thereof, or a vector that expresses PsaA or an epitope thereof, and (ii) (a) PspA or an epitope thereof or a vector that expresses PspA or an epitope thereof, or (b) PspC or an epitope thereof or a vector that expresses PspC or an epitope thereof, or (c) PspA or an epitope thereof and PspC or an epitope thereof, or a vector that expresses PspA or an epitope thereof and PspC or an epitope thereof, or a first vector that expresses PspA or an epitope thereof and a second vector that expresses PspC or an epitope

thereof, or (iii) a vector that expresses PsaA or an epitope thereof and PspA or an epitope thereof and/or PspC or an epitope thereof. The composition can include a carrier and/or diluents. The composition can further comprise an adjuvant. The adjuvant can be alum, e.g., aluminum phosphate and/or aluminum hydroxide, for instance, as a gel, Sponin, Quil A, and the water-in-oil adjuvant, Freund's with killed tubercle bacilli (Freund's complete) or without bacilli (Freund's incomplete); *see also* PCT/US98/23472 with respect to adjuvants that can be used in the practice of this invention.

It is a further object of the invention to provide methods for administering inventive compositions, e.g., combinations of at least two of PspC and/or PspA and/or PsaA, or a fragment and/or epitope thereof; immunological, immunogenic or vaccine compositions comprising at least two of PspC and/or PspA and/or PsaA, or a fragment and/or epitope(s) thereof, or a vector that so expresses such immunogen(s) or epitope(s); as well as uses of PspC and/or PspA and/or PsaA, or a fragment or/and epitope thereof, or a vector that so expresses such immunogen(s) or epitope(s) to formulate such compositions.

In a preferred embodiment of the invention, recombinantly produced PspA and PsaA are formulated together with an adjuvant, such as the B subunit of Cholera toxin B (CTB) or alum. CTB and alum function as an adjuvant. The PspA and/or PsaA and/or PspC combination, together with an adjuvant such as CTB and/or alum, are preferably administered intranasally.

It is a further object of the invention to provide methods of use of immunogenic combinations of PspA and/or PsaA and/or PspC or epitope thereof or vector (s) that so express the protein(s), to reduce nasal carriage of pneumococci. It is believed that reduction of nasal carriage will result in reduced transmission of pneumococci to individuals at risk for pneumococcal infection.

Documents cited in this disclosure, including the above-referenced applications, provide typical additional ingredients for such compositions, such that undue experimentation is not required by the skilled artisan to formulate a composition from this disclosure. Such compositions should preferably contain quantities of the pneumococcal PspA and/or PsaA and/or PspC or epitopes thereof or vector(s) that so express the protein(s) sufficient to elicit a suitable response. Determination of the optimal amounts of each protein to use in the combination can

be determined empirically, with a minimum of experimentation. Those skilled in the art will recognize, for example, that such determination can be based upon amounts of immunogen administered to experimental animals in the examples below and in the documents cited herein.

The terms "comprising", "comprises", "comprises of" and the like have the meaning ascribed to these terms under U.S. law and can mean "including", "includes" and the like.

Throughout this specification, reference is made to various documents so as to describe more fully the state of the art to which this invention pertains. These documents, as well as documents cited by these documents, are each hereby incorporated herein by reference. And, these and other objects and embodiments are described in or are obvious from and within the scope of the invention, from the following Detailed Description.

DETAILED DESCRIPTION

The present invention provides an immunological, immunogenic or vaccine composition comprising combinations of at least two of PspC and/or PspA and/or PsaA, and/or a portion thereof (such as epitopic region(s), and/or polypeptide(s) and/or fragment(s) thereof, of at least two of PspC and/or PspA and/or PsaA) e.g. PsaA (or a fragment thereof) and PspA (or a fragment thereof) and/or PspC (or a fragment thereof) and/or vector or vectors expressing such combinations and a pharmaceutically acceptable carrier or diluent. Thus, the invention provides a composition comprising: (i) PsaA or an epitope thereof, or a vector that expresses PsaA or an epitope thereof, and (ii) (a) PspA or an epitope thereof or a vector that expresses PspA or an epitope thereof, or (b) PspC or an epitope thereof or a vector that expresses PspC or an epitope thereof, or (c) PspA or an epitope thereof and PspC or an epitope thereof, or a vector that expresses PspA or an epitope thereof and PspC or an epitope thereof, or a first vector that expresses PspA or an epitope thereof and a second vector that expresses PspC or an epitope thereof, or (iii) a vector that expresses PsaA or an epitope thereof and PspA or an epitope thereof and/or PspC or an epitope thereof. The composition can include a carrier and/or diluents. The composition can further comprise an adjuvant. An immunological composition elicits an immunological response - local or systemic. The response can, but need not be, protective. An immunogenic composition containing the pneumococcal protein

combination likewise elicits a local or systemic immunological response which can, but need not be, protective. A vaccine composition elicits a local or systemic protective response. Accordingly, the terms "immunological composition" and "immunogenic composition" include a "vaccine composition" (as the two former terms can be protective compositions).

As to epitopes of interest, reference is made to documents cited herein and Kendrew, THE ENCYCLOPEDIA OF MOLECULAR BIOLOGY (Blackwell Science Ltd., 1995) and Sambrook, Fritsch and Maniatis, Molecular Cloning, A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, 1982. An epitope of interest is an immunologically relevant region of an immunogen or immunologically active fragment thereof, e.g., from a pathogen or toxin of veterinary or human interest. One skilled in the art can determine an epitope or immunodominant region of a peptide or polypeptide and ergo the coding DNA therefor from the knowledge of the amino acid and corresponding DNA sequences of the peptide or polypeptide, as well as from the nature of particular amino acids (e.g., size, charge, etc.) and the codon dictionary, without undue experimentation.

The DNA sequence preferably encodes at least regions of the peptide that generate an antibody response or a T cell response. One method to determine T and B cell epitopes involves epitope mapping. The protein of interest is synthetized in short overlapping peptides (PEPSCAN). The individual peptides are then tested for their ability to bind to an antibody elicited by the native protein or to induce T cell or B cell activation. Janis Kuby, Immunology, (1992) pp.79-80.

Another method for determining an epitope of interest is to choose the regions of the protein that are hydrophilic. Hydrophilic residues are often on the surface of the protein and are therefore often the regions of the protein which are accessible to the antibody. Janis Kuby, Immunology, (1992) p. 81.

Still another method for choosing an epitope of interest which can generate a T cell response is to identify from the protein sequence potential HLA anchor binding motifs which are peptide sequences which are known to be likely to bind to the MHC molecule.

The peptide which is a putative epitope of interest, to generate a T cell response, should be presented in a MHC complex. The peptide preferably contains

appropriate anchor motifs for binding to the MHC molecules, and should bind with high enough affinity to generate an immune response.

Some guidelines in determining whether a protein is an epitopes of interest which will stimulate a T cell response, include: Peptide length--the peptide should be at least 8 or 9 amino acids long to fit into the MHC class I complex and at least 13-25 amino acids long to fit into a class II MHC complex. This length is a minimum for the peptide to bind to the MHC complex. It is preferred for the peptides to be longer than these lengths because cells may cut the expressed peptides. The peptide should contain an appropriate anchor motif which will enable it to bind to the various class I or class II molecules with high enough specificity to generate an immune response (See Bocchia, M. et al, Specific Binding of Leukemia Oncogene Fusion Protein Peptides to HLA Class I Molecules, Blood 85:2680-2684; Englehard, VH, Structure of peptides associated with class I and class II MHC molecules Ann. Rev. Immunol. 12:181 (1994)). This can be done, without undue experimentation, by comparing the sequence of the protein of interest with published structures of peptides associated with the MHC molecules.

Further, the skilled artisan can ascertain an epitope of interest by comparing the protein sequence with sequences listed in the protein database.

Even further, another method is simply to generate or express portions of a protein of interest, generate monoclonal antibodies to those portions of the protein of interest, and then ascertain whether those antibodies inhibit growth in vitro of the pathogen from which the from which the protein was derived. The skilled artisan can use the other guidelines set forth in this disclosure and in the art for generating or expressing portions of a protein of interest for analysis as to whether antibodies thereto inhibit growth in vitro.

The invention therefore also provides a method of inducing an immunological response in a host mammal comprising administering to the host an inventive immunogenic, immunological or vaccine composition, e.g., a composition comprising a combination of pneumococcal proteins or immunogens, PspC and/or PspA and/or PsaA, or an epitope or fragment thereof, and/or vector or vectors expressing such combinations and a pharmaceutically acceptable carrier or diluent. The composition can further comprise an adjuvant and the method can further include administering an adjuvant. Surprisingly, the combination of PspA and PsaA is synergistic, and results

in an improved immunological response and reduced carriage of pneumococci compared to immunization with either immunogens alone.

The determination of the amount of each pneumococcal protein or immunogen, e.g. PspA and/or PsaA and/or PspC, and optional additional adjuvant in the inventive compositions and the preparation of those compositions can be in accordance with standard techniques well known to those skilled in the pharmaceutical or veterinary arts. In particular, the amount of immunogen and adjuvant in the inventive compositions and the dosages administered are determined by techniques well known to those skilled in the medical or veterinary arts taking into consideration such factors as the particular immunogengen, the adjuvant (if present), the age, sex, weight, species and condition of the particular animal or patient, and the route of administration. For instance, dosages of particular PspA and/or PsaA and/or PspC immunogens for suitable hosts in which an immunological response is desired, can be readily ascertained by those skilled in the art from this disclosure, as is the amount of any adjuvant typically administered therewith. Thus, the skilled artisan can readily determine the amount of immunogen and optional adjuvant in compositions and to be administered in methods of the invention. Typically, an adjuvant is commonly used as 0.001 to 50 wt% solution in phosphate buffered saline, and the immunogen is present on the order of micrograms to milligrams, such as about 0.0001 to about 5 wt%, preferably about 0.0001 to about 1 wt%, most preferably about 0.0001 to about 0.05 wt% (see, e.g., Examples below or in applications cited herein).

Typically, however, the immunogen is present in an amount on the order of micrograms to milligrams, or, about 0.001 to about 20 wt%, preferably about 0.01 to about 10 wt%, and most preferably about 0.05 to about 5 wt%.

Of course, for any composition to be administered to an animal or human, including the components thereof, and for any particular method of administration, it is preferred to determine therefor: toxicity, such as by determining the lethal dose (LD) and LD50 in a suitable animal model e.g., rodent such as mouse; and, the dosage of the composition(s), concentration of components therein and timing of administering the composition(s), which elicit a suitable immunological response, such as by titrations of sera and analysis thereof for antibodies or immunogens, e.g., by ELISA analysis. Such determinations do not require undue experimentation from the knowledge of the skilled artisan, this disclosure and the documents cited herein.

And, the time for sequential administrations can be ascertained without undue experimentation.

Examples of compositions of the invention include liquid preparations for orifice, e.g., oral, nasal, anal, vaginal, perioral, intragastric, mucosal (e.g., perlingual, alveolar, gingival, olfactory or respiratory mucosa) etc., administration such as suspensions, syrups or elixirs; and, preparations for parenteral, subcutaneous, intradermal, intramuscular or intravenous administration (e.g., injectable administration), such as sterile suspensions or emulsions. Such compositions may be in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose or the like. The compositions can also be lyophilized. The compositions can contain auxiliary substances such as wetting or emulsifying agents, pH buffering agents, gelling or viscosity enhancing additives, preservatives, flavoring agents, colors, and the like, depending upon the route of administration and the preparation desired. Standard texts, such as "REMINGTON'S PHARMACEUTICAL SCIENCE", 17th edition, 1985, incorporated herein by reference, may be consulted to prepare suitable preparations, without undue experimentation.

Compositions of the invention are conveniently provided as liquid preparations, e.g., isotonic aqueous solutions, suspensions, emulsions or viscous compositions which may be buffered to a selected pH. If digestive tract absorption is preferred, compositions of the invention can be in the "solid" form of pills, tablets, capsules, caplets and the like, including "solid" preparations which are time-released or which have a liquid filling, e.g., gelatin covered liquid, whereby the gelatin is dissolved in the stomach for delivery to the gut. If nasal or respiratory (mucosal) administration is desired, compositions may be in a form and dispensed by a squeeze spray dispenser, pump dispenser or aerosol dispenser. Aerosols are usually under pressure by means of a hydrocarbon. Pump dispensers can preferably dispense a metered dose or a dose having a particular particle size. Compositions of the invention can contain pharmaceutically acceptable flavors and/or colors for rendering them more appealing, especially if they are administered orally. The viscous compositions may be in the form of gels, lotions, ointments, creams and the like and will typically contain a sufficient amount of a thickening agent so that the viscosity is from about 2500 to 6500 cps, although more viscous compositions, even up to 10,000

cps may be employed. Viscous compositions have a viscosity preferably of 2500 to 5000 cps, since above that range they become more difficult to administer. However, above that range, the compositions can approach solid or gelatin forms which are then easily administered as a swallowed pill for oral ingestion.

Liquid preparations are normally easier to prepare than gels, other viscous compositions, and solid compositions. Additionally, liquid compositions are somewhat more convenient to administer, especially by injection or orally, to animals, children, particularly small children, and others who may have difficulty swallowing a pill, tablet, capsule or the like, or in multi-dose situations. Viscous compositions, on the other hand, can be formulated within the appropriate viscosity range to provide longer contact periods with mucosa, such as the lining of the stomach or nasal mucosa.

Obviously, the choice of suitable carriers and other additives will depend on the exact route of administration and the nature of the particular dosage form, e.g., liquid dosage form (e.g., whether the composition is to be formulated into a solution, a suspension, gel or another liquid form), or solid dosage form (e.g., whether the composition is to be formulated into a pill, tablet, capsule, caplet, time release form or liquid-filled form).

Solutions, suspensions and gels, normally contain a major amount of water (preferably purified water) in addition to the immunogen, and optional adjuvant. Minor amounts of other ingredients such as pH adjusters (e.g., a base such as NaOH), emulsifiers or dispersing agents, buffering agents, preservatives, wetting agents, jelling agents, (e.g., methylcellulose), colors and/or flavors may also be present. The compositions can be isotonic, i.e., it can have the same osmotic pressure as blood and lacrimal fluid.

The desired isotonicity of the compositions of this invention may be accomplished using sodium chloride, or other pharmaceutically acceptable agents such as dextrose, boric acid, sodium tartrate, propylene glycol or other inorganic or organic solutes. Sodium chloride is preferred particularly for buffers containing sodium ions.

Viscosity of the compositions may be maintained at the selected level using a pharmaceutically acceptable thickening agent. Methylcellulose is preferred because it is readily and economically available and is easy to work with. Other suitable

thickening agents include, for example, xanthan gum, carboxymethyl cellulose, hydroxypropyl cellulose, carbomer, and the like. The preferred concentration of the thickener will depend upon the agent selected. The important point is to use an amount that will achieve the selected viscosity. Viscous compositions are normally prepared from solutions by the addition of such thickening agents.

A pharmaceutically acceptable preservative can be employed to increase the shelf-life of the compositions. Benzyl alcohol may be suitable, although a variety of preservatives including, for example, parabens, thimerosal, chlorobutanol, or benzalkonium chloride may also be employed. A suitable concentration of the preservative will be from 0.02% to 2% based on the total weight although there may be appreciable variation depending upon the agent selected.

Those skilled in the art will recognize that the components of the compositions must be selected to be chemically inert with respect to the pneumococcal immunogens and optional additional adjuvant. This will present no problem to those skilled in chemical and pharmaceutical principles, or problems can be readily avoided by reference to standard texts or by simple experiments (not involving undue experimentation), from this disclosure and the documents cited herein.

The immunologically effective compositions of this invention are prepared by mixing the ingredients following generally accepted procedures. For example the selected components may be simply mixed in a blender, or other standard device to produce a concentrated mixture which may then be adjusted to the final concentration and viscosity by the addition of water or thickening agent and possibly a buffer to control pH or an additional solute to control tonicity. Generally the pH may be from about 3 to 7.5. Compositions can be administered in dosages and by techniques well known to those skilled in the medical and veterinary arts taking into consideration such factors as the age, sex, weight, and condition of the particular patient or animal, and the composition form used for administration (e.g., solid vs. liquid). Dosages for humans or other mammals can be determined without undue experimentation by the skilled artisan, from this disclosure, the documents cited herein, the Examples below (e.g., from the Examples involving mice and from the applications cited herein, e.g., under "Related Applications", especially since adminstion can be in a manner and dose analogous to PspA or PspC).

Suitable regimes for initial administration and booster doses or for sequential administrations also are variable, may include an initial administration followed by subsequent administrations; but nonetheless, may be ascertained by the skilled artisan, from this disclosure, the documents cited herein, including applications cited herein, and the Examples below. The compositions can be administered alone, or can be co-administered or sequentially administered with other compositions of the invention or with other prophylactic or therapeutic compositions. The skilled artisan can readily adjust concentrations known for PspA or PspC to account for a combination of at least two of PspC and/or PspA and/or PsaA, or an epitope or fragment thereof.

The PspC immunogen (PspC or an epitope or fragment thereof), as well as a PspA immunogen (PspA or an epitope or fragment thereof) as well as a PsaA immunogen (PsaA or an epitope or fragment thereof) can be expressed recombinantly, e.g., in *E. coli* or in another vector or plasmid for either *in vivo* expression or *in vitro* expression. The methods for making and/or administering a vector or recombinant or plasmid for expression of PspC and/or PspA and/or PsaA, or a epitope or fragment thereof, either *in vivo* or *in vitro* can be any desired method, e.g., a method which is by or analogous to the methods disclosed in: U.S. Patent Nos. 4,603,112, 4,769,330, 5,174,993, 5,505,941, 5,338,683, 5,494,807, 4,722,848, 5,942,335, 5,364,773, 5,762,938, 5,770,212, 5,942,235, 5,756,103, 5,766,599, 6,004,777, 5,990,091, 6,033,904, 5,869,312, 5,382,425, WO 94/16716, WO 96/39491, Paoletti, "Applications of pox virus vectors to vaccination: An update," PNAS USA 93:11349-11353, October 1996, Moss, "Genetically engineered poxviruses for recombinant gene expression, vaccination, and safety," PNAS USA 93:11341-11348, October 1996, Smith et al., U.S. Patent No. 4,745,051 (recombinant baculovirus), Richardson, C.D. (Editor), Methods in Molecular Biology 39, "Baculovirus Expression Protocols" (1995 Humana Press Inc.), Smith et al., "Production of Huma Beta Interferon in Insect Cells Infected with a Baculovirus Expression Vector," Molecular and Cellular Biology, Dec., 1983, Vol. 3, No. 12, p. 2156-2165; Pennock et al., "Strong and Regulated Expression of *Escherichia coli* B-Galactosidase in Infect Cells with a Baculovirus vector," Molecular and Cellular Biology Mar. 1984, Vol. 4, No. 3, p. 399-406; EPA 0 370 573, U.S. application Serial No. 920,197, filed October 16, 1986, EP Patent publication No. 265785, U.S. Patent No. 4,769,331 (recombinant herpesvirus), Roizman, "The function of herpes simplex virus genes: A primer for

genetic engineering of novel vectors," PNAS USA 93:11307-11312, October 1996, Andreansky et al., "The application of genetically engineered herpes simplex viruses to the treatment of experimental brain tumors," PNAS USA 93:11313-11318, October 1996, Robertson et al. "Epstein-Barr virus vectors for gene delivery to B lymphocytes," PNAS USA 93:11334-11340, October 1996, Frolov et al., "Alphavirus-based expression vectors: Strategies and applications," PNAS USA 93:11371-11377, October 1996, Kitson et al., J. Virol. 65, 3068-3075, 1991; U.S. Patent Nos. 5,591,439, 5,552,143 (recombinant adenovirus), Grunhaus et al., 1992, "Adenovirus as cloning vectors," Seminars in Virology (Vol. 3) p. 237-52, 1993, Ballay et al. EMBO Journal, vol. 4, p. 3861-65, Graham, Tibtech 8, 85-87, April, 1990, Prevec et al., J. Gen Virol. 70, 429-434, PCT WO91/11525, Felgner et al. (1994), J. Biol. Chem. 269, 2550-2561, Science, 259:1745-49, 1993 and McClements et al., "Immunization with DNA vaccines encoding glycoprotein D or glycoprotein B, alone or in combination, induces protective immunity in animal models of herpes simplex virus-2 disease," PNAS USA 93:11414-11420, October 1996, and U.S. Patents Nos 5,591,639, 5,589,466, and 5,580,859 relating to DNA expression vectors, *inter alia*. See also WO 98/33510; Ju et al., Diabetologia, 41:736-739, 1998 (lentiviral expression system); Sanford et al., U.S. Patent No. 4,945,050 (method for transporting substances into living cells and tissues and apparatus therefor); Fischbach et al. (Intracel), WO 90/01543 (method for the genetic expression of heterologous proteins by cells transfected); Robinson et al., seminars in IMMUNOLOGY, vol. 9, pp.271-283 (1997) (DNA vaccines); Szoka et al., U.S. Patent No. 4,394,448 (method of inserting DNA into living cells); and McCormick et al., U.S. Patent No. 5,677,178 (use of cytopathic viruses for therapy and prophylaxis of neoplasia).

The expression product generated by vectors or recombinants in this invention optionally can also be isolated and/or purified from infected or transfected cells; for instance, to prepare compositions for administration to patients. However, in certain instances, it may be advantageous to not isolate and/or purify an expression product from a cell; for instance, when the cell or portions thereof enhance the effect of the PsaA and or/PspA and/or PspC.

An inventive vector or recombinant expressing PspC, or an epitope or fragment thereof, and/or PspA, or an epitope or fragment thereof and/or PsaA, or an epitope or fragment thereof, can be administered in any suitable amount to achieve

expression at a suitable dosage level, e.g., a dosage level analogous to the aforementioned dosage levels (wherein the immunogen or epitope of interest is directly present). The inventive vector or recombinant can be administered to a patient or infected or transfected into cells in an amount of about at least 10^3 pfu; more preferably about 10^4 pfu to about 10^{10} pfu, e.g., about 10^5 pfu to about 10^9 pfu, for instance about 10^6 pfu to about 10^8 pfu. In plasmid compositions, the dosage should be a sufficient amount of plasmid to elicit a response analogous to compositions wherein PspC, or an epitope or fragment thereof, and/or PspA, or an epitope or fragment thereof and/or PsaA, or an epitope or fragment thereof are directly present; or to have expression analogous to dosages in such compositions; or to have expression analogous to expression obtained *in vivo* by recombinant compositions. For instance, suitable quantities of plasmid DNA in plasmid compositions can be 1 *u*g to 100 mg, preferably 0.1 to 10 mg, e.g., 500 micrograms, but lower levels such as 0.1 to 2 mg or preferably 1-10 *u*g may be employed. Documents cited herein regarding DNA plasmid vectors may be consulted for the skilled artisan to ascertain other suitable dosages for DNA plasmid vector compositions of the invention, without undue experimentation.

The present invention is additionally described by way of the following illustrative, non-limiting examples.

EXAMPLES

EXAMPLE 1

This Example illustrates the use of a mouse model for pneumococcal carriage. Three different strains S. pneumoniae (L82016, BG9163 and BG8826) were inoculated in 12 µl volumes into the nares of CBA mice over a period of several minutes using slow delivery from a 20 µ1 micropipette. After 7 days, the mice were sacrificed and their trachea was cut at the top of the throat. 50 µ1 of fluid was instilled and washed out through the nares. The area washed represents the pharynx and nasal tissues. Each of these strains was able to establish carriage in this tissue without concomitant sepsis or bacteremia. The results are shown in Table 1 below.

TABLE 1:

Table 1	Carriage	of three strains of *S.*	*pneumoniae* in the nasopharnyx of CBA/N mice			
Strain	L82106		BG9163		BG8826	
Capsule	6B		6B		23F	
Type LD_{50} (i.v.)	$\geq 10^7$		$\sim 3 \times 10^3$		$\geq 10^7$	
Mouse	#1	#2	#1	#2	#1	#2
Nasal	6,561	1,422	1,494	3,267	31,733	10,093
Blood	<3	<3	<3	<3	<3	<3
Lungs	600	<60	<60	<60	<60	180

Note: mice were inoculated with the indicated numbers of CFU i.n. and sacrificed 8 days later. Data expressed as CFU in the 50 µl nasal wash, in the 1 ml of lung homogenate, or in 50 µ1 of blood. Infection of CBA/N mice i.p. with 10^7 L82016 is invariably fatal.

After the first day of infection some low numbers of pneumococci were observed in the lung and blood of a small number of the mice. Later in the infection, pneumococci were only recoverable from the nasal wash. Colonization appeared to be stable for at least 3 weeks.

To identify the pneumococci from nasal washes they were plated on gentamicin plates. This antibiotic was chosen because it kills most bacteria found in the nose, but does not affect the growth of pneumococci. Individual colonies from each nasal wash were then picked and replated with an optochin disk to confirm that they were pneumococci. In some cases, the bacteria were capsule-typed to confirm their identity with the type of bacteria used to inoculate the mice. Control mice that received no bacteria yielded no bacteria that grew on 0.2% gentamycin plates and were sensitive to optochin. Challenge with as few as 2X107 bacteria will establish carriage in all mice. Lower doses, down to 103, yield comparable carriage in most mice, but as many as '/4 to 1/3 of mice inoculated with such a low dose fail to carry any pneumococci after 1 week. Accordingly, a suitable dose for conducting these studies appears to be between 107 and 106 CFU of L81905. The appropriate dose for other strains can be readily determined empirically, using the same general method as set forth above.

EXAMPLE 2

Ability of a combination of PspA and PsaA to reduce nasopharyngeal carriage by the *S. pneumoniae* strain L82016 (capsular type 6B).

PspA and PsaA genes were cloned from strain the Rxl/D39 family of strains and the proteins were expressed in *E. coli*. Mice were immunized with rPspA (pUAB055), PsaA (lot 11.12.98), a combination of both or neither. Both proteins were administered separately or mixed together as 500ng doses in 10 microliter volumes of lactated Ringer's solution. Mice were immunized every Monday and Friday for three consecutive weeks. Four micrograms for commercially obtained Cholera toxin B subunit, a mucosal adjuvant [Wu, 1997 #1101], was administered with each immunization for the first two weeks. Cholera toxin B subunit was not given during the third week of immunization. Control mice got Ringer's injection + CTB during the first two weeks and ringer's injection only during the third week. 14 days post infection mice were challenge i.n. with 4,650,000 colony forming units of strain L82016. L82016 is a capsular type 6B strain of S. pneumoniae.

Two weeks post infection of the mice, saliva and serum was collected from each mouse to assess antibody elicitation. The results are shown in Table 2 below.

TABLE 2:

Effect of intranasal immunization with PspA and/or PsaA on antibody production and resistance to nasal carrier of strain L82016 following i.n. challenge				
Parameter	Immunogen			
	CTB only	RpspA + CTB	RPsaA + CTB	RPsaA+ RPspA+ CTB+
Number of mice	5	4	4	4
Mean to CFU/nose	**5.04**	**4.05**	**2.18**	**1.31***
	Average saliva antibody in microgram/ml			
Anti-PspA (microgram/ml)	<0.001	**0.008**	<0.001	**0.008**
Anti-PspA (microgram/ml)	<0.16	<0.16	**0.819**	**0.680**
	Average serum antibody in microgram/ml			
Anti-PspA (microgram/ml)	<0.0008	**16.39**	<0.0008	**148.8**
Anti-PspA (microgram/ml)	<0.011	<0.011	**358**	**468**

EXAMPLE 3

Ability of a combination of pneumococcal surface proteins PspA and PsaA to protect against nasopharyngeal challenge with *S. pneumoniae* strain C134.

The experiment was performed exactly as for Example 1, above, except that the challenge consisted of 1,000,000 colony forming units of C134, a capsular type 23S strain of *S. pneumoniae*. The results are shown in Table 3 below.

TABLE 3:

Effect of intranasal immunization with PspA and/or PsaA on antibody production and resistance to nasal carrier of strain E 134 following i.n. challenge				
Parameter	Immunogen			
	CTB only	RpspA + CTB	RPsaA + CTB	RpsaA+ RPspA+ CTB+
Number of mice	11	10	10	10
Mean log CFU/nose Standard error	4.80	4.75	3.70	2.96*
	Average saliva antibody in microgram/ml			
Anti-PspA (microgram/ml)	<0.001	0.018	<0.001	0.003
Anti-PspA (microgram/ml)	<0.16	<0.16	1.22	1.10
	Average serum antibody in microgram/ml			
Anti-PspA (microgram/ml)	<0.0008	45.5	<0.0008	24.6
Anti-PspA (microgram/ml)	<0.011	<0.011	164	22.6

EXAMPLE 4

EFFECTS OF IMMUNIZATION ON NASAL CARRIAGE

The experiment was run essentially as for Example 2 above.

The results are shown in Table 4.

TABLE 4:

	Max Log CFU in lungs ± S.E. L82016 Cps. Type 6B	E134 Cps. Type 23
CTB alone	5.04	4.80
PspA	3.95*	4.75
PsaA	2.51**	3.70
PspA+PsaA	1.31	2.96*

* P<0.05

* *P<0.01

EXAMPLE 5

The experiment was run as for Example 2, except that alum was used as adjuvant and immunogens were administered sub-q. The results are shown in Table 5.

TABLE 5:

Immunogen	Max Log CFU in lungs
Alum only	6.24
PspA	3.92
PsaA	6.58
PspA+PsaA	3.46*

*P<0.01

* * *

Having thus described in detail preferred embodiments of the present invention, it is to be understood that the invention defined by the appended claims is not to be limited to particular details set forth in the above description as many

apparent variations thereof are possible without departing from the spirit or scope of the present invention.

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WHAT IS CLAIMED IS:

1. An immunological combination composition comprising a pharmaceutically acceptable carrier or diluent, and an immunologically active ingredient,

wherein the immunologically active ingredient comprises:

(i) Pneumococcal surface adhesion protein A (PsaA) or an epitope thereof or a vector that expresses PsaA or an epitope thereof, and

(ii) (a) Pneumococcal surface protein A (PspA) or an epitope thereof or a vector that expresses PspA or an epitope thereof, or

(b) Pneumococcal surface protein C (PspC) or an epitope thereof or a vector that expresses PspC or an epitope thereof, or

(c) PspA or an epitope thereof and PspC or an epitope thereof, or a vector that expresses PspA or an epitope thereof and PspC or an epitope thereof, or a first vector that expresses PspA or an epitope thereof and a second vector that expresses PspC or an epitope thereof;

or, the immunologically active ingredient comprises:

(iii) a vector that expresses PsaA or an epitope thereof, and PspA or an epitope thereof, and/or PspC or an epitope thereof.

2. The compositions of claim 1, wherein the pneumococcal surface protein immunogens are recombinantly produced.

3. The composition of claim 1 comprising PsaA and PspA.

4. The composition of claim 3, further comprising an adjuvant.

5. The immunological composition of claim 4, wherein the adjuvant is cholera toxin subunit B or alum.

6. A method of inducing an immunological response in an animal comprising the step of administering to the animal the immunological composition of any one of claims 4.

7. The method of claim 6, wherein the administering is intranasal.

8. A method of immunizing a host against pneumococcal infection, which method

comprises administering to the host an immunologically effective amount of PspA together with an immunologically effective amount of PsaA.

9. The method of claim 8, wherein the administering is intranasal.

10. An immunogenic composition for intranasal administration to a host susceptible.

to pneumococcal carriage to elicit a protective immunological response against colonization with *S. pneumoniae* in the nasopharnyx, which comprises an immunizing amount of a combination of two or more pneumococcal surface protein immunogens, wherein the combination includes PspA and PsaA, or immunogenic fragments thereof.

11. The immunogenic composition of claim 10, wherein the pneumococcal surface protein immunogens are recombinants produced.

12. The composition of claim 11, further comprising an adjuvant.

13. The composition of claim 12, wherein the adjuvant is cholera toxin B (CTB) or alum.