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(54) Title: MULTICOMPONENT IMMUNOGENIC COMPOSITION FOR THE PREVENTION OF BETA-HEMOLYTIC STREPTOCOCCAL (BHS) DISEASE

(57) Abstract: A number of β-hemolytic streptococci polynucleotides and polypeptides, particularly Streptococcus pyogenes polypeptides and polynucleotides, are described. Two or more of the polypeptides of the invention can be formulated for use as immunogenic compositions. Also disclosed are methods for immunizing against and reducing infection caused by β-hemolytic streptococci.

Fig 12

![Graph showing CFU/mL data for different groups with statistical significance](image-url)
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MULTICOMPONENT IMMUNOGENIC COMPOSITION FOR THE PREVENTION OF BETA-HEMOLYTIC STREPTOCOCCAL (BHS) DISEASE

FIELD OF THE INVENTION

This invention relates generally to β-hemolytic streptococcal (BHS) polypeptides and polynucleotides, particularly Streptococcus pyogenes polypeptides and polynucleotides, and their use in multicomponent immunogenic compositions to prevent BHS disease. More specifically, the invention relates to polypeptides of Streptococcus pyogenes which are surface localized. The invention further relates to immunogenic compositions, and methods for immunizing against and reducing β-hemolytic streptococcal infection comprising combinations of two or more of polypeptides.

BACKGROUND OF THE INVENTION

Traditional phenotypic criteria for classification of streptococci include both hemolytic reactions and Lancefield serological groupings. However, with taxonomic advances, it is now known that unrelated species of β-hemolytic (defined as the complete lysis of sheep erythrocytes in agar plates) streptococci (BHS) may produce identical Lancefield antigens and that strains genetically related at the species level may have heterogeneous Lancefield antigens. In spite of these exceptions to the traditional rules of streptococcal taxonomy, hemolytic reactions and Lancefield serological tests can still be used to divide streptococci into broad categories as a first step in identification of clinical isolates. Ruoff, K.L., R.A. Whiley, and D. Beighton. 1999. Streptococcus. In P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Yolken (eds.), Manual of Clinical Microbiology. American Society of Microbiology Press, Washington D.C.

β-hemolytic isolates with Lancefield group A, C, or G antigen can be subdivided into two groups: large-colony (>0.5 mm in diameter) and small-colony (<0.5 mm in diameter) formers. Large-colony-forming group A (Streptococcus pyogenes), C, and G strains are "pyogenic" streptococci replete with a variety of effective virulence mechanisms. Streptococcus agalactiae (group B) is still identified reliably by its production of Lancefield group B antigen or other phenotypic traits.
Similarities between BHS species include not only virulence factors, but also disease manifestations. Included in the latter are pneumonia, arthritis, abscesses, rhinopharyngitis, metritis, puerperal sepsis, neonatal septicemia, wound infections, meningitis, peritonitis, cellulitis, pyoderma, necrotizing fasciitis, toxic shock syndrome, septicemia, infective endocarditis, pericarditis, glomerulonephritis, and osteomyelitis.

*Streptococcus pyogenes* are Gram-positive diplococci that colonize the pharynx and skin of humans, sites that then serve as the primary reservoir for this organism. An obligate parasite, this bacterium is transmitted by either direct contact of respiratory secretions or by hand-to-mouth. The majority of *Streptococcus pyogenes* infections are relatively mild illnesses, such as pharyngitis or impetigo. Currently, there are anywhere from twenty million to thirty-five million cases of pharyngitis alone in the U.S., costing about $2 billion for physician visits and other related expenses. Additionally, nonsuppurative sequelae such as rheumatic fever, scarlet fever, and glomerulonephritis result from *Streptococcus pyogenes* infections. Globally, acute rheumatic fever (ARF) is the most common cause of pediatric heart disease (1997. Case definitions for Infectious Conditions Under Public Health Surveillance. CDC).

From the initial portals of entry, pharynx, and skin, *Streptococcus pyogenes* can disseminate to other parts of the body where bacteria are not usually found, such as the blood, deep muscle and fat tissue, or the lungs, and can cause invasive infections. Two of the most severe but least common forms of invasive *Streptococcus pyogenes* disease are necrotizing fasciitis and streptococcal toxic shock syndrome (STSS). Necrotizing fasciitis (described in the media as "flesh-eating bacteria") is a destructive infection of muscle and fat tissue. STSS is a rapidly progressing infection causing shock and injury to internal organs such as the kidneys, liver, and lungs. Much of this damage is due to a toxemia rather than localized damage due to bacterial growth.

In 1995, invasive *Streptococcus pyogenes* infections and STSS became mandated reportable diseases. In contrast to the millions of individuals that acquire pharyngitis and impetigo, the U.S. Centers for Disease Control and Prevention (CDC) mandated case reporting indicates that in 1997 there were from 15,000 to 20,000 cases of invasive *Streptococcus pyogenes* disease in the United States, resulting in over 2,000 deaths (1997. Case definitions for Infectious Conditions Under Public Health Surveillance. CDC). Other reports estimate invasive disease to be as high as 10-20 cases per 100,000 individuals per
year (Stevens, D. L. 1995. Streptococcal toxic-shock syndrome: spectrum of disease, pathogenesis, and new concepts in treatment. Emerg Infect Dis. 1:69-78). More specifically, of the 15,000 to 20,000 cases of invasive disease, 1,100 to 1,500 are cases of necrotizing fasciitis and 1,000 to 1,400 are cases of STSS, with a 20% and 60% mortality rate, respectively. Also included in serious invasive disease are cases of myositis, which carries a fatality rate of 80% to 100%. An additional 10% to 15% of individuals die with other forms of invasive group A streptococcal disease. These numbers have increased since case reporting was initiated in 1995 and reflect a general trend that has occurred over the past decade or two. Additionally, it is commonly agreed that the stringency of the case definitions results in lower and, thus, misleading numbers, in that many cases are successfully resolved due to early diagnosis and treatment before the definition has been met.

While *Streptococcus pyogenes* remains sensitive to penicillin and its derivatives, treatment does not necessarily eradicate the organism. Approximately 5% to 20% of the human population are carriers depending on the season (Stevens, D. L. 1995. Streptococcal toxic-shock syndrome: spectrum of disease, pathogenesis, and new concepts in treatment. Emerg Infect Dis. 1:69-78), despite antibiotic therapy. The reasons for this are not totally clear and may involve a variety of mechanisms. In cases of serious invasive infections, treatment often requires aggressive surgical intervention. For those cases involving STSS or related disease, clindamycin (a protein synthesis inhibitor) is the preferred antibiotic as it penetrates tissues well and prevents exotoxin production. There are reports of some resistance to tetracycline, sulfa, and most recently, erythromycin. Clearly, there remains a need for compositions to prevent and treat β-hemolytic infection.

Numerous virulence factors have been identified for *Streptococcus pyogenes*, some secreted and some surface localized. Although it is encapsulated, the capsule is composed of hyaluronic acid and is not suitable as a candidate antigen for inclusion in immunogenic compositions, since it is commonly expressed by mammalian cells and is nonimmunogenic (Dale, J. B., R. G. Washburn, M. B. Marques, and M. R. Wessels. 1996. Hyaluronate capsule and surface M protein in resistance to opsonization of group A streptococci. Infect Immun. 64:1495-501). The T antigen and Group Carbohydrate are other candidates, but may also elicit cross-reactive antibodies to heart tissue. Lipoteichoic acid is present on the surface of *Streptococcus pyogenes*, but raises safety concerns similar to LPS.
The most abundant surface proteins fall into a family of proteins referred to as M or "M-like" proteins because of their structural similarity. While members of this class have similar biological roles in inhibiting phagocytosis, they each have unique substrate binding properties. The best characterized protein of this family is the helical M protein. Antibodies directed to homologous M strains have been shown to be opsonic and protective (Dale, J. B., R. W. Baird, H. S. Courtney, D. L. Hasty, and M. S. Bronze. 1994. Passive protection of mice against group A streptococcal pharyngeal infection by lipoteichoic acid. J Infect Dis. 169:319-23, Dale, J. B., M. Simmons, E. C. Chiang, and E. Y. Chiang. 1996. Recombinant, Ellen, R. P., and R. J. Gibbons. 1972. M protein-associated adherence of Streptococcus pyogenes to epithelial surfaces: prerequisite for virulence. Infect Immun. 5:826-830.).

Complicating the use of M protein as a candidate antigen is the fact that there have been approximately 100 different serotypes of M protein identified with several more untyped. Typically, the Class I M serotypes, exemplified by serotypes M1, M3, M6, M12, and M18, are associated with pharyngitis, scarlet fever, and rheumatic fever and do not express immunoglobulin binding proteins. Class II M serotypes, such as M2 and M49, are associated with the more common localized skin infections and the sequelae glomerulonephritis, and do express immunoglobulin binding proteins (Podbielski, A., A. Flosdorff, and J. Weber-Heynemann. 1995. The group A streptococcal virR49 gene controls expression of four structural vir regulon genes. Infect Immun. 63:9-20). It is important to note that there is little, if any, heterologous cross-reactivity of antibodies to M serotypes. Equally important is the role these antibodies play in rheumatic fever. Specific regions of M protein elicit antibodies that cross react with host heart tissue, causing or at least correlating with cellular damage (Cunningham, M. W., and A. Quinn. 1997. Immunological crossreactivity between the class I epitope of streptococcal M protein and myosin. Adv Exp Med Biol. 418:887-921, Quinn, A., K. Ward, V. A. Fischetti, M. Hemric, and M. W. Cunningham. 1998. Immunological relationship between the class I epitope of streptococcal M protein and myosin. Infect Immun. 66:4418-24.).


Numerous secreted proteins have been described, several of which are considered to be toxins. Most Streptococcus pyogenes isolates from cases of serious invasive disease and streptococcal toxic shock syndrome (STSS) produce streptococcal pyogenic exotoxins (SPE) A and C (Cockerill, F. R., 3rd, R. L. Thompson, J. M. Musser, P. M. Schlievert, J. Talbot, K. E. Holley, W. S. Harmen, D. M. I1strup, P. C. Kohner, M. H. Kim, B. Frankfort, J. M. Manahan, J. M. Steckelberg, F. Roberson, and W. R. Wilson. 1998. Molecular, serological, and clinical features of 16 consecutive cases of invasive streptococcal disease. Southeastern Minnesota Streptococcal Working Group. Clin Infect Dis. 26:1448-58). Other pyogenic exotoxins have also been identified in the genomic Streptococcus pyogenes sequence completed at the University of Oklahoma, submitted to GenBank and assigned accession

Given the number of known virulence factors produced by Streptococcus pyogenes, it is clear that an important characteristic for a successful β-hemolytic streptococcal immunogenic composition would be its ability to stimulate a response that would prevent or limit colonization early in the infection process. This protective response would either block
adherence and/or enhance the clearance of cells through opsonophagocytosis. Antibodies to M protein have been shown to be opsonic and provide a mechanism to overcome the anti-phagocytic properties of the protein (Jones, K. F., and V. A. Fischetti. 1988. The importance of the location of antibody binding on the M6 protein for opsonization and phagocytosis of group A M6 streptococci. J Exp Med. 167: 1114-23) in much the same way that anti-serotype B capsular antibodies have demonstrated protection from disease caused by *Haemophilus influenzae* B (Madore, D. V. 1998. Characterization of immune response as an indicator of *Haemophilus influenzae* type b vaccine efficacy. Pediatr Infect Dis J. 17:S207-10). In addition, antibodies specific to Protein F have been shown to block adherence and internalization by tissue culture cells (Molinari, G., S. R. Talay, P. Valentin-Weigand, M. Rohde, and G. S. Chhatwal. 1997. The fibronectin-binding protein of *Streptococcus pyogenes*, SfbI, is involved in the internalization of group A streptococci by epithelial cells. Infect Immun. 65:1357-63).

There remains a need to develop immunogenic compositions and methods to prevent or ameliorate infections caused by β-hemolytic streptococci, including groups A, B, C and G. There also remains a need to provide immunogenic compositions which provide immunity to a broad range of BHS bacteria.

**SUMMARY OF THE INVENTION**

To meet these and other needs, and in view of its purposes, the present invention provides immunogenic compositions for the protecting of susceptible mammals against colonization or infection by β-hemolytic streptococci including Group A, B, C, and/or D streptococci, including those from *Streptococcus pyogenes*. These immunogenic compositions comprise a mixture of two or more polypeptides as described more fully below. The invention also provides methods of preventing or ameliorating such colonization, in a susceptible mammal by administering an effective amount of the immunogenic composition to generate antibodies to the specific polypeptides contained within the immunogenic composition. The invention further provides *Streptococcus pyogenes* polypeptides and polynucleotides, recombinant materials, and methods for their production. Another aspect of the invention relates to methods for using such *Streptococcus pyogenes* polypeptides and polynucleotides. The polypeptides and polynucleotides can also be used in the manufacture of a medicament for preventing or ameliorating an infection caused by β-hemolytic streptococci.
The polypeptides utilized in the immunogenic compositions of the invention include
isolated polypeptides comprising at least one of an amino acid sequence of any of Figs. 2, 4,
6, 8, or 10. The invention also includes amino acid sequences that have at least 90% identity
to any of the foregoing amino acid sequences, and mature polypeptides of these. The
invention further includes immunogenic fragments and biological equivalents of these
polypeptides. Also provided are antibodies that immunospecifically bind to the polypeptides
of the invention.

The polynucleotides of the invention include isolated polynucleotides that comprise
nucleotide sequences that encode a polypeptide of the invention. These polynucleotides
include isolated polynucleotides comprising at least one of a nucleotide sequence of any of
figs. 1, 3, 5, 7, or 9, and also include other nucleotide sequences that, as a result of the
degeneracy of the genetic code, also encode a polypeptide of the invention. The invention
also includes isolated polynucleotides comprising a nucleotide sequence that has at least 90%
identity to a nucleotide sequence that encodes a polypeptide of the invention, and isolated
polynucleotides comprising a nucleotide sequences that has at least 90% identity to any of the
foregoing nucleotide sequences. In addition, the isolated polynucleotides of the invention
include nucleotide sequences that hybridize under stringent hybridization conditions to a
nucleotide sequence that encodes a polypeptide of the invention, nucleotide sequences that
hybridize under stringent hybridization conditions to a nucleotide sequence of any of the
foregoing sequences, and nucleotide sequences that are fully complementary to these
polynucleotides. Furthermore, the invention includes expression vectors and host cells
comprising these polynucleotides.

The invention also provides immunogenic compositions which comprise an
immunogenic amount of at least two or more components (selected from SCP (Figure 2 (SEQ
ID NO:2) and the peptides coded for by ORF 554 (peptidylpropyl isomerase (Figure 4 (SEQ
ID NO:4)), ORF 1218 (hypothetical protein (Figure 6 (SEQ ID NO:6)), ORF 1358 (putative
adhesion protein (Figure 8 (SEQ ID NO:8)), and ORF 2459 (surface lipoprotein (Figure 10
(SEQ ID NO: 10)) each of which comprises a polypeptide of the invention in an amount
effective to prevent or ameliorate a β-hemolytic streptococcal colonization or infection in a
susceptible mammal. Each component may comprise the polypeptide itself, or may comprise
the polypeptide and any other substance (e.g., one or more chemical agents, proteins, etc.)
that can aid in the prevention and/or amelioration of β-hemolytic streptococcal colonization
or infection. These immunogenic compositions can further comprise at least a portion of the
polypeptide, optionally conjugated or linked to a peptide, polypeptide, or protein, or to a polysaccharide.

The invention also includes methods of protecting a susceptible mammal against β-hemolytic streptococcal colonization or infection. In one embodiment, the method comprises administering to a mammal an effective amount of a two or more immunogenic composition comprising an immunogenic amount of a polypeptide of the invention, which amount is effective to prevent or ameliorate β-hemolytic streptococcal colonization or infection in the susceptible mammal. Such combinations of components, it has been found, are effective to provide such protection to a broad range of groups, and generally provide a greater immune response than the individual components administered separately. The immunogenic compositions of the invention can be administered by any conventional route, for example, by subcutaneous or intramuscular injection, oral ingestion, or intranasally.

The invention further provides immunogenic compositions. In one embodiment, the immunogenic composition comprises at least one polypeptide of the invention. In another embodiment, the immunogenic composition comprises at least one polynucleotide of the invention.

It is to be understood that the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.

**BRIEF DESCRIPTION OF THE DRAWINGS**

Fig. 1 presents the nucleic acid sequence encoding for C5a peptidase ("SCP": SEQ ID NO:1).

Fig. 2 presents the amino acid sequence of SCP (SEQ ID NO:2).

Fig. 3 presents the nucleic acid sequence of ORF 554 encoding for peptidylpropyl isomerase (SEQ ID NO:3).

Fig. 4 presents the amino acid sequence of peptidylpropyl isomerase (SEQ ID NO:4).

Fig. 5 presents the nucleic acid sequence of ORF 1218 encoding for a hypothetical protein (SEQ ID NO:5).

Fig. 6 presents the amino acid sequence of a hypothetical protein (SEQ ID NO:6).
Fig. 7 presents the nucleic acid sequence of ORF 1358 encoding for a putative adhesion protein (SEQ ID NO:7).

Fig. 8 presents the amino acid sequence of a putative adhesion protein (SEQ ID NO:8).

Fig. 9 presents the nucleic acid sequence of ORF 2459 encoding for a surface lipoprotein (SEQ ID NO:9).

Fig. 10 presents the amino acid sequence of a surface lipoprotein (SEQ ID NO:10).

Fig. 11 graphically presents percentage killing compared to media of the three component ("Trivax" = SCP, peptidylpropyl isomerase (ORF 554), and putative adhesion protein (ORF 1358)) and one component ("554" = peptidylpropyl isomerase (ORF 554)) immunogenic compositions examined in Example 2.

Figs. 12-16 graphically demonstrate the passive immunity transfer results of Example 3. CFUs = colony forming units.

**DETAILED DESCRIPTION OF THE INVENTION**

The present invention provides immunogenic compositions to prevent or ameliorate infections caused by β-hemolytic streptococci, including groups A, B, C and G. Two or more of the polypeptides enumerated herein are combined together to make an immunogenic composition.

Specifically, in one embodiment, an immunogenic composition of this invention comprises a mixture of two or more polypeptides, each polypeptide encoded by a nucleic acid sequence having at least 90% identity to a nucleic acid sequence selected from the group consisting of:

(a) C5a peptidase ("SCP") (Figure 1 (SEQ ID NO:1));

(b) open reading frame ("ORF") 554 (Figure 3 (SEQ ID NO:3));

(c) ORF 1218 (Figure 5 (SEQ ID NO:5));

(d) ORF 1358 (Figure 7 (SEQ ID NO:7)); and
(e) ORF 2459 (Figure 9 (SEQ ID NO:9)).

In another embodiment, an immunogenic composition of this invention comprises a mixture of two or more polypeptides, each polypeptide having at least 90% identity to an amino acid sequence selected from the group consisting of:

5  (a) SCP (Figure 2 (SEQ ID NO:2));

(b) peptidylpropyl isomerase (Figure 4 (SEQ ID NO:4));

(c) hypothetical protein (Figure 6 (SEQ ID NO:6));

(d) putative adhesion protein (Figure 8 (SEQ ID NO:8)); and

(e) surface lipoprotein (Figure 10 (SEQ ID NO:10)).

In yet another embodiment, an immunogenic composition of this invention comprises a mixture of:

(a) an SCP polypeptide encoded by a nucleic acid sequence having at least 90% identity to the nucleic acid sequence of Figure 1 (SEQ ID NO:1);

(b) a peptidylpropyl isomerase polypeptide encoded by a nucleic acid sequence having at least 90% identity to the nucleic acid sequence of Figure 3 (SEQ ID NO:3); and

(c) at least one other polypeptide encoded by a nucleic acid sequence having at least 90% identity to an nucleic acid sequence selected from the group consisting of (i) Figure 5 (SEQ ID NO:5); (ii) Figure 7 (SEQ ID NO:7); and (iii) Figure 9 (SEQ ID NO:9).

In still another embodiment, an immunogenic composition of this invention comprises a mixture of:

(a) an SCP polypeptide having at least 90% identity to the amino acid sequence of Figure 2 (SEQ ID NO:2);

(b) a peptidylpropyl isomerase polypeptide having at least 90% identity to the amino acid sequence of Figure 4 (SEQ ID NO:4); and
(c) at least one other polypeptide having at least 90% identity to an amino acid sequence of the group consisting of (i) Figure 6 (SEQ ID NO:6); (ii) Figure 8 (SEQ ID NO:8); and (iii) Figure 10 (SEQ ID NO: 10).

The terms "polynucleotide", "nucleic acid" and "nucleic acid fragment" are used interchangeably herein. These terms encompass nucleotides connected by phosphodiester linkages. A "polynucleotide" may be a ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) polymer that is single- or double-stranded, that optionally contains synthetic, non-natural or altered nucleotide bases. A polynucleotide in the form of a polymer of DNA may comprise one or more segments of cDNA, genomic DNA, synthetic DNA, or mixtures thereof. Nucleotide bases are indicated herein by a single letter code: adenine (A), guanine (G), thymine (T), cytosine (C), inosine (I) and uracil (U).

The streptococcal polynucleotides described herein may be obtained using standard cloning and screening techniques. These polynucleotides may be obtained, for example, from genomic DNA, from a cDNA library derived from mRNA, from a genomic DNA library, or can be synthesized using well known and commercially available techniques, such as e.g. by PCR from a cDNA library or via RT-PCR (reverse transcription - polymerase chain reaction).

There are several methods available and well known to those skilled in the art to obtain full-length cDNAs or to extend short cDNAs, such as e.g. those based on the method of rapid amplification of cDNA ends (RACE). See Frohman et al, Proc. Natl. Acad. Sci. USA 85, 8998-9002, 1988. Recent modifications of the technique, exemplified by the MARATHON™ technology (Clontech Laboratories Inc.) for example, have significantly simplified the search for longer cDNAs. In the MARATHON™ technology, cDNAs have been prepared from mRNA extracted from a chosen tissue and an "adaptor" sequence ligated onto each end. Nucleic acid amplification (PCR) is then carried out to amplify the "missing" 5' end of the cDNA using a combination of gene specific and adaptor specific oligonucleotide primers. The PCR reaction is then repeated using "nested" primers, that is, primers designed to anneal within the amplified product (typically an adaptor specific primer that anneals further 3' in the adaptor sequence and a gene specific primer that anneals further 5' in the known gene sequence). The products of this reaction can then be analyzed by DNA sequencing and a full-length cDNA constructed either by joining the product directly to the
existing cDNA to give a complete sequence, or by carrying out a separate full-length PCR using the new sequence information for the design of the 5’ primer.

The term "recombinant" means, for example, that a polynucleotide is made by an artificial combination of two or more otherwise separated polynucleotide segments, e.g., by chemical synthesis or by the manipulation of isolated polynucleotides using genetic engineering techniques. A "recombinant DNA construct" comprises any of the isolated polynucleotides of the present invention operably linked to at least one regulatory element.

Orthologues and allelic variants of the streptococcal polynucleotides can readily be identified using methods well known in the art. Allelic variants and orthologs of the polynucleotides can comprise a nucleotide sequence that is typically at least about 90-95% or more identical to any one or more of the nucleotide sequences shown in odd numbered Figures 1-9 (odd numbered SEQ ID NO.: 'S 1-9), or fragments thereof. The allelic variants and orthologs of these polynucleotides can encode a polypeptide that comprises an amino acid sequence that is at least 90% identical to the amino acid sequence set forth in any one or more of even numbered Figures 2-10 (even numbered SEQ ID NO.: 'S 2-10). Such polynucleotides can readily be identified as being able to hybridize under stringent conditions, to any one or more of the polynucleotides having a nucleotide sequence set forth in Figures 1-9 (odd numbered SEQ ID NO.: 'S 1-9), or fragments thereof.

It is well understood by one skilled in the art that many levels of sequence identity are useful in identifying related polynucleotide and polypeptide sequences. Sequence alignments and percent identity calculations can be performed using the MEGALIGN™ program of the LASERGENE™ bioinformatics computing suite (DNASTAR Inc., Madison, Wis.). Multiple alignment of the sequences can be performed using the Clustal method of alignment (Higgins and Sharp, Gene, 73(1):237-44, 1988) with the default parameters of e.g. GAP PENALTY=10 and GAP LENGTH PENALTY=10. Default parameters for pairwise alignments using the Clustal method can be e.g. KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5.

A polypeptide sequence of the invention may be identical to the recited sequence, that is, 100% identical, or it may include up to a certain integer number of amino acid alterations as compared to the reference sequence such that the % identity is less than 100%. Such alterations include at least one amino acid deletion, substitution, including conservative and
non-conservative substitution, or insertion. The alterations may occur at the amino- or
carboxy-terminal positions of the reference polypeptide sequence or anywhere between those
terminal positions, interspersed either individually among the amino acids in the reference
amino acid sequence or in one or more contiguous groups within the reference amino acid
sequence.

Thus, the invention also provides isolated polypeptides having sequence identity to
the amino acid sequences contained in the cited sequences. Depending on the particular
sequence, the degree of sequence identity is preferably greater than 90% (e.g., 90%, 95%,
97%, 99% or more). These homologous proteins include mutants and allelic variants.

"Identity," as known in the art, is a relationship between two or more polypeptide
sequences or two or more polynucleotide sequences, as determined by comparing the
sequences. In the art, "identity" also means the degree of sequence relatedness between
polypeptide or polynucleotide sequences, as the case may be, as determined by the match
between strings of such sequences. "Identity" and "similarity" can be readily calculated by
known methods, including but not limited to those described in (Computational Molecular
Biology, Lesk, A. M., ed., Oxford University Press, New York, 1988; Biocomputing:
Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G.,
Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds.,
Math., 48: 1073 (1988). Preferred methods to determine identity are designed to give the
largest match between the sequences tested. Methods to determine identity and similarity are
codified in publicly available computer programs. Preferred computer program methods to
determine identity and similarity between two sequences include, but are not limited to, the
GCG program package (Devereux, J., et al. 1984), BLASTP, BLASTN, and FASTA
(Altschul, S. F., et al., 1990. The BLASTX program is publicly available from NCBI and
other sources (BLAST Manual, Altschul, S., et al., NCBI NLM NIH Bethesda, Md. 20894;
Altschul, S., et al., 1990). The well known Smith Waterman algorithm may also be used to
determine identity.

For example, the number of amino acid alterations for a given % identity can be
determined by multiplying the total number of amino acids in one of even numbered Figures
2-10 (SEQ ID NO: S2, 4, 6, 8 and 10) by the numerical percent of the respective percent identity (divided by 100) and then subtracting that product from said total number of amino acids in the one of even numbered Figures 2-10 (SEQ ID NO: S2, 4, 6, 8 and 10), or:

\[ n_a \leq x_a - y \]

wherein \( n_a \) is the number of amino acid alterations, \( x_a \) is the total number of amino acids in the one of even numbered Figures 2-10 (SEQ ID NO: S2, 4, 6, 8 and 10), and \( y \) is, for instance, 0.90 for 90%, 0.95 for 95%, 0.97 for 97% etc., and wherein any non-integer product of \( x_a \) and \( y \) is rounded down to the nearest integer prior to subtracting it from \( x_a \).

The present invention also contemplates isolated polypeptides that are substantially conserved across strains of β-hemolytic streptococci. Further, isolated polypeptides that are substantially conserved across strains of β-hemolytic streptococci and that are effective in preventing or ameliorating a β-hemolytic streptococcal colonization or infection in a susceptible subject are also contemplated by the present invention. As used herein, the term "conserved" refers to, for example, the number of amino acids that do not undergo insertions, substitution and/or deletions as a percentage of the total number of amino acids in a protein. For example, if a protein is 90% conserved and has, for example, 263 amino acids, then there are 237 amino acid positions in the protein at which amino acids do not undergo substitution. Likewise, if a protein is 95% conserved and has, for example, about 280 amino acids, then there are 14 amino acid positions at which amino acids may undergo substitution and 266 (i.e., 280 minus 14) amino acid positions at which the amino acids do not undergo substitution. According to an embodiment of the present invention, the isolated polypeptide is preferably at least about 90% conserved across the strains of β-hemolytic streptococci, more preferably at least about 95% conserved across the strains, even more preferably at least about 97% conserved across the strains, and most preferably at least about 99% conserved across the strains, without limitation.

Modifications and changes can be made in the structure of the polypeptides and still obtain polypeptides having β-hemolytic streptococci and/or Streptococcus pyogenes activity and/or antigenicity. For example, certain amino acids can be substituted for other amino acids in a sequence without appreciable loss of activity and/or antigenicity. Because it is the interactive capacity and nature of a polypeptide that defines that polypeptide's biological functional activity, certain amino acid sequence substitutions can be made in a polypeptide
sequence (or, of course, its underlying DNA coding sequence) and nevertheless obtain a polypeptide with like properties.

The invention includes any isolated polypeptide which is a biological equivalent that provides the desired reactivity as described herein. The term "desired reactivity" refers to reactivity that would be recognized by a person skilled in the art as being a useful result for the purposes of the invention. Examples of desired reactivity are described herein, including without limitation, desired levels of protection, desired antibody titers, desired opsonophagocytic activity and/or desired cross-reactivity, such as would be recognized by a person skilled in the art as being useful for the purposes of the present invention. The desired opsonophagocytic activity is indicated by a percent killing of bacteria as measured by decrease in colony forming units (CFU) in OPA versus a negative control. Without being limited thereto, the desired opsonophagocytic activity is preferably at least about 15%, more preferably at least about 20%, even more preferably at least about 40%, even more preferably at least about 50% and most preferably at least about 60%.

The invention includes polypeptides that are variants of the polypeptides comprising an amino acid sequence of even numbered Figures 2-10 (SEQ ID NO: S 2, 4, 6, 8 and 10). "Variant" as the term is used herein, includes a polypeptide that differs from a reference polypeptide, but retains essential properties. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical (i.e., biologically equivalent). A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, or deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polypeptides may be made by direct synthesis or by mutagenesis techniques.

In making such changes, the hydropathic index of amino acids can be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a polypeptide is generally understood in the art (Kyte & Doolittle, 1982). It is known that certain amino acids can be substituted for other amino acids having a similar hydropathic index or score and still result in a polypeptide with similar biological activity. Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and
charge characteristics. Those indices are listed in parentheses after each amino acid as follows: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cysteine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

It is believed that the relative hydropathic character of the amino acid residue determines the interaction of the secondary and tertiary structure of the resultant polypeptide, which in turn defines the interaction of the polypeptide with other molecules, such as enzymes, substrates, receptors, antibodies, antigens, and the like. It is known in the art that an amino acid can be substituted by another amino acid having a similar hydropathic index and still obtain a functionally equivalent polypeptide. In such changes, the substitution of amino acids whose hydropathic indices are within ±2 is preferred, those which are within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred.

Substitution of like amino acids can also be made on the basis of hydrophilicity, particularly where the biological functional equivalent polypeptide or peptide thereby created is intended for use in immunological embodiments. U.S. Patent Number 4,554,101, incorporated herein by reference, states that the greatest local average hydrophilicity of a polypeptide, as governed by the hydrophilicity of its adjacent amino acids, correlates with its immunogenicity and antigenicity, i.e., with a biological property of the polypeptide.

As detailed in U.S. Patent Number 4,554,101, incorporated herein by reference, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 ±1); glutamate (+3.0 ±1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); proline (-0.5 ±1); threonine (-0.4); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); and tryptophan (-3.4). It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent and in particular, an immunologically equivalent, polypeptide. In such changes, the substitution of amino acids whose hydrophilicity values are within ±2 is preferred, those which are within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred.
As outlined above, amino acid substitutions are generally, therefore, based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions which take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine, and isoleucine. As shown in Table I below, suitable amino acid substitutions include the following:

**TABLE 1:**

<table>
<thead>
<tr>
<th>Original Residue</th>
<th>Exemplary Residue Substitution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala</td>
<td>Gly; Ser</td>
</tr>
<tr>
<td>Arg</td>
<td>Lys</td>
</tr>
<tr>
<td>Asn</td>
<td>Gln; His</td>
</tr>
<tr>
<td>Asp</td>
<td>Glu</td>
</tr>
<tr>
<td>Cys</td>
<td>Ser</td>
</tr>
<tr>
<td>Gln</td>
<td>Asn</td>
</tr>
<tr>
<td>Glu</td>
<td>Asp</td>
</tr>
<tr>
<td>Gly</td>
<td>Ala</td>
</tr>
<tr>
<td>His</td>
<td>Asn; Gln</td>
</tr>
<tr>
<td>Ile</td>
<td>Leu; Val</td>
</tr>
<tr>
<td>Leu</td>
<td>Ile; Val</td>
</tr>
<tr>
<td>Lys</td>
<td>Arg</td>
</tr>
<tr>
<td>Met</td>
<td>Met; Leu; Tyr</td>
</tr>
<tr>
<td>Ser</td>
<td>Thr</td>
</tr>
<tr>
<td>Thr</td>
<td>Ser</td>
</tr>
<tr>
<td>Trp</td>
<td>Tyr</td>
</tr>
<tr>
<td>Tyr</td>
<td>Trp; Phe</td>
</tr>
<tr>
<td>Val</td>
<td>Ile; Leu</td>
</tr>
</tbody>
</table>

Thus, the invention includes functional or biological equivalents of the polypeptides of the sequences in even numbered Figures 2-10 (SEQ ID NO:’S 2, 4, 6, 8 and 10) that contain one or more amino acid substitutions.

Biological or functional equivalents of a polypeptide can also be prepared using site-specific mutagenesis. Site-specific mutagenesis is a technique useful in the preparation of second generation polypeptides, or biologically, functionally equivalent polypeptides, derived from the sequences thereof, through specific mutagenesis of the underlying DNA. As noted above, such changes can be desirable where amino acid substitutions are desirable. The technique further provides a ready ability to prepare and test sequence variants, for example,
incorporating one or more of the foregoing considerations, by introducing one or more nucleotide sequence changes into the DNA. Site-specific mutagenesis allows the production of mutants through the use of specific oligonucleotide sequences which encode the DNA sequence of the desired mutation, as well as a sufficient number of adjacent nucleotides, to provide a primer sequence of sufficient size and sequence complexity to form a stable duplex on both sides of the deletion junction being traversed. Typically, a primer of about 17 to 25 nucleotides in length is preferred, with about 5 to 10 residues on both sides of the junction of the sequence being altered.

In general, the technique of site-specific mutagenesis is well known in the art. As will be appreciated, the technique typically employs a phage vector which can exist in both a single-stranded and double-stranded form. Typically, site-directed mutagenesis in accordance herewith is performed by first obtaining a single-stranded vector which includes within its sequence a DNA sequence which encodes all or a portion of the Streptococcus pyogenes polypeptide sequence selected. An oligonucleotide primer bearing the desired mutated sequence is prepared, for example, by well known techniques (e.g., synthetically). This primer is then annealed to the single-stranded vector, and extended by the use of enzymes, such as E. coli polymerase I Klenow fragment, in order to complete the synthesis of the mutation-bearing strand. Thus, a heteroduplex is formed wherein one strand encodes the original non-mutated sequence and the second strand bears the desired mutation. This heteroduplex vector is then used to transform appropriate cells, such as E. coli cells, and clones are selected which include recombinant vectors bearing the mutation. Commercially available kits provide the necessary reagents.

The polypeptides and polypeptide antigens of the invention are understood to include any polypeptide comprising substantial sequence similarity, structural similarity, and/or functional similarity to a polypeptide comprising an amino acid sequence of any of even numbered Figures 2-10 (SEQ ID NO:`S 2, 4, 6, 8 and 10). In addition, a polypeptide or polypeptide antigen of the invention is not limited to a particular source. Thus, the invention provides for the general detection and isolation of the polypeptides from a variety of sources.

The polypeptides of the invention may be in the form of the "mature" protein or may be a part of a larger protein such as a fusion protein. It is often advantageous to include an additional amino acid sequence which contains, for example, secretory or leader sequences,
pro-sequences, sequences which aid in purification such as multiple histidine residues, or an additional sequence for stability during recombinant production.

The term "immunogenic composition" as used herein refers to any type of biological agent in an administrable form capable of stimulating an immune response in a subject inoculated with the immunogenic composition. An immune response may include induction of antibodies and/or induction of a T-cell response. The term "protection," when used in reference to an immunogenic composition, refers herein to the amelioration (either partial or complete) of any of the symptoms associated with the disease or condition in question. Thus, protection of subjects from infection by a Streptococcus species such as \textit{S. dysgalactiae} (including the subspecies \textit{Dysgalactiae} and \textit{Equisimihs}) by the present immunogenic compositions generally results in a diminishing of bacterial growth and/or one or more of the clinical symptoms associated with streptococcal infection, including arthritis, endocarditis, meningitis, polyserositis, bronchopneumonia, meningitis, permanent hearing loss and septic shock.

The methods disclosed herein may include inducing an immune response against one or more pathogens that include a species of Streptococcus (e.g., \textit{Streptococcus dysgalactiae}, \textit{S. dysgalactiae} sub. \textit{Equisimihs}, \textit{S. dysgalactiae} sub. \textit{Dysgalactiae}, \textit{S. pyogenes}, \textit{S. agalactiae}, \textit{S. anginosus}, \textit{S. constellatus}, \textit{S. equisimihs} and \textit{S. intermedius}.) For example, the methods may include inducing polyclonal antibody production against one or more streptococcal pathogens such as e.g. \textit{S. dysgalactiae} sub. \textit{Equisimihs}.

As discussed above, immunogenic compositions comprise two or more polypeptides of the invention. To do so, one or more polypeptides are adjusted to an appropriate concentration and can be formulated with any suitable adjuvant, diluent, pharmaceutically acceptable carrier, or any combination thereof. As used herein the phrase "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, excipients and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Physiologically acceptable vehicles may be used as carriers and/or diluents. A pharmaceutically acceptable vehicle is understood to designate a compound or a combination of compounds entering into a pharmaceutical or immunogenic composition which does not cause side effects and which makes it possible, for example, to facilitate the administration of the active compound, to
increase its life and/or its efficacy in the body, to increase its solubility in solution or alternatively to enhance its preservation. These pharmaceutically acceptable vehicles are well known and will be adapted by persons skilled in the art according to the nature and the mode of administration of the active compound chosen. These include, but are not limited to, water, Ringer's solution, an appropriate isotonic medium, glycerol, ethanol and other conventional solvents, phosphate buffered saline, and the like.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, polyethylene glycol and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, and the like. In many cases, isotonic agents are included in the composition, for example, sugars, polyalcohols such as manitol, sorbitol and/or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the polypeptides in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the
active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Immunogenic compositions as described herein also comprise, in certain embodiments, one or more adjuvants. An adjuvant is a substance that enhances the immune response when administered together with an immunogen or antigen. A number of cytokines or lymphokines have been shown to have immune modulating activity, and thus are useful as adjuvants, including, but not limited to, the interleukins 1-α, 1-β, 2, 4, 5, 6, 7, 8 and 10, 12 (see, e.g., U.S. Pat. No. 5,723,127), 13, 14, 15, 16, 17 and 18 (and its mutant forms); the interferons-α, β and γ; granulocyte-macrophage colony stimulating factor (GM-CSF) (see, e.g., U.S. Pat. No. 5,078,996 and ATCC Accession Number 39900); macrophage colony stimulating factor (M-CSF); granulocyte colony stimulating factor (G-CSF); and the tumor necrosis factors α and β. Still other adjuvants that are useful with the immunogenic compositions described herein include chemokines, including without limitation, MCP-I, MIP-1α, MIP-1β, and RANTES; adhesion molecules, such as a selectin, e.g., L-selectin, P-selectin and E-selectin; mucin-like molecules, e.g., CD34, GlyCAM-1 and MadCAM-1; a member of the integrin family such as LFA-I, VLA-I, Mac-1 and pi50.95; a member of the immunoglobulin superfamily such as PECAM, ICAMs, e.g., ICAM-I, ICAM-2 and ICAM-3, CD2 and LFA-3; co-stimulatory molecules such as CD40 and CD40L; growth factors including vascular growth factor, nerve growth factor, fibroblast growth factor, epidermal growth factor, B7.2, PDGF, BL-I, and vascular endothelial growth factor; receptor molecules including Fas, TNF receptor, Fit, Apo-1, p55, WSL-I, DR3, TRAMP, Apo-3, AIR, LARD, NGFR, DR4, DR5, KILLER, TRAIL-R2, TRICK2, and DR6; and Caspase (ICE).

Suitable adjuvants used to enhance an immune response further include, without limitation, MPL™ (3-O-deacylated monophosphoryl lipid A, Corixa, Hamilton, MT), which is described in U.S. Pat. No. 4,912,094. Also suitable for use as adjuvants are synthetic lipid A analogs or aminoalkyl glucosamine phosphate compounds (AGP), or derivatives or analogs thereof, which are available from Corixa (Hamilton, MT), and which are described in U.S. Pat. No. 6,113,918. One such AGP is 2-[(R)-3-Tetradecanoyloxytetradecanoylaminol] ethyl 2-Deoxy-4-O-phosphono-3-O-[(R)-3-tetradecanoyloxytetradecanoyl]-2-[(R)-3-tetradecanoyloxytetradecanoyl-amino]-b-D-glucopyranoside, which is also known as 529 (formerly known as RC529). This 529 adjuvant is formulated as an aqueous form (AF) or as a stable emulsion (SE).
Still other adjuvants include muramyl peptides, such as N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanine-2-(r-2' dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE); oil-in-water emulsions, such as MF59 (U.S. Pat. No. 6,299,884) (containing 5% Squalene, 0.5% Tween 80, and 0.5% Span 85 (optionally containing various amounts of MTP-PE) formulated into submicron particles using a microfluidizer such as Model HOY microfluidizer (Microfluidics, Newton, MA)), and SAF (containing 10% Squalene, 0.4% Tween 80, 5% pluronic-blocked polymer L 121, and thr-MDP, either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion); aluminum salts (alum), such as aluminum hydroxide, aluminum phosphate, aluminum sulfate; Amphigen; Avridine; L121/squalene; D-lactide-poly(lactide/glycoside); pluronic polyols; killed Bordetella; saponins, such as Stimulon™ QS-21 (Antigenics, Framingham, MA), described in U.S. Pat. No. 5,057,540, ISCOMATRIX (CSL Limited, Parkville, Australia), described in U.S. Pat. No. 5,254,339, and immunostimulating complexes (ISCOMS); Mycobacterium tuberculosis; bacterial lipopolysaccharides; synthetic polynucleotides such as oligonucleotides containing a CpG motif (e.g., U.S. Pat. No. 6,207,646); IC-31 (Intercell AG, Vienna, Austria), described in European Patent Nos. 1,296,713 and 1,326,634; a pertussis toxin (PT) or mutant thereof, a cholera toxin or mutant thereof (e.g., U.S. Pat. Nos. 7,285,281, 7,332,174, 7,361,355 and 7,384,640); or an E. coli heat-labile toxin (LT) or mutant thereof, particularly LT-K63, LT-R72 (e.g., U.S. Pat. Nos. 6,149,919, 7,115,730 and 7,291,588).

The polypeptide can also include at least a portion of the polypeptide, optionally conjugated or linked to a peptide, polypeptide, or protein, or to a polysaccharide. It is also anticipated that the immunogenic compositions can contain other components, such as polysaccharides, alone or conjugated to proteins which can elicit an immune response.

Various tests are used to assess the in vitro immunogenicity of the polypeptides comprising the immunogenic compositions of the invention. For example, an in vitro opsonic assay is conducted by incubating together a mixture of Streptococcus sp. cells, heat inactivated serum containing specific antibodies to the polypeptide in question, and an exogenous complement source. Opsonophagocytosis proceeds during incubation of freshly isolated polymorphonuclear cells (PMN’s) and the antibody/complement/ Streptococcus sp. cell mixture. Bacterial cells that are coated with antibody and complement are killed upon
opsonophagocytosis. Colony forming units (cfu) of surviving bacteria that escape from opsonophagocytosis are determined by plating the assay mixture. Titers are reported as the reciprocal of the highest dilution that gives $\geq 50\%$ bacterial killing, as determined by comparison to assay controls. Specimens that demonstrate less than 50% killing at the lowest serum dilution tested (1:8), are reported as having an opsonophagocytosis antibody (OPA) titer of 4. The method described above is a modification of Gray's method (Gray, Conjugate Vaccines Supplement, p. 694-697,1990).

A test serum control, which contains test serum plus bacterial cells and heat inactivated complement, is included for each individual serum. This control is used to assess whether the presence of antibiotics or other serum components are capable of killing the bacterial strain directly (i.e. in the absence of complement or PMN's). A human serum with known opsonic titer is used as a positive human serum control. The opsonic antibody titer for each unknown serum is calculated as the reciprocal of the initial dilution of serum giving 50% cfu reduction compared to the control without serum.

A whole cell ELISA assay can also be used to assess in vitro immunogenicity and surface exposure of the polypeptide antigen, wherein the bacterial strain of interest is coated onto a plate, such as a 96 well plate, and test sera from an immunized animal is reacted with the bacterial cells. If any antibody specific for the test polypeptide antigen is reactive with a surface exposed epitope of the polypeptide antigen, it can be detected by standard methods known to one skilled in the art. A similar approach is to monitor the antigen on the cell surface using Flow Cytometry and antigen specific antibodies.

Any polypeptide demonstrating the desired in vitro activity may then be tested in an in vivo animal challenge model. In some embodiments, immunogenic compositions are used in the immunization of an animal (e.g., a mouse) by methods and routes of immunization known to those of skill in the art (e.g., intranasal, parenteral, intramuscular, oral, rectal, vaginal, transdermal, intraperitoneal, intravenous, subcutaneous, etc.). Following immunization of the animal with a Streptococcal immunogenic composition, the animal is challenged with one or more Streptococcal species and assayed for resistance to *Streptococcus spp.* infection.

Combination immunogenic compositions are provided by including two or more of the polypeptides of the invention, as well as by combining one or more of the polypeptides of
the invention with one or more known *Streptococcus pyogenes* polypeptides, including, but not limited to, the M proteins, adhesins, and the like.

Once formulated, the immunogenic compositions of the invention can be administered directly to the subject, delivered *ex vivo* to cells derived from the subject, or *in vitro* for expression of recombinant proteins. For delivery directly to the subject, administration may be by any conventional form, such as intranasally, parenterally, orally, intraperitoneally, intravenously, subcutaneously, or topically applied to any mucosal surface such as intranasal, oral, eye, lung, vaginal, or rectal surface, such as by an aerosol spray.

It is advantageous to formulate oral or parenteral compositions in unit dosage form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

Injectable preparations, for example sterile injectable aqueous or oleaginous suspensions, are formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation can also be a sterile injectable solution or suspension in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butandiol.

For parenteral administration, immunogenic compositions of the invention can be administered as injectable dosages in a physiologically acceptable diluent with a pharmaceutically acceptable carrier that can be a sterile liquid such as water oils, saline, glycerol, or ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents, surfactants, pH buffering substances and the like can be present in compositions. Other components can include those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, and mineral oil. In general, glycols such as propylene glycol or polyethylene glycol are preferred liquid carriers, particularly for injectable solutions.
Typically, compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared. The preparation also can be emulsified or encapsulated in liposomes or micro particles such as polylactide, polyglycolide, or copolymer for enhanced adjuvant effect, as discussed above (see Langer, Science 249: 1527 (1990) and Hanes, Advanced Drug Delivery Reviews 28:97 (1997)). The immunogenic compositions of this invention can be administered in the form of a depot injection or implant preparation, which can be formulated in such a manner as to permit a sustained or pulsatile release of the active ingredient.

The subjects are generally human. An immunologically effective amount of the immunogenic composition in an appropriate number of doses is administered to the subject to elicit an immune response. Immunologically effective amount, as used herein, means the administration of that amount to a mammalian host (preferably human), either in a single dose or as part of a series of doses, sufficient to at least cause the immune system of the individual treated to generate an immune response that reduces the clinical impact of the bacterial infection. The term "immune response" or "immunological response" includes the development of a humoral (antibody-mediated) and/or a cellular (mediated by antigen-specific T cells or their secretion products) response. Protection may be conferred by a single dose of the immunogenic composition, or may require the administration of several doses, in addition to booster doses at later times to maintain protection. This may range from a minimal decrease in bacterial burden to prevention of the infection. Ideally, the treated individual will not exhibit the more serious clinical manifestations of the β-hemolytic streptococcal infection. The dosage amount can vary depending upon specific conditions of the individual, such as age and weight. This amount can be determined in routine trials by means known to those skilled in the art.

In prophylactic applications, immunogenic compositions are administered to a subject susceptible to, or otherwise at risk of, beta hemolytic streptococcal infection in an amount sufficient to eliminate or reduce the risk, lessen the severity, or delay the outset of the disease, including biochemical, histologic and/or behavioral symptoms of disease associated with the infection, its complications and intermediate pathological phenotypes presenting during development of the disease. In therapeutic applications, compositions are administered to a patient suspected of, or already suffering from such a disease in an amount
sufficient to cure, or at least partially arrest, the symptoms of the disease (biochemical, histologic and/or behavioral), including its complications and intermediate pathological phenotypes in development of the disease.

It has been observed that there is no single peptide sequence that provides protection for all strains of BHS, including groups A, B, C, and G. As shown in Table II (presented in Example 1 below), below, each antigen provides an immune response against a subset of these groups.

Generally, any combination of two or more surface-expressed antigens from BHS will be expected to provide the enhanced immune response described above. Such could include the antigens discussed above BHS capsular antigens, M protein, ABC transporter, or any other surface exposed antigen. However, it has been found that the following antigens exhibit particularly beneficial properties for the production of immunogenic compositions:

SCP (C5a Peptidase)

peptidylpropyl isomerase (encoded by ORF 554)

putative adhesion protein (encoded by ORF 1358)

surface lipoprotein (encoded by ORF 2459)

hypothesical protein (encoded by ORF 1218)

Combinations of two or more of these antigens into a single multicomponent immunogenic composition provide enhanced protection against one or more groups of BHS and produce an enhanced immune response to them.

EXAMPLES

The following examples are illustrative and the present invention is not intended to be limited thereto.

EXAMPLE 1--ANTIBODY BINDING

The binding of antibodies to bacteria, a process known as opsonization, can lead to uptake and killing of the bacteria by phagocytic cells. Screening of such antibodies is used in
to determine the effectiveness of antibodies raised against particular serotypes in killing bacteria expressing or not expressing that serotype on the surface.

For each serotype screened, antibodies were raised in mice against the antibodies encoded for by the recited ORF. The antibodies were then screened against various BHS strains. Screening of the antibodies was performed by fluorescence activated cell sorting (FACS). Briefly, heat killed streptococci were incubated with a recited antibody on ice for 45 minutes, followed by two washes. The streptococci were then incubated with a goat anti-mouse-Alexa-488 antibody (Molecular Probes, Eugene, OR) for 30 minutes on ice, followed by two washes. Cells thus treated were run on a FACS machine (e.g. see DeMaster et al, Infect. Immun, 70(1): 350-359, 2002.) The results are summarized in Table 2.

In the course of screening those anti-beta hemolytic-streptococcal antisera and monoclonal antibodies against various beta hemolytic streptococcal (BHS) strains, it was noted that some antisera and antibodies are cross-reactive against many BHS strains, including members of *Streptococcus pyogenes* (Group A streptococci), *Streptococcus agalactiae* (Group B streptococci) and Group C and Group G streptococci (which include the streptococcal species *Streptococcus anginosus, Streptococcus constellatus, Streptococcus intermedii, Streptococcus dysgalactiae sub. Equisimilhs and Streptococcus dysgalactiae sub. Dysgalactiae*). This cross-reactivity also means that the polypeptides recited or encoded by the relevant ORF may be used in an immunogenic composition to induce an immune response effective to protect against infection by Group A or Group B Streptococcus, as well as by Group C or Group G Streptococcus.

In Table 2, the symbol "+" means that the antibodies react to the antigen at least three-fold over background; the symbol "+/−" means that the antibodies react to the antigen between two-fold and three-fold over background; and the symbol "−" means that the detection of antibody signal is at or below background.
| Strain | Species | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T | U | V | W | X | Y | Z |
| G925   | unc     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| G925   | GS9CS   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| G925   | GS9CS   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| G925   | GS9CS   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| G925   | GS9CS   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| G925   | GS9CS   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| G925   | GS9CS   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| G925   | GS9CS   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| G925   | GS9CS   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| G925   | GS9CS   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| G925   | GS9CS   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| G925   | GS9CS   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| G925   | GS9CS   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| G925   | GS9CS   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| G925   | GS9CS   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| G925   | GS9CS   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| G925   | GS9CS   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| G925   | GS9CS   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| G925   | GS9CS   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| G925   | GS9CS   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| G925   | GS9CS   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| G925   | GS9CS   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| G925   | GS9CS   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
EXAMPLE 2—USE OF A THREE COMPONENT IMMUNOGENIC COMPOSITION TO PRODUCE IMMUNE SERA

A trivalent immunogenic composition consisting of SCP, the polypeptide encoded by ORF 554, and the polypeptide encoded by ORF 1358 adjuvanted with aluminum phosphate was prepared, and the immunogenic composition was used to produce hyperimmune rabbit serum by three subcutaneous inoculations separated by 2-4 weeks, followed by exsanguination; a monovalent immunogenic composition consisting of similarly adjuvanted polypeptide encoded by ORF 554 was used as a control. The sera were screened for opsonophagocytic activity (OPA) against *S. pyogenes* SF370 at various dilutions. Briefly, the bacteria were incubated with 10 ul of sera for one hour in the presence of complement (baby rabbit complement), and then diluted 1:10 and plated on blood agar plates. The results are presented in Figure 11.

As shown, it can be seen that the Trivax elicits increased opsonophagocytic activity than the 554 immunogenic composition, which is indicative of a much better killing of the bacteria.

EXAMPLE 3—PASSIVE IMMUNITY TRANSFER

Antibodies were raised against each of the following antigens as described above: SCP and polypeptides encoded by ORFs 554, 1358, 2459, and 1218. These antibodies were then injected into infant rats without fully functional immune systems. The treated rats are then subsequently challenged with *S. pyogenes*, and recovered bacteria were counted four hours post-challenge. The negative control was PBS, and the positive human control was 385 sera.

The results are shown in Figures 12-16. Briefly, the results demonstrated that antibodies elicited by each of the antigens significantly reduced bacteremia in the infant rats.
Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.
What is claimed is:

1. An immunogenic composition comprising a mixture of two or more polypeptides, each polypeptide encoded by a nucleic acid sequence having at least 90% identity to a nucleic acid sequence selected from the group consisting of:
   
   (a) C5a peptidase ("SCP") (Figure 1 (SEQ ID NO: I));
   
   (b) open reading frame ("ORF") 554 (Figure 3 (SEQ ID NO:3));
   
   (c) ORF 1218 (Figure 5 (SEQ ID NO:5));
   
   (d) ORF 1358 (Figure 7 (SEQ ID NO:7)); and
   
   (e) ORF 2459 (Figure 9 (SEQ ID N0:9)).

2. The immunogenic composition of claim 1, which further comprises a physiologically-acceptable vehicle.

3. The immunogenic composition of claim 1, which further comprises an effective amount of an adjuvant.

4. The immunogenic composition of claim 1 wherein each polypeptide is capable of generating an antibody that specifically recognizes said polypeptide, and wherein the amount of said immunogenic composition is effective to prevent or ameliorate colonization or infection by β-hemolytic streptococci in a susceptible mammal.

5. The immunogenic composition of claim 1, which further comprises a physiologically-acceptable vehicle.

6. The immunogenic composition of claim 1, which further comprises an effective amount of an adjuvant.

7. The immunogenic composition of claim 4, wherein the β-hemolytic streptococci is Group A streptococci, Group B streptococci, Group C streptococci, or Group G streptococci.

8. The immunogenic composition of claim 4, wherein the β-hemolytic streptococci is *Streptococcus pyogenes*. 


9. An immunogenic composition comprising a mixture of two or more polypeptides, each polypeptide having at least 90% identity to an amino acid sequence selected from the group consisting of:

(a) SCP (Figure 2 (SEQ ID NO:2));

(b) peptidylpropyl isomerase (Figure 4 (SEQ ID NO:4));

(c) hypothetical protein (Figure 6 (SEQ ID NO:6));

(d) putative adhesion protein (Figure 8 (SEQ ID NO:8)); and

(e) surface lipoprotein (Figure 10 (SEQ ID NO:10)).

10. The immunogenic composition of claim 9, which further comprises a physiologically-acceptable vehicle.

11. The immunogenic composition of claim 9, which further comprises an effective amount of an adjuvant.

12. The immunogenic composition of claim 9, wherein each polypeptide is capable of generating an antibody that specifically recognizes said polypeptide, and wherein the amount of said immunogenic composition is effective to prevent or ameliorate colonization or infection by β-hemolytic streptococci in a susceptible mammal.

13. The immunogenic composition of claim 12, which further comprises a physiologically-acceptable vehicle.

14. The immunogenic composition of claim 12, which further comprises an effective amount of an adjuvant.

15. The immunogenic composition of claim 12, wherein the β-hemolytic streptococci is Group A streptococci, Group B streptococci, Group C streptococci, or Group G streptococci.

16. The immunogenic composition of claim 15, wherein the β-hemolytic streptococci is *Streptococcus pyogenes*. 
17. A method of protecting a susceptible mammal against colonization or infection by β-hemolytic streptococci comprising administering to the mammal an effective amount of the immunogenic composition of claim 1, wherein each polypeptide is capable of generating an antibody specific to said polypeptide, and wherein the amount of said immunogenic composition is effective to prevent or ameliorate colonization or infection by β-hemolytic streptococci in the susceptible mammal.

18. The method of claim 17, wherein the immunogenic composition is administered by subcutaneous injection, by intramuscular injection, by oral ingestion, intranasally, or combinations thereof.

19. The method of claim 17 wherein the β-hemolytic streptococci is Group A streptococci, Group B streptococci, Group C streptococci, or Group G streptococci.

20. The method of claim 19 wherein the β-hemolytic streptococci is *Streptococcus pyogenes*.

21. The method of claim 17 wherein the mammal is a human.

22. A method of protecting a susceptible mammal against colonization or infection by β-hemolytic streptococci comprising administering to the mammal an effective amount of the immunogenic composition of claim 9, wherein each polypeptide is capable of generating an antibody specific to said polypeptide, and wherein the amount of said immunogenic composition is effective to prevent or ameliorate colonization or infection by β-hemolytic streptococci in the susceptible mammal.

23. The method of claim 22, wherein the immunogenic composition is administered by subcutaneous injection, by intramuscular injection, by oral ingestion, intranasally, or combinations thereof.

24. The method of claim 22 wherein the β-hemolytic streptococci is Group A streptococci, Group B streptococci, Group C streptococci, or Group G streptococci.

25. The method of claim 24 wherein the β-hemolytic streptococci is *Streptococcus pyogenes*.

26. The method of claim 22 wherein the mammal is a human.
27. An immunogenic composition comprising a mixture of:

(a) an SCP polypeptide encoded by a nucleic acid sequence having at least 90% identity to the nucleic acid sequence of Figure 1 (SEQ ID NO:1);

(b) a peptidylpropyl isomerase polypeptide encoded by a nucleic acid sequence having at least 90% identity to the nucleic acid sequence of Figure 3 (SEQ ID NO:3); and

(c) at least one other polypeptide encoded by a nucleic acid sequence having at least 90% identity to an nucleic acid sequence selected from the group consisting of (i) Figure 5 (SEQ ID NO:5); (ii) Figure 7 (SEQ ID NO:7); and (iii) Figure 9 (SEQ ID NO:9).

28. The immunogenic composition of claim 27, which further comprises a physiologically-acceptable vehicle.

29. The immunogenic composition of claim 27, which further comprises an effective amount of an adjuvant.

30. The immunogenic composition of claim 27 wherein each polypeptide is capable of generating an antibody that specifically recognizes said polypeptide, and wherein the amount of said immunogenic composition is effective to prevent or ameliorate colonization or infection by β-hemolytic streptococci in a susceptible mammal.

31. The immunogenic composition of claim 30, which further comprises a physiologically-acceptable vehicle.

32. The immunogenic composition of claim 30, which further comprises an effective amount of an adjuvant.

33. The immunogenic composition of claim 30, wherein the β-hemolytic streptococci is Group A streptococci, Group B streptococci, Group C streptococci, or Group G streptococci.

34. The immunogenic composition of claim 33, wherein the β-hemolytic streptococci is Streptococcus pyogenes.
35. An immunogenic composition comprising a mixture of:

(a) an SCP polypeptide having at least 90% identity to the amino acid sequence of Figure 2 (SEQ ID NO:2);

(b) a peptidylpropyl isomerase polypeptide having at least 90% identity to the amino acid sequence of Figure 4 (SEQ ID NO:4); and

(c) at least one other polypeptide having at least 90% identity to an amino acid sequence selected from the group consisting of (i) Figure 6 (SEQ ID NO:6); (ii) Figure 8 (SEQ ID NO:8); and (iii) Figure 10 (SEQ ID NO:10).

36. The immunogenic composition of claim 35, which further comprises a physiologically-acceptable vehicle.

37. The immunogenic composition of claim 36, which further comprises an effective amount of an adjuvant.

38. The immunogenic composition of claim 35 wherein each polypeptide is capable of generating an antibody that specifically recognizes said polypeptide, and wherein the amount of said immunogenic composition is effective to prevent or ameliorate colonization or infection by β-hemolytic streptococci in a susceptible mammal.

39. The immunogenic composition of claim 38, which further comprises a physiologically-acceptable vehicle.

40. The immunogenic composition of claim 38, which further comprises an effective amount of an adjuvant.

41. The immunogenic composition of claim 38, wherein the β-hemolytic streptococci is Group A streptococci, Group B streptococci, Group C streptococci, or Group G streptococci.

42. The immunogenic composition of claim 41, wherein the β-hemolytic streptococci is Streptococcus pyogenes.

43. A method of protecting a susceptible mammal against colonization or infection by β-hemolytic streptococci comprising administering to the mammal an effective
amount of the immunogenic composition of claim 27, wherein each polypeptide is capable of generating an antibody specific to said polypeptide, and wherein the amount of said immunogenic composition is effective to prevent or ameliorate colonization or infection by β-hemolytic streptococci in the susceptible mammal.

44. The method of claim 43, wherein the immunogenic composition is administered by subcutaneous injection, by intramuscular injection, by oral ingestion, intranasally, or combinations thereof.

45. The method of claim 43 wherein the β-hemolytic streptococci is Group A streptococci, Group B streptococci, Group C streptococci, or Group G streptococci.

46. The method of claim 45 wherein the β-hemolytic streptococci is Streptococcus pyogenes.

47. The method of claim 43 wherein the mammal is a human.

48. A method of protecting a susceptible mammal against colonization or infection by β-hemolytic streptococci comprising administering to the mammal an effective amount of the immunogenic composition of claim 35, wherein each polypeptide is capable of generating an antibody specific to said polypeptide, and wherein the amount of said immunogenic composition is effective to prevent or ameliorate colonization or infection by β-hemolytic streptococci in the susceptible mammal.

49. The method of claim 48, wherein the immunogenic composition is administered by subcutaneous injection, by intramuscular injection, by oral ingestion, intranasally, or combinations thereof.

50. The method of claim 48 wherein the β-hemolytic streptococci is Group A streptococci, Group B streptococci, Group C streptococci, or Group G streptococci.

51. The method of claim 50 wherein the β-hemolytic streptococci is Streptococcus pyogenes.

52. The method of claim 48 wherein the mammal is a human.
53. An immunogenic composition comprising a mixture of:

(a) an SCP polypeptide having at least 90% identity to the amino acid sequence of Figure 2 (SEQ ID NO:2);

(b) a peptidylpropyl isomerase polypeptide having at least 90% identity to the amino acid sequence of Figure 4 (SEQ ID NO:4); and

(c) a putative adhesion polypeptide having at least 90% identity to the amino acid sequence of Figure 8 (SEQ ID NO:8).
CCAGATAAAGACACGAGGAGCTCTAAGATGTCGTCAACACACCCAGAAA
AAACCTGTACCTGAGATACCCAGCAGCTGGTTCTCTCTATGCCCTGCTTACAAACAGAGATATGCTTCTACAGTTCTCTTTCTTTG

Fig. 1 (continued)
>SPy_2010 | C5A peptidase precursor
LRKQKLPFDKLALIAIMSTILLSQNLASSQISDIAKANTVETFDPATEQAVETPVQPTAVSEEAPSS
KEKTPQTPDEAAETADNDLAPQAPKADTPATPKATIRLDNDPSQVKTLQEKAGK
GATTVVAVIGDFRNHEAWRLDKTQKARYQSKEDLEKAKKEH0ITYGEWVNDKXYHD
YSKDQKTAQKREKHAVTHVSGILSVGAPSETKEPYRLAGEAPQYLLLMEINEWTLADYAR
NYAQIIDAIVNLAKVKINMSFGNAALAYANLPDETKKAFDYAKSKGVSIVTSANAGDSFG
GKTRPLADHDVYVGVTGAADSTLTivasSPDKLTETATVKTADQDDKEMPVLSTNR
FEPNKYAYANRMGEFFDKVKGKIALIERGIDFKDKIANAKAGAAGVGLVYINQD
KGFPFELPNVQMPAAFSRKRDLQLLKENVFQKQTITFNATPKVLPSTASGTLRSFSSWGLT
ADGNFKPDIAPQDILSSSVANNKYAKLSGTSMSAPLVTAVGIMGLLQKQYETQYDPMTS
PERLDLASKLMSSTAYLDEDEKAYFSPROVGAGVDAKSAATMVTDKDNSSSKVHLN
NVSDKFEVTVVHNKSDKPQELLYQATVQTDKVDGLFAALAPKALYETSWQQKITIPANSS
KQVTIFKQDSQFSDLLAPMKNGYFLEGFVRFKQDPKKEELMISPYIGFRQDVGNDSALE
KPYIDSKDGGYYHEANSDAKQDLGDGLQFYALKNNFTALTSESNPWTI1IAKVEGVEN
IEDIESSEITETIFAGTFQAQDDSHYIHRHANGKPYAAISPNGDNRQDVQFQGFR
NAKNLVAELDKEGHNVWETSVEQVQVKNYNNDLASTLGSTRFEKTRDWGDKKGKQVAN
GTYYKRKVRFSSGKEAQETFDFVIVNTEFPEATSATFDSTEDRRTEFLASKFKTSQPVY
REIAYTYMDEDLPTTEYISPNEDGTFTLFEEAEMEGATVPLKMSDFTYYVEDMNAGIT
YTPVTLKLGHSNKQEPQDGSQDAPDKKEKPEQDGSQAPDKKPKKEKPEQDGSQFTPDK
KPKEQDGSGQTPDKMKPEKPERDSSGQTPQKQPSRTLEKRSSRALKASTK
KDQLPTNDKDNRLHLLKLVMYTFGLGLVAHIFKTKRTE
Fig. 4

SPy_1390 [peptidylprolyl isomerase]
MKNSNKLIAVSVTASVMALAAQGSTNNITKVISMRGTISVIDSYNETKNDTSNKAMLNLVISRVEAQYGDKVSKEVEKAYHTAEQYGASFAALQAQLTPTETFQIRSSKLVEYAVKKEAKKELTTQEEYKAYYESYPTMAVENITLDNEETAKSVLEELKAEQADFTAIAKEKTTPEKKVYTKEFDSGATNVPDVKAKASSLNEGGISIDVISVLDPSTSYYKKFYIVKVTKKAEKSDWQEEYKRLAIIPAEKSKDMNFQNKVIANALDKANVKIKDKAFANILALQYANLGQKTKAASESSTTSESSKAEEFPSESEQTQTSSAEEPTETEAQTQEPAQQ
>1218  SPy0843 | hypothetical protein
MKKHKLTVATTLLTVTHQVEVLKHELPQVTQASSSSGADAYESSGSKSLKINE
TSGPDDRTVDLFSHDRTPERRKINDLAKGPQREQLKAVTENTKSEKQITSGSQLEQSK
SLSLNVTPSNWIECIFIKGNLVLGSKGVEKLSQTDHLVPLSQAAGTQLIQVAS
FAFTDFKTAIAEYTSRAGENGISQLDLDGEKILIINEGEVFNSYLLKVTIPTGYKHIGQ
DAFVDNKNIAEVLPLESLETISDHYAFAHLKQIDLPNLAIAEGELAFFDNQITGKLSP
RQLMRLEAERAFKSNHIKTEIEFGRGNLSKVIAGEAFQDNDLSQMLDPLEKIESEAFIGNP
GDDHYNNRVRVWLWTKSKGNPSGLATENYVNPDSLWQESPEIDYTKWLEEDFTYQKNSVT
GFSNKGILQKVKNKNEIPQKHWGVTITEIGHNAFRNVDFQNKTLKYLDEEVLKPSTIR
KIGAFAPQSNLKSFEADLLEIEKEGAFMNRIETLEKLKDLVTIGDAAFNHAYIIV
LPESVQIEIRSAFROQNGANLIFMGSKVKTLGEMAFLSNRLEHLDLSEQKQLITEVPQAF
SDNAKVEVLLFASLKTIREEAFKNNHKLQLEAVASLSHIANALDDNDGEQDFNVVVK
THHNSYALADGEHFIVDVPSSTIVDLEKILKLIEGELDSTLRQTGTQFRDNTGAKA
LLSNLRQGEKQKFLQEAQFLLGRVDLDKAIAKAEKALVKKATKNGQLLERSINKAVL
AYNNSAIAKANKLRLEKELDLLTLVEGKPLAQAMVQCVVLLKLPPLPEYYYIQLNVY
FDSGSLIYALDSMTIGEGQKDANYGFLNVDNEGYYHALAVATLADYEGLDIKTILN
SKLSQFLTSIRQVFTAAAYHRAGIFQA1QNAAEEAEOQILFKPGLTSEKSSSSESANSKDRGL
QSNFKTNRGRHSAIPLRTGSKSFVYGILGTYTSVALLSLITAIKKKK 
Fig. 8

1358  SPy_0714 | putative adhesion protein, SF370
MKKKILMMMLLSVFFAWQLTQAKQVLAEGKVVTFFYPYFETYFKGIVAIGNGDVFMMLK
AGTEPHDFEPSTDKQADAFVYMDMNETWSDVKKSLTSKVTIVKGTGNMLLVA
GAGHDHPHEDADKHEHNMKXHEEGHNAFDHPHVLWPSYRTTVENIRDSLSDKYPEKAE
NFKANAATYIEKVELDKDYLAAALSDAQKSFVTQAAGYMALDYGRLNQISINGVTPDA
EPSAKRIALTLSKYVKKYGIKYIYFEEENASSQKAVTLRAGVKAAVLSQPLGLTEKEMKA
GQTYFTVRKRLRLLRTDAGKEILPEKDDTGTKTVNIGYFKEKDRQLSDWGSWQSQ
VYPYLVQGDLQVWYAKKSKGKMTTAAEYKYTTGGYKTDVEQIKINGKKKMTFVNRG
EKTFTFYAGKEILTYPKGNRGVRFMEAKEADAGEFKYVQFSDAIAPKEKAKHFHLYW
GGDSQKLEHLPWTPYYGSDLSGREGIAQEINAH
>SPy_2000 | surface lipoprotein
gTGTCAAATACCTAAATACCTATCCTATATCCGTTATATTTGACTGGGCTTTTTTATA
GTTCATGTCAACAAACAAAGCCCTACAAACAAACAAACAGTGCGAATACAGGCTCAGGAAAA
GACGATCTGCTGTTATGCGGCGCTACCTCTCTCTGTAAGATGCTGACTGTTG
TCGTTCTGACTGTCGACTGATGATTTTAAAATTTCTGAAAATGAGCTGACTGCTATG
GTAAAGTTATACTATAGTATGAAAAAGCAGTGAGGAAAGCTGGGACTACCTTCTG
AAAGAAGTGTGGAGTAATGTGGGAAAAATCATGTCATACTACTATCCATGAGGCCTGATCC
ACATCTTGACAGTGATGATGATGAAAATCCCAATGCTTCCCTAAAAAACATCATATGAA
TATAAAGACGAATACCTCAGGCTAGCCCTACTGCGTAAAGATGATATGCGGAGAAA
CGCTATTCTTTGTTGTAACCCCTATTGCGAATTGGGAACAAAACATCTATTAAAAATG
ACTTGGGCTCTTTGATGAAAAACACACGACATGACGCTTTAGAATCTCGTGTGATGAT
ATGATCTACGCAACGCGGCAAGACTTGCTGATAAAAAAGCTCAAAGGCAACCCGCTTCTTG
ATATCCCTCAAAATGAGTGTCCGGCCTTTACATTCTTTATGGAAGGCGTCATCCT
GATTCTCCGTATGTTATCCATGTAGGAATGATGTCACTAGTGATCCAGCAATCCGAAA
GCTTGCTATTGTTATATGAAAAATGGCCAAAGATCTGACTAGTTGTTATGGAATGTTG
AAACCAGCTTATTTCAATTATTGATAAAAAACACATTTTTGGAATCCAAAAACGCCATTAA
GATAAATAAAGTACCTAAAGCTAAAATATTGACAAAAAGCGGATGGAAGAACAAGCA
GACGGTAGGCTTAAAAAGTGATGACTTACCCCTGCTGTACTACAATG
GACCAATTGCGAGCGGCAACTAGCCGTAGTAGAGCAGCAAGCCAAAGCCCTAGGATT
ACTATTTAACCATTTCAAGCTCTATGAACTTGGAATAATGGCAACAGTAATGACTGCTAGC
TTACTTTATGCGGAGAAGGATGTCACCGCGACAGATTATGTAAGTGAGCATCATCAGACC
CTACGGGAAAAAGTTGTTAAATTAATCGCTTTTAAACAAATCTACCGTGACTGAGTAC
CTTGCAAAAGCAGATACTCTCTGACTGAAACCTAAGGCTAAATTTTGAGATTTGAA
CAGTTGGGCTAAAAACAGGCTTCTCGAATCTCGGAGATTTGCCAATAAGTTATGTTG
AGCCCTAATCATTTATATGTAATAAGCTCAATGCTATGTTGAAACAGGCTCACC
AGTCATGGCTAGTGGCTATTTATGACTAAACATTGCGGACTTGGGATGAAATCA
ACTAAGTAA
Fig. 10

>SPY_2000 |surface lipoprotein
VSKEYLYFSSTLFLTLVLILVACQQQPQTRKERQRKRKRQKRDELVSMGAKLPHEDFKDR
YGVRHEGNIHSTLLKRSPELIRGELSKYTHLSFGSLDLDLDLDKFSNGEPVTADD
VKFTYDMLKADGRAPDLTFIKNVEVVGKQVNHLTIAHSTFAQLTEIPIFVPKHYNDK
YKSNFPGSPYPVYMKERAGEQAIFVRNYPYWHGKKPYFKKWTWVLLDENTALAALESGDVD
MIYATPELAKKKVGLLIDSBDVRSGLSLFYVKKGVITDSPDGPYVGNVTSDPAIRK
ALTIGNLRQKVLDTMLNGYKPGYSGII1DKTPFWNPCKAIKDKNVAKAKILLTLQAGWKEQA
DGSRKKGDLDAADLTYPNTNDQLRANLAVEVAEQAKALGGITIKLAKASNWDEMATKSHDSA
LLYAGGRHHAQFYESHHPSLAGKGNWITFNYNPTVTKYLDKAMTSDDLKANEYWKLA
QNDGKTGASTLGDPNVWLVSLNHTYIGDKRINVGKQGVBSHGHDWSLLNIAEWTWDES
TK

Fig. 11

% killing of S. pyogenes SF370

![Graph showing % killing of S. pyogenes SF370 with TriVax and 554 dilutions compared to media.](image-url)
Fig 12

- PBS: p = 0.0028
- Preimmune: p = 0.0343

Group

CFUs/40ul blood (log)

3 4 5

PBS Preimmune SCP vaccinated
Fig 14
Fig 16

- p = 0.0147

CFUs/40ul blood (log)

Group

- 1218 pre
- 1218 vac
- PBS
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07K14/315 C07K16/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim</th>
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<td>CHENG QI ET AL: &quot;Immunization with C5a peptidase or peptidase-type III polysaccharide conjugate vaccines enhances clearance of group B streptococci from lungs of infected mice&quot; INFECTION AND IMMUNITY, vol. 70, no. 11, November 2002 (2002-11), pages 6409-6415, XP002567423 ISSN: 0019-9567 the whole document</td>
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See patent family annex

Further documents are listed in the continuation of Box C

Date of the actual completion of the international search
25 February 2010

Date of mailing of the international search report
17/03/2010

Name and mailing address of the ISA/
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Authorized officer
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<td>WO 2006/042027 A2 (CHIRON CORP [US]; BENSI GIULIANO [IT]; GRANDI GUIDO [IT]; NORAIS NATHA) 20 April 2006 (2006-04-20) abstract; examples 18, 19; sequences 57, 714</td>
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