MUTANT FORMS OF STREPTOLYSIN O

[01] This application claims the priority benefit of US61/016193 filed on 21st December 2007 and US61/088381 filed on 13th August 2008.

FIELD OF THE INVENTION

[02] The invention relates to the fields of immunology and vaccinology.

BACKGROUND OF THE INVENTION

[03] Streptolysin O (SLO; GAS25) is one of the most important virulence factors of the human pathogen Streptococcus pyogenes (GAS). Because of its capacity to invoke an early and strong immune response in humans, it is routinely used as a diagnostic marker of GAS infection.

[04] SLO belongs to the family of the highly homologous thiol-activated cytolysins (TACYs), which exert their cytolytic activity through interaction with cholesterol on the cell membrane, self-oligomerization, and formation of pores. Furthermore, their capacity to activate directly the classical complement pathway by binding to the Fc region of human IgG may result in direct complement-mediated attack on host cells. TACYs can also interfere with host defense and immune cell function by means of the induction of cytokines and inflammatory mediators. WO 2995/108419 describes immunogenic compositions comprising isolated mutant cytolysin proteins, including Streptolysin O with mutations at residues L249 to I266. WO 93/005155 and Pinkney et al, 1989, Infection and Immunity, vol 57, no. 8, pages 2553-2558 each disclose additional Streptolysin O variants.

[05] Some TACYs can passively and actively protect laboratory animals. See FEMS Lett. 182, 197-205, 2000. However, the use of these toxins as vaccine candidates has been hampered by their complex pattern of harmful side effects. There is, therefore, a need in the art for SLO proteins which are not toxic.

BRIEF DESCRIPTION OF THE FIGURES

[06] **FIG. 1**. Three-dimensional computer model of SLO. Prolines are represented as space-filled. Pro427 is colored in white.

[07] **FIG. 2**. Graph showing results of hemolytic assay using *E. coli* extracts containing wild-type SLO and SLO mutant P427L.

[08] **FIG. 3**. Photomicrograph of SDS-polyacrylamide gel showing purified SLO mutant P427L.

[09] **FIG. 4**. Graph showing results of hemolytic assay using purified wild-type SLO and SLO mutant P427L.

[10] **FIG. 5**. Photomicrograph of SDS-polyacrylamide gel of *E. coli* lysate supernatants. Lane A, *E. coli* negative control; lane B, rSLO wild-type, without tag; lane C, rSLO P427L, without tag; and lane D, purified rSLO wild-type, without tag (5mg).

[11] **FIG. 6**. Graph demonstrating that under the same conditions, SLO mutant P427L is 1000 times less hemolytic than wild-type SLO.

[12] **FIG. 7**. Graph demonstrating effects of cholesterol on hemolysis by wild-type SLO and SLO mutant P427L.

[13] **FIG. 8**. Photomicrographs of SDS-PAGE analysis of total *tag-less* proteins in cell extracts. **FIG. 8A**, expression of SLO wild-type and P427L *tag-less* proteins; **FIG. 8B**, expression of SLO P427L + W535, P427L + C530G, and P427L + C530G + W535F *tag-less* proteins.

[14] **FIG. 9**. Photomicrograph of SDS-PAGE analysis of total His-tagged proteins in cell extracts.

[15] **FIG. 10**. Photomicrograph of SDS-PAGE analysis of purified His-tagged proteins.

[16] **FIG. 11**. Photomicrographs of SDS-PAGE analysis of purified *tag-less* proteins. **FIG. 11A**, Lanes: A, SLO wild-type *tag-less*; B, SLO P427L *tag-less*; molecular weight markers (116-66.2-45-35-25-18.4-14.4); black arrow indicates SLO protein purified from mutants and wild-type clones. **FIG. 11B**, lane A, SLO Wild Type *tag-less* (3µg), lane B, SLO P427L-W535F *tag-less* (3µg); molecular weight markers (116-66.2-45-35-25-18.4-14.4); black arrow indicates SLO protein purified from mutants and wild-type clones.

[17] **FIG. 12**. Photomicrograph of SDS-PAGE analysis of purified *tag-less* SLO wild-type protein. Samples of different purification lots of wild-type SLO were analyzed under reducing and non-reducing conditions.

[18] **FIG. 13**. Graph showing results of hemolysis tests of His-tagged SLO mutants.

[19] **FIG. 14**. Graph showing inhibition of SLO-induced hemolytic activity by anti-SLO antiserum.

[20] **FIG. 15**. Graph showing titration of anti-SLO antiserum inhibition of SLO hemolysis.

[21] **FIG. 16.** Graph showing SLO hemolytic activity titration.

[22] **FIG. 17.** Graph showing titration of hemolytic activity of wild-type SLO, chemically detoxified wild-type SLO, and SLO mutants (P427L; P427L + W535F).

[23] **FIG. 18.** Graph showing titration of hemolytic activity of wild-type SLO and SLO mutants (P427L; P427L + W535F).

[24] **FIG. 19.** Graph showing titration of hemolytic activity of wild-type SLO and chemically detoxified wild-type SLO.

[25] **FIG. 20**. Graph showing dilution of antiserum against SLO mutant P427L + W535F required to obtain 50% reduction of SLO hemolytic activity (50 ng/ml SLO).

[26] **FIG. 21**. Graph showing dilution of antiserum against SLO mutant P427L + W535F required to obtain 50% reduction of SLO hemolytic activity (100 ng/ml SLO).

[27] **FIG. 22**. Titration curve showing that hemolysis inhibition assays were performed with toxin concentrations which allow 100% haemolysis.

[28] **FIG. 23**. Alignment of SLO proteins. M1_SF370, SEQ ID NO:1; M12_2096, SEQ ID NO:2; M12_9429, SEQ ID NO:3; M1_5005, SEQ ID NO:4; M2, SEQ ID NO:5; M28, SEQ ID NO:6; M6, SEQ ID NO:7; M18, SEQ ID NO:8; M5, SEQ ID NO:9; M3, SEQ ID NO:10; M3_SSI, SEQ ID NO:11; and M4, SEQ ID NO:12.

[29] **FIG. 24**. Alignment of wild-type SLO and His-tagged SLO mutants. SLO_M1strainSF370, SEQ ID NO:13; SLO WT_histagged, SEQ ID NO:14; SLOmut.P427L_histagged, SEQ ID NO:15; SLOmut.C530G_histagged, SEQ ID NO:16; SLOmut.DeltaA248_histagged, SEQ ID NO:17; SLOmut.W535F_histagged, SEQ ID NO:18; and SLOmutW535F&D482N_histagged, SEQ ID NO:19.

[30] **FIG. 25**. Graph comparing reduction of SLO hemolytic activity by antiserum against wild-type SLO and antiserum against SLO mutant P427L + W535F.

[31] **FIG. 26**. Graph showing *in vivo* protective properties of recombinant SLO mutant P427L + W535F using either alum or MF59 as an adjuvant.

DETAILED DESCRIPTION OF THE INVENTION

[32] The invention provides mutants of streptolysin O (SLO; GAS25) which are non-toxic but which still maintain the ability to induce protection against *S. pyogenes*. Mutant forms of SLO are useful, *inter alia*, in vaccine compositions, to induce protection against *S. pyogenes*.

Mutant SLO proteins

[33] Mutant forms of SLO according to the invention have at least 50% less hemolytic activity than wild-type SLO (*e.g.*, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99, or 100%) relative to wild-type SLO as determined by a hemolytic assay but are immunogenic, *e.g.*, they confer protection against GAS lethal challenge in a mouse model (*e.g.*, see Example 7). SLO mutants of the invention include SLO mutants P427L (SEQ ID NO:20), W535F (SEQ ID NO:21), C530G (SEQ ID NO:22), ΔA248 (SEQ ID NO:23), W535F + D482N (SEQ ID NO:24), P427L + W535F (SEQ ID NO:25), P427L + C530G (SEQ ID NO:26), and P427L + C530G + W535F (SEQ ID NO:27). The invention also includes His-tagged versions of these mutants. Examples are shown in **FIG. 24**.

[34] SLO mutants of the invention include those with an amino acid alteration (*i.e.*, a substitution, deletion, or insertion) at one or more of amino acids P427, W535, C530, A248, and D482 numbered according to the wild-type SLO sequence shown in SEQ ID NO:1. **FIG. 23** provides an alignment of wild-type GAS25 sequences from different M types.

[35] SLO mutants of the invention include single, double, or triple amino acid alterations ("single mutants," "double mutants," "triple mutants") at positions P427, W535, C530, A248, and/or D482. Thus, SLO mutants can comprise the following:

i. P427L (SEQ ID NO:20), P427R, P427N, P427C, P427Q, P427E, P427G, P427H, P427I, P427L, P427K, P427M, P427F, P427A, P427S, P427T, P427W, P427Y, or P427V;

ii. W535F (SEQ ID NO:21), W535R, W535N, W535D, W535C, W535Q, W535E, W535G, W535I, W535L, W535K, W535M, W535A, W535P, W535S, W535T, W535Y, or W535V;

iii. C530G (SEQ ID NO:22), C530R, C530N, C530D, C530S, C530Q, C530E, C530A, C530H, C530I, C530L, C530K, C530M, C530F, C530P, C530T, C530W, C530Y, or C530V;

iv. D482L, D482R, D482N, D482C, D482Q, D482E, D482G, D482H, D482I, D482L, D482K, D482M, D482F, D482A, D482S, D482T, D482W, D482Y, or D482V;

v. A248L, A248R, A248N, A248C, A248Q, A248E, A248G, A248H, A248I, A248L, A248K, A248M, A248F, A248S, A248T, A248W, A248Y, or A248V

vi. ΔP427; or ΔW535; or ΔC530; or ΔD482; or ΔA248 (SEQ ID NO:23); and

vii. combinations thereof, such as W535F + D482N (SEQ ID NO:24), P427L + W535F (SEQ ID NO:25), P427L + C530G (SEQ ID NO:26), and P427L + C530G + W535F (SEQ ID NO:27).

[36] Double mutants of the invention include P427L + W535F (SEQ ID NO25), P427L + C530G (SEQ ID NO:26), P427L + A248L, P427L + D482L, W535F + C530G, W535F + A248L, W535F + D482L, C530G + A248L, and A248L + D482L. Triple mutants include P427L + C530G + A248L, P427L + C530G + D482L, P427L + A248L + D482L, P427L + C530G + W535F (SEQ ID NO:27), W535F + C530G + A248L, W535F + C530G + D482L, W535F + A248L + D482L, and C530G + A248L + D482L.

[37] Mutant SLO proteins of the invention also include fusion polypeptides which comprise a mutant SLO protein as disclosed above and another GAS antigen. GAS antigens are disclosed, *e.g.*, in WO 02/34771 and include, but are not limited to, GAS39 (spy0266; gi-15674446), GAS40 (spy0269; gi-15674449), GAS42 (spy0287; gi-15674461), GAS45 (M5005_spy0249; gi-71910063), GAS57 (spy0416; gi-15674549), GAS58 (spy0430; gi-15674556), GAS84 (spy1274; gi-15675229), GAS95 (spt1733; gi-15675582), GAS117

(spy0448; gi-15674571), GAS130 (spy0591; gi-15674677), GAS137 (spy0652; gi-15674720), GAS159 (spy1105; gi-15675088), GAS193 (spy2025; gi-15675802), GAS202 (spy1309; gi-15675258), GAS217 (spy0925; gi-15674945), GAS236 (spy1126; gi-15675106), GAS253 (spy1524; gi-15675423), GAS277 (spy1939; gi-15675742), GAS294 (spy1173; gi-15675145), GAS309 (spy0124; gi-15674341), GAS366 (spy1525; gi-15675424), GAS372 (spy1625; gi-15675501), GAS384 (spy1874; gi-15675693), GAS389 (spy1981; gi-15675772), GAS504 (spy1751; gi-15675600), GAS509 (spy1618; gi-15675496), GAS290 (spy1959; gi-15675757), GAS511 (spy1743; gi-15675592), GAS527 (spy1204; gi-15675169), GAS529 (spy1280; gi-15675233), and GAS533 (spy1877; gi-15675696). Further GAS antigens include, but are not limited to GAS68 (Spy0163; gi13621456), GAS84 (Spy1274; gi13622398), GAS88 (Spy1361; gi13622470), GAS89 (Spy1390; gi13622493), GAS98 (Spy1882; gi13622916), GAS99 (Spy1979; gi13622993), GAS102 (Spy2016, gi13623025), GAS146 (Spy0763; gi13621942), GAS195 (Spy2043; gi13623043), GAS561 (Spy1134; gi13622269), GAS179 (Spy1718, gi13622773) and GAS681 (spy1152; gi1362228).

Nucleic acid molecules encoding mutant SLO proteins

[38] The invention includes nucleic acid molecules which encode mutant SLO proteins. The invention also includes nucleic acid molecules comprising nucleotide sequences having at least 50% sequence identity to such molecules. Depending on the particular sequence, the degree of sequence identity is preferably greater than 50% (*e.g.*, 60%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more). Identity between nucleotide sequences is preferably determined by the Smith-Waterman homology search algorithm as implemented in the MPSRCH program (Oxford Molecular), using an affine gap search with parameters gap open penalty = 12 and gap extension penalty = 1.

[39] The invention also provides nucleic acid molecules which can hybridize to these molecules. Hybridization reactions can be performed under conditions of different

"stringency." Conditions which increase stringency of a hybridization reaction are widely known and published in the art. See, *e.g.*, page 7.52 of Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 1989. Examples of relevant conditions include (in order of increasing stringency): incubation temperatures of 25°C, 37 °C, 50 °C, 55 °C, and 68 °C; buffer concentrations of 10X SSC, 6X SSC, 1X SSC, and 0.1X SSC (where SSC is 0.15 M NaCl and 15 mM citrate buffer) and their equivalents using other buffer systems; formamide concentrations of 0%, 25%, 50%, and 75%; incubation times from 5 minutes to 24 hours; 1, 2, or more washing steps; wash incubation times of 1, 2, or 15 minutes; and wash solutions of 6X SSC, 1X SSC, 0.1X SSC, or de-ionized water. Hybridization techniques and their optimization are well known in the art. See, *e.g.*, Sambrook, 1989; Ausubel *et al.*, eds., Short Protocols in Molecular Biology, 4th ed., 1999; U.S. Patent 5,707,829; Ausubel *et al.*, eds., Current Protocols in Molecular Biology, Supplement 30, 1987.

[40] In some embodiments, nucleic acid molecules of the invention hybridize to a target under low stringency conditions; in other embodiments, nucleic acid molecules of the invention hybridize under intermediate stringency conditions; in preferred embodiments, nucleic acid molecules of the invention hybridize under high stringency conditions. An example of a low stringency hybridization condition is 50°C and 10X SSC. An example of an intermediate stringency hybridization condition is 55°C and 1X SSC. An example of a high stringency hybridization condition is 68°C and 0.1X SSC.

Production of mutant SLO proteins

Recombinant production

[41] The redundancy of the genetic code is well-known. Thus, any nucleic acid molecule (polynucleotide) which encodes wild-type SLO protein or a SLO mutant protein of the invention can be used to produce that protein recombinantly. Examples of nucleotide sequences which encode wild-type SLO, SLO mutant P427L, W535F, C530G, ΔA248, W535F + D482N, P427L + W535F, P427L + C530G, and P427L + C530G + W535F are

provided in the sequence listing (see also SEQ ID NOS:28, 29, 30, 31, 32, 33, 34, 35, and 36, respectively. Nucleic acid molecules encoding wild-type SLO also can be isolated from the appropriate *S. pyogenes* bacterium using standard nucleic acid purification techniques or can be synthesized using an amplification technique, such as the polymerase chain reaction (PCR), or by using an automatic synthesizer. *See* Caruthers *et al.*, Nucl. Acids Res. Symp. Ser. 215 223, 1980; Horn *et al.* Nucl. Acids Res. Symp. Ser. 225 232, 1980; Hunkapiller *et al.*, Nature 310, 105-11, 1984; Grantham *et al.*, Nucleic Acids Res. 9, r43-r74, 1981.

[42] cDNA molecules can be made with standard molecular biology techniques, using mRNA as a template. cDNA molecules can thereafter be replicated using molecular biology techniques well known in the art. An amplification technique, such as PCR, can be used to obtain additional copies of polynucleotides of the invention, using either genomic DNA or cDNA as a template.

[43] If desired, polynucleotides can be engineered using methods generally known in the art to alter antigen-encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the polypeptide or mRNA product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides can be used to engineer the nucleotide sequences. For example, site directed mutagenesis can be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, introduce mutations, and so forth.

[44] Sequence modifications, such as the addition of a purification tag sequence or codon optimization, can be used to facilitate expression. For example, the N-terminal leader sequence may be replaced with a sequence encoding for a tag protein such as polyhistidine ("HIS") or glutathione S-transferase ("GST"). Such tag proteins may be used to facilitate purification, detection, and stability of the expressed protein. Codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the

rate of protein expression or to produce an RNA transcript having desirable properties, such as a half life which is longer than that of a transcript generated from the naturally occurring sequence. These methods are well known in the art and are further described in WO05/032582.

Expression vectors

[45] A nucleic acid molecule which encodes a mutant SLO protein can be inserted into an expression vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods which are well known to those skilled in the art can be used to construct expression vectors containing coding sequences and appropriate transcriptional and translational control elements. These methods include *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination.

Host cells

[46] Host cells for producing mutant SLO proteins can be prokaryotic or eukaryotic. *E. coli* is a preferred host cell, but other suitable hosts include *Lactococcus lactis, Lactococcus cremoris, Bacillus subtilis, Vibrio cholerae, Salmonella typhi, Salmonella typhimurium, Neisseria lactamica, Neisseria cinerea, Mycobacteria* (*e.g.*, *M. tuberculosis*), yeasts, baculovirus, mammalian cells, etc.

[47] A host cell strain can be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed polypeptide in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post translational processing which cleaves a "prepro" form of the polypeptide also can be used to facilitate correct insertion, folding and/or function. Different host cells which have specific cellular machinery and characteristic mechanisms for post translational activities are available from the American Type Culture Collection (ATCC; 10801

University Boulevard, Manassas, VA 20110-2209) and can be chosen to ensure the correct modification and processing of a foreign protein. See WO 01/98340.

[48] Expression constructs can be introduced into host cells using well-established techniques which include, but are not limited to, transferrin-polycation-mediated DNA transfer, transfection with naked or encapsulated nucleic acids, liposome-mediated cellular fusion, intracellular transportation of DNA-coated latex beads, protoplast fusion, viral infection, electroporation, "gene gun" methods, and DEAE- or calcium phosphate-mediated transfection.

[49] Host cells transformed with expression vectors can be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a transformed cell can be secreted or contained intracellularly depending on the nucleotide sequence and/or the expression vector used. Those of skill in the art understand that expression vectors can be designed to contain signal sequences which direct secretion of soluble antigens through a prokaryotic or eukaryotic cell membrane.

Purification

[50] Signal export sequences can be included in a recombinantly produced mutant SLO protein so that the antigen can be purified from cell culture medium using known methods. Alternatively, recombinantly produced mutant SLO proteins of the invention can be isolated from engineered host cells and separated from other components in the cell, such as proteins, carbohydrates, or lipids, using methods well-known in the art. Such methods include, but are not limited to, size exclusion chromatography, ammonium sulfate fractionation, ion exchange chromatography, affinity chromatography, and preparative gel electrophoresis. A preparation of purified mutant SLO proteins is at least 80% pure; preferably, the preparations are 90%, 95%, or 99% pure. Purity of the preparations can be assessed by any means known in the art, such as SDS-polyacrylamide gel electrophoresis. Where appropriate, mutant SLO proteins can be solubilized, for example, with urea.

Chemical synthesis

[51] Mutant SLO proteins can be synthesized, for example, using solid phase techniques. See, *e.g.*, Merrifield, J. Am. Chem. Soc. 85, 2149 54, 1963; Roberge *et al.*, Science 269, 202 04, 1995. Protein synthesis can be performed using manual techniques or by automation. Automated synthesis can be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Optionally, fragments of a mutant SLO protein can be separately synthesized and combined using chemical methods to produce a full-length molecule.

Antibodies

[52] The invention provides antibodies which bind specifically to a mutant SLO protein of the invention but which do not bind wild-type SLO protein. The term "antibody" includes intact immunoglobulin molecules, as well as fragments thereof which are capable of binding an antigen. These include hybrid (chimeric) antibody molecules (*e.g.*, Winter *et al.*, *Nature 349*, 293-99, 1991; U.S. Patent 4,816,567); F(ab')2 and F(ab) fragments and Fv molecules; non-covalent heterodimers (*e.g.*, Inbar *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 69*, 2659-62, 1972; Ehrlich *et al.*, *Biochem 19*, 4091-96, 1980); single-chain Fv molecules (sFv) (*e.g.*, Huston *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 85*, 5897-83, 1988); dimeric and trimeric antibody fragment constructs; minibodies (*e.g.*, Pack *et al.*, *Biochem 31*, 1579-84, 1992; Cumber *et al.*, *J. Immunology 149B*, 120-26, 1992); humanized antibody molecules (*e.g.*, Riechmann *et al.*, *Nature 332*, 323-27, 1988; Verhoeyan *et al.*, *Science 239*, 1534-36, 1988; and U.K. Patent Publication No. GB 2,276,169, published 21 September 1994); and any functional fragments obtained from such molecules, as well as antibodies obtained through non-conventional processes such as phage display. Preferably, the antibodies are monoclonal antibodies. Methods of obtaining monoclonal antibodies are well known in the art.

[53] Typically, at least 6, 7, 8, 10, or 12 contiguous amino acids are required to form an epitope. However, epitopes which involve non-contiguous amino acids may require

more, *e.g.*, at least 15, 25, or 50 amino acids. Various immunoassays (*e.g.*, Western blots, ELISAs, radioimmunoassays, immunohistochemical assays, immunoprecipitations, or other immunochemical assays known in the art) can be used to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays are well known in the art. Such immunoassays typically involve the measurement of complex formation between an immunogen and an antibody which specifically binds to the immunogen. A preparation of antibodies which specifically bind to a mutant SLO protein typically provides a detection signal at least 5-, 10-, or 20-fold higher than a detection signal provided with other proteins when used in an immunochemical assay and does not provide a detectable signal if contacted with wild-type SLO protein. Preferably, the antibodies do not detect other proteins in immunochemical assays and can immunoprecipitate the particular antigen from solution.

Generation of antibodies

[54] Mutant SLO proteins or non-SLO polypeptide antigens (described below) can be used to immunize a mammal, such as a mouse, rat, rabbit, guinea pig, monkey, or human, to produce polyclonal antibodies. If desired, an antigen can be conjugated to a carrier protein, such as bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin. Depending on the host species, various adjuvants can be used to increase the immunological response. Such adjuvants include, but are not limited to, Freund's adjuvant, mineral gels (*e.g.*, aluminum hydroxide), and surface active substances (*e.g.* lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol). Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are especially useful.

[55] Monoclonal antibodies which specifically bind to an antigen can be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These techniques include, but are not limited to, the hybridoma technique, the human B cell hybridoma technique, and the EBV hybridoma technique

(Kohler *et al.*, *Nature 256*, 495 497, 1985; Kozbor *et al.*, *J. Immunol. Methods 81*, 31 42, 1985; Cote *et al.*, *Proc. Natl. Acad. Sci. 80*, 2026 2030, 1983; Cole *et al.*, *Mol. Cell Biol. 62*, 109 120, 1984).

[56] In addition, techniques developed for the production of "chimeric antibodies," the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used (Morrison *et al.*, *Proc. Natl. Acad. Sci. 81*, 6851 6855, 1984; Neuberger *et al.*, *Nature 312*, 604 608, 1984; Takeda *et al.*, *Nature 314*, 452 454, 1985). Monoclonal and other antibodies also can be "humanized" to prevent a patient from mounting an immune response against the antibody when it is used therapeutically. Such antibodies may be sufficiently similar in sequence to human antibodies to be used directly in therapy or may require alteration of a few key residues. Sequence differences between rodent antibodies and human sequences can be minimized by replacing residues which differ from those in the human sequences by site directed mutagenesis of individual residues or by grating of entire complementarity determining regions.

[57] Alternatively, humanized antibodies can be produced using recombinant methods, as described below. Antibodies which specifically bind to a particular antigen can contain antigen binding sites which are either partially or fully humanized, as disclosed in U.S. 5,565,332.

[58] Alternatively, techniques described for the production of single chain antibodies can be adapted using methods known in the art to produce single chain antibodies which specifically bind to a particular antigen. Antibodies with related specificity, but of distinct idiotypic composition, can be generated by chain shuffling from random combinatorial immunoglobin libraries (Burton, *Proc. Natl. Acad. Sci. 88*, 11120 23, 1991).

[59] Single-chain antibodies also can be constructed using a DNA amplification method, such as PCR, using hybridoma cDNA as a template (Thirion *et al.*, 1996, *Eur. J. Cancer Prev.*

5, 507-11). Single-chain antibodies can be mono- or bispecific, and can be bivalent or tetravalent. Construction of tetravalent, bispecific single-chain antibodies is taught, for example, in Coloma & Morrison, *Nat. Biotechnol. 15*, 159-63, 1997. Construction of bivalent, bispecific single-chain antibodies is taught in Mallender & Voss, *J. Biol. Chem. 269*, 199-206, 1994.

[60] A nucleotide sequence encoding a single-chain antibody can be constructed using manual or automated nucleotide synthesis, cloned into an expression construct using standard recombinant DNA methods, and introduced into a cell to express the coding sequence, as described below. Alternatively, single-chain antibodies can be produced directly using, for example, filamentous phage technology (Verhaar *et al.*, *Int. J. Cancer 61*, 497-501, 1995; Nicholls *et al.*, *J. Immunol. Meth. 165*, 81-91, 1993).

[61] Antibodies which specifically bind to a particular antigen also can be produced by inducing in vivo production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature (Orlandi *et al.*, *Proc. Natl. Acad. Sci. 86*, 3833 3837, 1989; Winter *et al.*, *Nature 349*, 293 299, 1991).

[62] Chimeric antibodies can be constructed as disclosed in WO 93/03151. Binding proteins which are derived from immunoglobulins and which are multivalent and multispecific, such as the "diabodies" described in WO 94/13804, also can be prepared.

[63] Antibodies can be purified by methods well known in the art. For example, antibodies can be affinity purified by passage over a column to which the relevant antigen is bound. The bound antibodies can then be eluted from the column using a buffer with a high salt concentration.

Pharmaceutical Compositions

[64] The invention also provides compositions for use as medicaments (*e.g.*, as immunogenic compositions or vaccines). Compositions of the invention are useful for preventing

and/or treating disease caused as a result of *S. pyogenes* infection and comprise at least one active agent, which can be a polypeptide, a nucleic acid molecule, or an antibody. Said disease may be, for example, bacteremia, meningitis, puerperal fever, scarlet fever, erysipelas, pharyngitis, impetigo, necrotizing fasciitis, myositis or toxic shock syndrome.

[65] Compositions containing mutant SLO proteins are preferably immunogenic compositions, and are more preferably vaccine compositions. The pH of such compositions preferably is between 6 and 8, preferably about 7. The pH can be maintained by the use of a buffer. The composition can be sterile and/or pyrogen free. The composition can be isotonic with respect to humans.

[66] Vaccines according to the invention may be used either prophylactically or therapeutically, but will typically be prophylactic. Accordingly, the invention includes a method for the therapeutic or prophylactic treatment of a *Streptococcus pyogenes* infection. The animal is preferably a mammal, most preferably a human. The methods involve administering to the animal a therapeutic or prophylactic amount of the immunogenic compositions of the invention.

[67] Some compositions of the invention comprise a polypeptide mutant SLO protein as described herein. Other compositions of the invention comprise a nucleic acid molecule which encodes the mutant SLO protein(s) and, optionally, other antigens which can be included in the composition (see below). See, *e.g.*, Robinson & Torres (1997) Seminars in Immunology 9:271-283; Donnelly *et al.* (1997) Ann. Rev Immunol 15:617-648; Scott-Taylor & Dalgleish (2000) Expert Opin Investig Drugs 9:471-480; Apostolopoulos & Plebanski (2000) Curr Opin Mol Ther 2:441-447; Ilan (1999) Curr Opin Mol Ther 1:116-120; Dubensky *et al.* (2000) Mol Med 6:723-732; Robinson & Pertmer (2000) Adv Virus Res 55:1-74; Donnelly *et al.* (2000) Am J Respir Crit Care Med 162(4 Pt 2):S190-193; Davis (1999) Mt. Sinai J. Med. 66:84-90. Typically the nucleic acid molecule is a DNA molecule, *e.g.*, in the form of a plasmid.

[68] In some embodiments, compositions of the invention can include one or more additional active agents. Such agents include, but are not limited to, (a) another mutant SLO protein of the invention, (b) a polypeptide antigen which is useful in a pediatric vaccine, (c) a polypeptide antigen which is useful in a vaccine for elderly or immunocompromised individuals, (d) a nucleic acid molecule encoding (a)-(c), and an antibody which specifically binds to (a)-(c).

Additional antigens

[69] Compositions of the invention may be administered in conjunction with one or more additional antigens for use in therapeutic or prophylactic methods of the present invention. Suitable antigens include those listed below. Additionally, the compositions of the present invention may be used to treat or prevent infections caused by any of the below-listed pathogens. In addition to combination with the antigens described below, the compositions of the invention may also be combined with an adjuvant as described herein.

[70] Antigens for use with the invention include, but are not limited to, one or more of the following antigens set forth below, or antigens derived from one or more of the pathogens set forth below:

A. Bacterial Antigens

[71] Bacterial antigens suitable for use in the invention include proteins, polysaccharides, lipopolysaccharides, and outer membrane vesicles which may be isolated, purified or derived from a bacteria. In addition, bacterial antigens may include bacterial lysates and inactivated bacteria formulations. Bacteria antigens may be produced by recombinant expression. Bacterial antigens preferably include epitopes which are exposed on the surface of the bacteria during at least one stage of its life cycle. Bacterial antigens are preferably conserved across multiple serotypes. Bacterial antigens include antigens

derived from one or more of the bacteria set forth below as well as the specific antigens examples identified below.

[72] *Neisseria meningitides*: *Meningitides* antigens may include proteins (such as those identified in References 1 – 7), saccharides (including a polysaccharide, oligosaccharide or lipopolysaccharide), or outer-membrane vesicles (References 8, 9, 10, 11) purified or derived from *N. meningitides* serogroup such as A, C, W135, Y, and/or B. *Meningitides* protein antigens may be selected from adhesions, autotransporters, toxins, Fe acquisition proteins, and membrane associated proteins (preferably integral outer membrane protein).

[73] *Streptococcus pneumoniae*: *Streptococcus pneumoniae* antigens may include a saccharide (including a polysaccharide or an oligosaccharide) and/or protein from *Streptococcus pneumoniae*. Saccharide antigens may be selected from serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, and 33F. Protein antigens may be selected from a protein identified in WO 98/18931, WO 98/18930, US Patent No. 6,699,703, US Patent No. 6,800,744, WO 97/43303, and WO 97/37026. *Streptococcus pneumoniae* proteins may be selected from the Poly Histidine Triad family (PhtX), the Choline Binding Protein family (CbpX), CbpX truncates, LytX family, LytX truncates, CbpX truncate-LytX truncate chimeric proteins, pneumolysin (Ply), PspA, PsaA, Sp128, Sp101, Sp130, Sp125 or Sp133.

[74] *Streptococcus pyogenes* (Group A Streptococcus): Group A *Streptococcus* antigens may include a protein identified in WO 02/34771 or WO 2005/032582 (including, but not limited to, GAS39 (spy0266; gi-15674446), GAS40 (spy0269; gi-15674449), GAS42 (spy0287; gi-15674461), GAS45 (M5005_spy0249; gi-71910063), GAS57 (spy0416; gi-15674549), GAS58 (spy0430; gi-15674556), GAS84 (spy1274; gi-15675229), GAS95 (spt1733; gi-15675582), GAS117 (spy0448; gi-15674571), GAS130 (spy0591; gi-15674677), GAS137 (spy0652; gi-15674720), GAS159 (spy1105; gi-15675088), GAS193 (spy2025; gi-15675802), GAS202 (spy1309; gi-15675258), GAS217 (spy0925; gi-15674945), GAS236 (spy1126; gi-15675106), GAS253 (spy1524; gi-15675423),

GAS277 (spy1939; gi-15675742), GAS294 (spy1173; gi-15675145), GAS309 (spy0124; gi-15674341), GAS366 (spy1525; gi-15675424), GAS372 (spy1625; gi-15675501), GAS384 (spy1874; gi-15675693), GAS389 (spy1981; gi-15675772), GAS504 (spy1751; gi-15675600), GAS509 (spy1618; gi-15675496), GAS290 (spy1959; gi-15675757), GAS511 (spy1743; gi-15675592), GAS527 (spy1204; gi-15675169), GAS529 (spy1280; gi-15675233), and GAS533 (spy1877; gi-15675696)), fusions of fragments of GAS M proteins (including those described in WO 02/094851, and Dale, Vaccine (1999) 17:193-200, and Dale, Vaccine 14(10): 944-948), fibronectin binding protein (Sfb1), Streptococcal heme-associated protein (Shp), and Streptolysin S (SagA). Further GAS antigens include GAS68 (Spy0163; gi13621456), GAS84 (Spy1274; gi13622398), GAS88 (Spy1361; gi13622470), GAS89 (Spy1390; gi13622493), GAS98 (Spy1882; gi13622916), GAS99 (Spy1979; gi13622993), GAS102 (Spy2016, gi13623025), GAS146 (Spy0763; gi13621942), GAS195 (Spy2043; gi13623043), GAS561 (Spy1134; gi13622269), GAS179 (Spy1718, gi13622773) and GAS681 (spy1152; gi1362228).

[75] *Moraxella catarrhalis*: *Moraxella* antigens include antigens identified in WO 02/18595 and WO 99/58562, outer membrane protein antigens (HMW-OMP), C-antigen, and/or LPS.

[76] *Bordetella pertussis*: Pertussis antigens include petussis holotoxin (PT) and filamentous haemagglutinin (FHA) from *B. pertussis*, optionally also combination with pertactin and/or agglutinogens 2 and 3 antigen.

[77] *Staphylococcus aureus*: *Staphylococcus aureus* antigens include *S. aureus* type 5 and 8 capsular polysaccharides optionally conjugated to nontoxic recombinant *Pseudomonas aeruginosa* exotoxin A, such as StaphVAX™, or antigens derived from surface proteins, invasins (leukocidin, kinases, hyaluronidase), surface factors that inhibit phagocytic engulfment (capsule, Protein A), carotenoids, catalase production, Protein A, coagulase, clotting factor, and/or membrane-damaging toxins (optionally detoxified) that lyse eukaryotic cell membranes (hemolysins, leukotoxin, leukocidin).

[78] *Staphylococcus epidermis*: *S. epidermidis* antigens include slime-associated antigen (SAA).

[79] *Clostridium tetani* (Tetanus): Tetanus antigens include tetanus toxoid (TT), preferably used as a carrier protein in conjunction/conjugated with the compositions of the present invention.

[80] *Cornynebacterium diphtheriae* (Diphtheria): Diphtheria antigens include diphtheria toxin, preferably detoxified, such as CRM197. Additionally antigens capable of modulating, inhibiting or associated with ADP ribosylation are contemplated for combination/co-administration/conjugation with the compositions of the present invention. The diphtheria toxoids may be used as carrier proteins.

[81] *Haemophilus influenzae B* (Hib): Hib antigens include a Hib saccharide antigen.

[82] *Pseudomonas aeruginosa*: *Pseudomonas* antigens include endotoxin A, Wzz protein, P. aeruginosa LPS, more particularly LPS isolated from PAO1 (O5 serotype), and/or Outer Membrane Proteins, including Outer Membrane Proteins F (OprF) (Infect Immun. 2001 May; 69(5): 3510-3515).

[83] *Legionella pneumophila*. Bacterial antigens may be derived from *Legionella pneumophila*.

[84] *Streptococcus agalactiae* (Group B Streptococcus): Group B *Streptococcus* antigens include a protein or saccharide antigen identified in WO 02/34771, WO 03/093306, WO 04/041157, or WO 2005/002619 (including proteins GBS 80, GBS 104, GBS 276 and GBS 322, and including saccharide antigens derived from serotypes Ia, Ib, Ia/c, II, III, IV, V, VI, VII and VIII).

[85] *Neiserria gonorrhoeae*: *Gonorrhoeae* antigens include Por (or porin) protein, such as PorB (see Zhu *et al.*, Vaccine (2004) 22:660 – 669), a transferring binding protein, such as TbpA and TbpB (See Price *et al.*, Infection and Immunity (2004) 71(1):277 – 283), a

opacity protein (such as Opa), a reduction-modifiable protein (Rmp), and outer membrane vesicle (OMV) preparations (see Plante *et al.*, J Infectious Disease (2000) 182:848 – 855), also see *e.g.* WO99/24578, WO99/36544, WO99/57280, WO02/079243).

[86] *Chlamydia trachomatis*: *Chlamydia trachomatis* antigens include antigens derived from serotypes A, B, Ba and C (agents of trachoma, a cause of blindness), serotypes L1, L2 & L3 (associated with *Lymphogranuloma venereum*), and serotypes, D-K. Chlamydia trachomas antigens may also include an antigen identified in WO 00/37494, WO 03/049762, WO 03/068811, or WO 05/002619, including PepA (CT045), LcrE (CT089), ArtJ (CT381), DnaK (CT396), CT398, OmpH-like (CT242), L7/L12 (CT316), OmcA (CT444), AtosS (CT467), CT547, Eno (CT587), HrtA (CT823), and MurG (CT761).

[87] *Treponema pallidum* (Syphilis): Syphilis antigens include TmpA antigen.

[88] *Haemophilus ducreyi* (causing chancroid): Ducreyi antigens include outer membrane protein (DsrA).

[89] *Enterococcus faecalis* or *Enterococcus faecium*: Antigens include a trisaccharide repeat or other *Enterococcus* derived antigens provided in US Patent No. 6,756,361.

[90] *Helicobacter pylori*: *H. pylori* antigens include Cag, Vac, Nap, HopX, HopY and/or urease antigen.

[91] *Staphylococcus saprophyticus*: Antigens include the 160 kDa hemagglutinin of *S. saprophyticus* antigen.

[92] *Yersinia enterocolitica* antigens include LPS (Infect Immun. 2002 August; 70(8): 4414).

[93] *E. coli*: *E. coli* antigens may be derived from enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAggEC), diffusely adhering *E. coli* (DAEC), enteropathogenic *E. coli* (EPEC), and/or enterohemorrhagic *E. coli* (EHEC).

[94] *Bacillus anthracis* (anthrax): *B. anthracis* antigens are optionally detoxified and may be selected from A-components (lethal factor (LF) and edema factor (EF)), both of which can share a common B-component known as protective antigen (PA).

[95] *Yersinia pestis* (plague): Plague antigens include F1 capsular antigen (Infect Immun. 2003 Jan; 71(1)): 374-383, LPS (Infect Immun. 1999 Oct; 67(10): 5395), *Yersinia pestis* V antigen (Infect Immun. 1997 Nov; 65(11): 4476-4482).

[96] *Mycobacterium tuberculosis*: Tuberculosis antigens include lipoproteins, LPS, BCG antigens, a fusion protein of antigen 85B (Ag85B) and/or ESAT-6 optionally formulated in cationic lipid vesicles (Infect Immun. 2004 October; 72(10): 6148), *Mycobacterium tuberculosis* (Mtb) isocitrate dehydrogenase associated antigens (Proc Natl Acad Sci U S A. 2004 Aug 24; 101(34): 12652), and/or MPT51 antigens (Infect Immun. 2004 July; 72(7): 3829).

[97] *Rickettsia*: Antigens include outer membrane proteins, including the outer membrane protein A and/or B (OmpB) (Biochim Biophys Acta. 2004 Nov 1;1702(2):145), LPS, and surface protein antigen (SPA) (J Autoimmun. 1989 Jun;2 Suppl:81).

[98] *Listeria monocytogenes*. Bacterial antigens may be derived from Listeria monocytogenes.

[99] *Chlamydia pneumoniae*: Antigens include those identified in WO 02/02606.

[100] *Vibrio cholerae*: Antigens include proteinase antigens, LPS, particularly lipopolysaccharides of *Vibrio cholerae* II, O1 Inaba O-specific polysaccharides, V. cholera O139, antigens of IEM108 vaccine (Infect Immun. 2003 Oct;71(10):5498-504), and/or Zonula occludens toxin (Zot).

[101] *Salmonella typhi* (typhoid fever): Antigens include capsular polysaccharides preferably conjugates (Vi, *i.e.* vax-TyVi).

[102] *Borrelia burgdorferi* (Lyme disease): Antigens include lipoproteins (such as OspA, OspB, Osp C and Osp D), other surface proteins such as OspE-related proteins (Erps), decorin-binding proteins (such as DbpA), and antigenically variable VI proteins. , such as antigens associated with P39 and P13 (an integral membrane protein, Infect Immun. 2001 May; 69(5): 3323-3334), VlsE Antigenic Variation Protein (J Clin Microbiol. 1999 Dec; 37(12): 3997).

[103] *Porphyromonas gingivalis*: Antigens include *P. gingivalis* outer membrane protein (OMP).

[104] *Klebsiella*: Antigens include an OMP, including OMP A, or a polysaccharide optionally conjugated to tetanus toxoid.

[105] Further bacterial antigens of the invention may be capsular antigens, polysaccharide antigens or protein antigens of any of the above. Further bacterial antigens may also include an outer membrane vesicle (OMV) preparation. Additionally, antigens include live, attenuated, and/or purified versions of any of the aforementioned bacteria. The antigens of the present invention may be derived from gram-negative or gram-positive bacteria. The antigens of the present invention may be derived from aerobic or anaerobic bacteria.

[106] Additionally, any of the above bacterial-derived saccharides (polysaccharides, LPS, LOS or oligosaccharides) can be conjugated to another agent or antigen, such as a carrier protein (for example CRM197). Such conjugation may be direct conjugation effected by reductive amination of carbonyl moieties on the saccharide to amino groups on the protein, as provided in US Patent No. 5,360,897 and Can J Biochem Cell Biol. 1984 May;62(5):270-5. Alternatively, the saccharides can be conjugated through a linker, such as, with succinamide or other linkages provided in Bioconjugate Techniques, 1996 and CRC, Chemistry of Protein Conjugation and Cross-Linking, 1993.

B. Viral Antigens

[107] Viral antigens suitable for use in the invention include inactivated (or killed) virus, attenuated virus, split virus formulations, purified subunit formulations, viral proteins which may be isolated, purified or derived from a virus, and Virus Like Particles (VLPs). Viral antigens may be derived from viruses propagated on cell culture or other substrate. Alternatively, viral antigens may be expressed recombinantly. Viral antigens preferably include epitopes which are exposed on the surface of the virus during at least one stage of its life cycle. Viral antigens are preferably conserved across multiple serotypes or isolates. Viral antigens include antigens derived from one or more of the viruses set forth below as well as the specific antigens examples identified below.

[108] *Orthomyxovirus*: Viral antigens may be derived from an *Orthomyxovirus*, such as Influenza A, B and C. *Orthomyxovirus* antigens may be selected from one or more of the viral proteins, including hemagglutinin (HA), neuraminidase (NA), nucleoprotein (NP), matrix protein (M1), membrane protein (M2), one or more of the transcriptase components (PB1, PB2 and PA). Preferred antigens include HA and NA.

[109] Influenza antigens may be derived from interpandemic (annual) flu strains. Alternatively influenza antigens may be derived from strains with the potential to cause pandemic a pandemic outbreak (*i.e.*, influenza strains with new haemagglutinin compared to the haemagglutinin in currently circulating strains, or influenza strains which are pathogenic in avian subjects and have the potential to be transmitted horizontally in the human population, or influenza strains which are pathogenic to humans).

[110] Paramyxoviridae viruses: Viral antigens may be derived from Paramyxoviridae viruses, such as Pneumoviruses (RSV), Paramyxoviruses (PIV) and Morbilliviruses (Measles).

[111] Pneumovirus: Viral antigens may be derived from a Pneumovirus, such as Respiratory syncytial virus (RSV), Bovine respiratory syncytial virus, Pneumonia virus of mice, and Turkey rhinotracheitis virus. Preferably, the Pneumovirus is RSV. Pneumovirus

antigens may be selected from one or more of the following proteins, including surface proteins Fusion (F), Glycoprotein (G) and Small Hydrophobic protein (SH), matrix proteins M and M2, nucleocapsid proteins N, P and L and nonstructural proteins NS1 and NS2. Preferred Pneumovirus antigens include F, G and M. See *e.g.*, J Gen Virol. 2004 Nov; 85(Pt 11):3229). Pneumovirus antigens may also be formulated in or derived from chimeric viruses. For example, chimeric RSV/PIV viruses may comprise components of both RSV and PIV.

[112] Paramyxovirus: Viral antigens may be derived from a Paramyxovirus, such as Parainfluenza virus types 1 – 4 (PIV), Mumps, Sendai viruses, Simian virus 5, Bovine parainfluenza virus and Newcastle disease virus. Preferably, the Paramyxovirus is PIV or Mumps. Paramyxovirus antigens may be selected from one or more of the following proteins: Hemagglutinin –Neuraminidase (HN), Fusion proteins F1 and F2, Nucleoprotein (NP), Phosphoprotein (P), Large protein (L), and Matrix protein (M). Preferred Paramyxovirus proteins include HN, F1 and F2. Paramyxovirus antigens may also be formulated in or derived from chimeric viruses. For example, chimeric RSV/PIV viruses may comprise components of both RSV and PIV. Commercially available mumps vaccines include live attenuated mumps virus, in either a monovalent form or in combination with measles and rubella vaccines (MMR).

[113] Morbillivirus: Viral antigens may be derived from a Morbillivirus, such as Measles. Morbillivirus antigens may be selected from one or more of the following proteins: hemagglutinin (H), Glycoprotein (G), Fusion factor (F), Large protein (L), Nucleoprotein (NP), Polymerase phosphoprotein (P), and Matrix (M). Commercially available measles vaccines include live attenuated measles virus, typically in combination with mumps and rubella (MMR).

[114] Picornavirus: Viral antigens may be derived from Picornaviruses, such as Enteroviruses, Rhinoviruses, Heparnavirus, Cardioviruses and Aphthoviruses. Antigens derived from Enteroviruses, such as Poliovirus are preferred.

[115] Enterovirus: Viral antigens may be derived from an Enterovirus, such as Poliovirus types 1, 2 or 3, Coxsackie A virus types 1 to 22 and 24, Coxsackie B virus types 1 to 6, Echovirus (ECHO) virus) types 1 to 9, 11 to 27 and 29 to 34 and Enterovirus 68 to 71. Preferably, the Enterovirus is poliovirus. Enterovirus antigens are preferably selected from one or more of the following Capsid proteins VP1, VP2, VP3 and VP4. Commercially available polio vaccines include Inactivated Polio Vaccine (IPV) and Oral poliovirus vaccine (OPV).

[116] Heparnavirus: Viral antigens may be derived from an Heparnavirus, such as Hepatitis A virus (HAV). Commercially available HAV vaccines include inactivated HAV vaccine.

[117] Togavirus: Viral antigens may be derived from a Togavirus, such as a Rubivirus, an Alphavirus, or an Arterivirus. Antigens derived from Rubivirus, such as Rubella virus, are preferred. Togavirus antigens may be selected from E1, E2, E3, C, NSP-1, NSPO-2, NSP-3 or NSP-4. Togavirus antigens are preferably selected from E1, E2 or E3. Commercially available Rubella vaccines include a live cold-adapted virus, typically in combination with mumps and measles vaccines (MMR).

[118] Flavivirus: Viral antigens may be derived from a Flavivirus, such as Tick-borne encephalitis (TBE), Dengue (types 1, 2, 3 or 4), Yellow Fever, Japanese encephalitis, West Nile encephalitis, St. Louis encephalitis, Russian spring-summer encephalitis, Powassan encephalitis. Flavivirus antigens may be selected from PrM, M, C, E, NS-1, NS-2a, NS2b, NS3, NS4a, NS4b, and NS5. Flavivirus antigens are preferably selected from PrM, M and E. Commercially available TBE vaccine include inactivated virus vaccines.

[119] Pestivirus: Viral antigens may be derived from a Pestivirus, such as Bovine viral diarrhea (BVDV), Classical swine fever (CSFV) or Border disease (BDV).

[120] Hepadnavirus: Viral antigens may be derived from a Hepadnavirus, such as Hepatitis B virus. Hepadnavirus antigens may be selected from surface antigens (L, M and S), core

antigens (HBc, HBe). Commercially available HBV vaccines include subunit vaccines comprising the surface antigen S protein.

[121] Hepatitis C virus: Viral antigens may be derived from a Hepatitis C virus (HCV). HCV antigens may be selected from one or more of E1, E2, E1/E2, NS345 polyprotein, NS 345-core polyprotein, core, and/or peptides from the nonstructural regions (Houghton *et al.*, Hepatology (1991) 14:381).

[122] Rhabdovirus: Viral antigens may be derived from a Rhabdovirus, such as a Lyssavirus (Rabies virus) and Vesiculovirus (VSV). Rhabdovirus antigens may be selected from glycoprotein (G), nucleoprotein (N), large protein (L), nonstructural proteins (NS). Commercially available Rabies virus vaccine comprise killed virus grown on human diploid cells or fetal rhesus lung cells.

[123] Caliciviridae; Viral antigens may be derived from Calciviridae, such as Norwalk virus, and Norwalk-like Viruses, such as Hawaii Virus and Snow Mountain Virus.

[124] Coronavirus: Viral antigens may be derived from a Coronavirus, SARS, Human respiratory Coronavirus, Avian infectious bronchitis (IBV), Mouse hepatitis virus (MHV), and Porcine transmissible gastroenteritis virus (TGEV). Coronavirus antigens may be selected from spike (S), envelope (E), matrix (M), nucleocapsid (N), and Hemagglutinin-esterase glycoprotein (HE). Preferably, the Coronavirus antigen is derived from a SARS virus. SARS viral antigens are described in WO 04/92360;

[125] Retrovirus: Viral antigens may be derived from a Retrovirus, such as an Oncovirus, a Lentivirus or a Spumavirus. Oncovirus antigens may be derived from HTLV-1, HTLV-2 or HTLV-5. Lentivirus antigens may be derived from HIV-1 or HIV-2. Retrovirus antigens may be selected from gag, pol, env, tax, tat, rex, rev, nef, vif, vpu, and vpr. HIV antigens may be selected from gag (p24gag and p55gag), env (gp160 and gp41), pol, tat, nef, rev vpu, miniproteins, (preferably p55 gag and gp140v delete). HIV antigens may be

derived from one or more of the following strains: HIVIIIb, HIVSF2, HIVLAV, HIVLAI, HIVMN, HIV-1CM235, HIV-1US4.

[126] Reovirus: Viral antigens may be derived from a Reovirus, such as an Orthoreovirus, a Rotavirus, an Orbivirus, or a Coltivirus. Reovirus antigens may be selected from structural proteins λ1, λ2, λ3, μ1, μ2, σ1, σ2, or σ3, or nonstructural proteins σNS, μNS, or σ1s. Preferred Reovirus antigens may be derived from a Rotavirus. Rotavirus antigens may be selected from VP1, VP2, VP3, VP4 (or the cleaved product VP5 and VP8), NSP 1, VP6, NSP3, NSP2, VP7, NSP4, or NSP5. Preferred Rotavirus antigens include VP4 (or the cleaved product VP5 and VP8), and VP7.

[127] Parvovirus: Viral antigens may be derived from a Parvovirus, such as Parvovirus B19. Parvovirus antigens may be selected from VP-1, VP-2, VP-3, NS-1 and NS-2. Preferably, the Parvovirus antigen is capsid protein VP-2.

[128] Delta hepatitis virus (HDV): Viral antigens may be derived HDV, particularly δ-antigen from HDV (see, *e.g.*, U.S. Patent No. 5,378,814).

[129] Hepatitis E virus (HEV): Viral antigens may be derived from HEV.

[130] Hepatitis G virus (HGV): Viral antigens may be derived from HGV.

[131] Human Herpesvirus: Viral antigens may be derived from a Human Herpesvirus, such as Herpes Simplex Viruses (HSV), Varicella-zoster virus (VZV), Epstein-Barr virus (EBV), Cytomegalovirus (CMV), Human Herpesvirus 6 (HHV6), Human Herpesvirus 7 (HHV7), and Human Herpesvirus 8 (HHV8). Human Herpesvirus antigens may be selected from immediate early proteins (α), early proteins (β), and late proteins (γ). HSV antigens may be derived from HSV-1 or HSV-2 strains. HSV antigens may be selected from glycoproteins gB, gC, gD and gH, fusion protein (gB), or immune escape proteins (gC, gE, or gI). VZV antigens may be selected from core, nucleocapsid, tegument, or envelope proteins. A live attenuated VZV vaccine is commercially available. EBV antigens may be selected from early antigen (EA) proteins, viral capsid antigen (VCA),

and glycoproteins of the membrane antigen (MA). CMV antigens may be selected from capsid proteins, envelope glycoproteins (such as gB and gH), and tegument proteins

[132] Papovaviruses: Antigens may be derived from Papovaviruses, such as Papillomaviruses and Polyomaviruses. Papillomaviruses include HPV serotypes 1, 2, 4, 5, 6, 8, 11, 13, 16, 18, 31, 33, 35, 39, 41, 42, 47, 51, 57, 58, 63 and 65. Preferably, HPV antigens are derived from serotypes 6, 11, 16 or 18. HPV antigens may be selected from capsid proteins (L1) and (L2), or E1 – E7, or fusions thereof. HPV antigens are preferably formulated into virus-like particles (VLPs). Polyomyavirus viruses include BK virus and JK virus. Polyomavirus antigens may be selected from VP1, VP2 or VP3.

[133] Further provided are antigens, compositions, methods, and microbes included in Vaccines, 4th Edition (Plotkin and Orenstein ed. 2004); Medical Microbiology 4th Edition (Murray *et al.* ed. 2002); Virology, 3rd Edition (W.K. Joklik ed. 1988); Fundamental Virology, 2nd Edition (B.N. Fields and D.M. Knipe, eds. 1991), which are contemplated in conjunction with the compositions of the present invention.

C. Fungal Antigens

[134] Fungal antigens for use in the invention may be derived from one or more of the fungi set forth below.

[135] Fungal antigens may be derived from Dermatophytres, including: Epidermophyton floccusum, Microsporum audouini, Microsporum canis, Microsporum distortum, Microsporum equinum, Microsporum gypsum, Microsporum nanum, Trichophyton concentricum, Trichophyton equinum, Trichophyton gallinae, Trichophyton gypseum, Trichophyton megnini, Trichophyton mentagrophytes, Trichophyton quinckeanum, Trichophyton rubrum, Trichophyton schoenleini, Trichophyton tonsurans, Trichophyton verrucosum, T. verrucosum var. album, var. discoides, var. ochraceum, Trichophyton violaceum, and/or Trichophyton faviforme.

[136] Fungal pathogens may be derived from Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger, Aspergillus nidulans, Aspergillus terreus, Aspergillus sydowi, Aspergillus flavatus, Aspergillus glaucus, Blastoschizomyces capitatus, Candida albicans, Candida enolase, Candida tropicalis, Candida glabrata, Candida krusei, Candida parapsilosis, Candida stellatoidea, Candida kusei, Candida parakwsei, Candida lusitaniae, Candida pseudotropicalis, Candida guilliermondi, Cladosporium carrionii, Coccidioides immitis, Blastomyces dermatidis, Cryptococcus neoformans, Geotrichum clavatum, Histoplasma capsulatum, Klebsiella pneumoniae, Paracoccidioides brasiliensis, Pneumocystis carinii, Pythiumn insidiosum, Pityrosporum ovale, Sacharomyces cerevisae, Saccharomyces boulardii, Saccharomyces pombe, Scedosporium apiosperum, Sporothrix schenckii, Trichosporon beigelii, Toxoplasma gondii, Penicillium marneffei, Malassezia spp., Fonsecaea spp., Wangiella spp., Sporothrix spp., Basidiobolus spp., Conidiobolus spp., Rhizopus spp, Mucor spp, Absidia spp, Mortierella spp, Cunninghamella spp, Saksenaea spp., Alternaria spp, Curvularia spp, Helminthosporium spp, Fusarium spp, Aspergillus spp, Penicillium spp, Monolinia spp, Rhizoctonia spp, Paecilomyces spp, Pithomyces spp, and Cladosporium spp.

[137] Processes for producing a fungal antigens are well known in the art (see US Patent No. 6,333,164). In a preferred method a solubilized fraction extracted and separated from an insoluble fraction obtainable from fungal cells of which cell wall has been substantially removed or at least partially removed, characterized in that the process comprises the steps of: obtaining living fungal cells; obtaining fungal cells of which cell wall has been substantially removed or at least partially removed; bursting the fungal cells of which cell wall has been substantially removed or at least partially removed; obtaining an insoluble fraction; and extracting and separating a solubilized fraction from the insoluble fraction.

D. STD Antigens

[138] The compositions of the invention may include one or more antigens derived from a sexually transmitted disease (STD). Such antigens may provide for prophylactis or

therapy for STD's such as chlamydia, genital herpes, hepatits (such as HCV), genital warts, gonorrhoea, syphilis and/or chancroid (See, WO00/15255). Antigens may be derived from one or more viral or bacterial STD's. Viral STD antigens for use in the invention may be derived from, for example, HIV, herpes simplex virus (HSV-1 and HSV-2), human papillomavirus (HPV), and hepatitis (HCV). Bacterial STD antigens for use in the invention may be derived from, for example, Neiserria gonorrhoeae, Chlamydia trachomatis, Treponema pallidum, Haemophilus ducreyi, E. coli, and Streptococcus agalactiae. Examples of specific antigens derived from these pathogens are described above.

E. Respiratory Antigens

[139] The compositions of the invention may include one or more antigens derived from a pathogen which causes respiratory disease. For example, respiratory antigens may be derived from a respiratory virus such as Orthomyxoviruses (influenza), Pneumovirus (RSV), Paramyxovirus (PIV), Morbillivirus (measles), Togavirus (Rubella), VZV, and Coronavirus (SARS). Respiratory antigens may be derived from a bacteria which causes respiratory disease, such as Streptococcus pneumoniae, Pseudomonas aeruginosa, Bordetella pertussis, Mycobacterium tuberculosis, Mycoplasma pneumoniae, Chlamydia pneumoniae, Bacillus anthracis, and Moraxella catarrhalis. Examples of specific antigens derived from these pathogens are described above.

F. Pediatric Vaccine Antigens

[140] The compositions of the invention may include one or more antigens suitable for use in pediatric subjects. Pediatric subjects are typically less than about 3 years old, or less than about 2 years old, or less than about 1 years old. Pediatric antigens may be administered multiple times over the course of 6 months, 1, 2 or 3 years. Pediatric antigens may be derived from a virus which may target pediatric populations and/or a virus from which pediatric populations are susceptible to infection. Pediatric viral antigens include antigens derived from one or more of Orthomyxovirus (influenza), Pneumovirus (RSV),

Paramyxovirus (PIV and Mumps), Morbillivirus (measles), Togavirus (Rubella), Enterovirus (polio), HBV, Coronavirus (SARS), and Varicella-zoster virus (VZV), Epstein Barr virus (EBV). Pediatric bacterial antigens include antigens derived from one or more of Streptococcus pneumoniae, Neisseria meningitides, Streptococcus pyogenes (Group A Streptococcus), Moraxella catarrhalis, Bordetella pertussis, Staphylococcus aureus, Clostridium tetani (Tetanus), Cornynebacterium diphtheriae (Diphtheria), Haemophilus influenzae B (Hib), Pseudomonas aeruginosa, Streptococcus agalactiae (Group B Streptococcus), and E. coli. Examples of specific antigens derived from these pathogens are described above.

G. Antigens suitable for use in Elderly or Immunocompromised Individuals

[141] The compositions of the invention may include one or more antigens suitable for use in elderly or immunocompromised individuals. Such individuals may need to be vaccinated more frequently, with higher doses or with adjuvanted formulations to improve their immune response to the targeted antigens. Antigens which may be targeted for use in Elderly or Immunocompromised individuals include antigens derived from one or more of the following pathogens: Neisseria meningitides, Streptococcus pneumoniae, Streptococcus pyogenes (Group A Streptococcus), Moraxella catarrhalis, Bordetella pertussis, Staphylococcus aureus, Staphylococcus epidermis, Clostridium tetani (Tetanus), Cornynebacterium diphtheriae (Diphtheria), Haemophilus influenzae B (Hib), Pseudomonas aeruginosa, Legionella pneumophila, Streptococcus agalactiae (Group B Streptococcus), Enterococcus faecalis, Helicobacter pylori, Clamydia pneumoniae, Orthomyxovirus (influenza), Pneumovirus (RSV), Paramyxovirus (PIV and Mumps), Morbillivirus (measles), Togavirus (Rubella), Enterovirus (polio), HBV, Coronavirus (SARS), Varicella-zoster virus (VZV), Epstein Barr virus (EBV), Cytomegalovirus (CMV). Examples of specific antigens derived from these pathogens are described above.

H. Antigens suitable for use in Adolescent Vaccines

[142] The compositions of the invention may include one or more antigens suitable for use in adolescent subjects. Adolescents may be in need of a boost of a previously administered pediatric antigen. Pediatric antigens which may be suitable for use in adolescents are described above. In addition, adolescents may be targeted to receive antigens derived from an STD pathogen in order to ensure protective or therapeutic immunity before the beginning of sexual activity. STD antigens which may be suitable for use in adolescents are described above.

I. Antigen Formulations

[143] In other aspects of the invention, methods of producing microparticles having adsorbed antigens are provided. The methods comprise: (a) providing an emulsion by dispersing a mixture comprising (i) water, (ii) a detergent, (iii) an organic solvent, and (iv) a biodegradable polymer selected from the group consisting of a poly(α-hydroxy acid), a polyhydroxy butyric acid, a polycaprolactone, a polyorthoester, a polyanhydride, and a polycyanoacrylate. The polymer is typically present in the mixture at a concentration of about 1% to about 30% relative to the organic solvent, while the detergent is typically present in the mixture at a weight-to-weight detergent-to-polymer ratio of from about 0.00001:1 to about 0.1:1 (more typically about 0.0001:1 to about 0.1:1, about 0.001:1 to about 0.1:1, or about 0.005:1 to about 0.1:1); (b) removing the organic solvent from the emulsion; and (c) adsorbing an antigen on the surface of the microparticles. In certain embodiments, the biodegradable polymer is present at a concentration of about 3% to about 10% relative to the organic solvent.

[144] Microparticles for use herein will be formed from materials that are sterilizable, non-toxic and biodegradable. Such materials include, without limitation, poly(α-hydroxy acid), polyhydroxybutyric acid, polycaprolactone, polyorthoester, polyanhydride, PACA, and polycyanoacrylate. Preferably, microparticles for use with the present invention are derived from a poly(α-hydroxy acid), in particular, from a poly(lactide) ("PLA") or a

copolymer of D,L-lactide and glycolide or glycolic acid, such as a poly(D,L-lactide-co-glycolide) ("PLG" or "PLGA"), or a copolymer of D,L-lactide and caprolactone. The microparticles may be derived from any of various polymeric starting materials which have a variety of molecular weights and, in the case of the copolymers such as PLG, a variety of lactide:glycolide ratios, the selection of which will be largely a matter of choice, depending in part on the coadministered macromolecule. These parameters are discussed more fully below.

[145] Further antigens may also include an outer membrane vesicle (OMV) preparation.

[146] Additional formulation methods and antigens (especially tumor antigens) are provided in U.S. Patent Serial No. 09/581,772.

J. Antigen References

[147] The following references include antigens useful in conjunction with the compositions of the present invention:

1 International patent application WO99/24578
2 International patent application WO99/36544.
3 International patent application WO99/57280.
4 International patent application WO00/22430.
5 Tettelin *et al.* (2000) Science 287:1809-1815.
6 International patent application WO96/29412.
7 Pizza *et al.* (2000) Science 287:1816-1820.
8 PCT WO 01/52885.
9 Bjune *et al.* (1991) Lancet 338(8775).
10 Fuskasawa *et al.* (1999) Vaccine 17:2951-2958.
11 Rosenqist *et al.* (1998) Dev. Biol. Strand 92:323-333.
12 Constantino *et al.* (1992) Vaccine 10:691-698.
13 Constantino *et al.* (1999) Vaccine 17:1251-1263.
14 Watson (2000) Pediatr Infect Dis J 19:331-332.
15 Rubin (20000) Pediatr Clin North Am 47:269-285,v.
16 Jedrzejas (2001) Microbiol Mol Biol Rev 65:187-207.
17 International patent application filed on 3rd July 2001 claiming priority from GB-0016363.4;WO 02/02606; PCT IB/01/00166.
18 Kalman *et al.* (1999) Nature Genetics 21:385-389.
19 Read *et al.* (2000) Nucleic Acids Res 28:1397-406.

Shirai *et al.* (2000) J. Infect. Dis 181(Suppl 3):S524-S527.
International patent application WO99/27105.
International patent application WO00/27994.
International patent application WO00/37494.
International patent application WO99/28475.
Bell (2000) Pediatr Infect Dis J 19:1187-1188.
Iwarson (1995) APMIS 103:321-326.
Gerlich *et al.* (1990) Vaccine 8 Suppl:S63-68 & 79-80.
Hsu *et al.* (1999) Clin Liver Dis 3:901-915.
Gastofsson *et al.* (1996) N. Engl. J. Med. 334-:349-355.
Rappuoli *et al.* (1991) TIBTECH 9:232-238.
Vaccines (1988) eds. Plotkin & Mortimer. ISBN 0-7216-1946-0.
Del Guidice *et al.* (1998) Molecular Aspects of Medicine 19:1-70.
International patent application WO93/018150.
International patent application WO99/53310.
International patent application WO98/04702.
Ross *et al.* (2001) Vaccine 19:135-142.
Sutter *et al.* (2000) Pediatr Clin North Am 47:287-308.
Zimmerman & Spann (1999) Am Fan Physician 59:113-118, 125-126.
Dreensen (1997) Vaccine 15 Suppl"S2-6.
MMWR Morb Mortal Wkly rep 1998 Jan 16:47(1):12, 9.
McMichael (2000) Vaccine19 Suppl 1:S101-107.
Schuchat (1999) Lancer 353(9146):51-6.
GB patent applications 0026333.5, 0028727.6 & 0105640.7.
Dale (1999) Infect Disclin North Am 13:227-43, viii.
Ferretti *et al.* (2001) PNAS USA 98: 4658-4663.
Kuroda *et al.* (2001) Lancet 357(9264):1225-1240; see also pages 1218-1219.
Ramsay *et al.* (2001) Lancet 357(9251):195-196.
Lindberg (1999) Vaccine 17 Suppl 2:S28-36.
Buttery & Moxon (2000) J R Coil Physicians Long 34:163-168.
Ahmad & Chapnick (1999) Infect Dis Clin North Am 13:113-133, vii.
Goldblatt (1998) J. Med. Microbiol. 47:663-567.
European patent 0 477 508.
U.S. Patent No. 5,306,492.
International patent application WO98/42721.
Conjugate Vaccines (eds. Cruse *et al.*) ISBN 3805549326, particularly vol. 10:48-114.
Hermanson (1996) Bioconjugate Techniques ISBN: 012323368 & 012342335X.
European patent application 0372501.
European patent application 0378881.
European patent application 0427347.
International patent application WO93/17712.
International patent application WO98/58668.
European patent application 0471177.

63 International patent application WO00/56360.
64 International patent application WO00/67161.

[148] Where a saccharide or carbohydrate antigen is used, it is preferably conjugated to a carrier protein in order to enhance immunogenicity. See Ramsay et al. (2001) Lancet 357(9251):195-196; Lindberg (1999) Vaccine 17 Suppl 2:S28-36; Buttery & Moxon (2000) J R Coll Physicians Lond 34:163-168; Ahmad & Chapnick (1999) Infect Dis Clin North Am 13:113-133, vii; Goldblatt (1998) J. Med. Microbiol. 47:563-567; European patent 0 477 508; US Patent No. 5,306,492; WO98/42721; Conjugate Vaccines (eds. Cruse et al.) ISBN 3805549326, particularly vol. 10:48-114; Hermanson (1996) Bioconjugate Techniques ISBN: 0123423368 or 012342335X.

[149] Preferred carrier proteins are bacterial toxins or toxoids, such as diphtheria or tetanus toxoids. The CRMl 97 diphtheria toxoid is particularly preferred.

[150] Other carrier polypeptides include the N. meningitidis outer membrane protein (EP-A- 0372501), synthetic peptides (EP-A-0378881 and EP-A 0427347), heat shock proteins (WO 93/17712 and WO 94/03208), pertussis proteins (WO 98/58668 and EP A 0471177), protein D from H. influenzae (WO 00/56360), cytokines (WO 91/01146), lymphokines, hormones, growth factors, toxin A or B from C. difficile (WO 00/61761), iron-uptake proteins (WO 01/72337), etc. Where a mixture comprises capsular saccharide from both serigraphs A and C, it may be preferred that the ratio (w/w) of MenA saccharide:MenC saccharide is greater than 1 (e.g., 2:1, 3:1, 4:1, 5:1, 10:1 or higher). Different saccharides can be conjugated to the same or different type of carrier protein. Any suitable conjugation reaction can be used, with any suitable linker where necessary.

[151] Toxic protein antigens may be detoxified where necessary e.g., detoxification of pertussis toxin by chemical and/or genetic means.

Pharmaceutically acceptable carriers

[152] Compositions of the invention will typically, in addition to the components mentioned above, comprise one or more "pharmaceutically acceptable carriers." These include any carrier which does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers typically are large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and lipid aggregates (such as oil droplets or liposomes). Such carriers are well known to those of ordinary skill in the art. A composition may also contain a diluent, such as water, saline, glycerol, etc. Additionally, an auxiliary substance, such as a wetting or emulsifying agent, pH buffering substance, and the like, may be present. A thorough discussion of pharmaceutically acceptable components is available in Gennaro (2000) Remington: The Science and Practice of Pharmacy. 20th ed., ISBN: 0683306472.

Immunoregulatory Agents

Adjuvants

[153] Vaccines of the invention may be administered in conjunction with other immunoregulatory agents. In particular, compositions will usually include an adjuvant. Adjuvants for use with the invention include, but are not limited to, one or more of the following set forth below:

A. Mineral Containing Compositions

[154] Mineral containing compositions suitable for use as adjuvants in the invention include mineral salts, such as aluminum salts and calcium salts. The invention includes mineral salts such as hydroxides (*e.g.* oxyhydroxides), phosphates (*e.g.* hydroxyphosphates, orthophosphates), sulfates, etc. (*e.g.* see chapters 8 & 9 of Vaccine Design... (1995) eds. Powell & Newman. ISBN: 030644867X. Plenum.), or mixtures of different mineral compounds (*e.g.* a mixture of a phosphate and a hydroxide adjuvant, optionally with an

excess of the phosphate), with the compounds taking any suitable form (*e.g.* gel, crystalline, amorphous, etc.), and with adsorption to the salt(s) being preferred. The mineral containing compositions may also be formulated as a particle of metal salt (WO00/23105).

[155] Aluminum salts may be included in vaccines of the invention such that the dose of Al^{3+} is between 0.2 and 1.0 mg per dose.

[156] In one embodiment the aluminum based adjuvant for use in the present invention is alum (aluminum potassium sulfate ($AlK(SO_4)_2$)), or an alum derivative, such as that formed in-situ by mixing an antigen in phosphate buffer with alum, followed by titration and precipitation with a base such as ammonium hydroxide or sodium hydroxide.

[157] Another aluminum-based adjuvant for use in vaccine formulations of the present invention is aluminum hydroxide adjuvant ($Al(OH)_3$) or crystalline aluminum oxyhydroxide (AlOOH), which is an excellent adsorbant, having a surface area of approximately $500m^2/g$. Alternatively, aluminum phosphate adjuvant ($AlPO_4$) or aluminum hydroxyphosphate, which contains phosphate groups in place of some or all of the hydroxyl groups of aluminum hydroxide adjuvant is provided. Preferred aluminum phosphate adjuvants provided herein are amorphous and soluble in acidic, basic and neutral media.

[158] In another embodiment the adjuvant of the invention comprises both aluminum phosphate and aluminum hydroxide. In a more particular embodiment thereof, the adjuvant has a greater amount of aluminum phosphate than aluminum hydroxide, such as a ratio of 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1 or greater than 9:1, by weight aluminum phosphate to aluminum hydroxide. More particular still, aluminum salts in the vaccine are present at 0.4 to 1.0 mg per vaccine dose, or 0.4 to 0.8 mg per vaccine dose, or 0.5 to 0.7 mg per vaccine dose, or about 0.6 mg per vaccine dose.

[159] Generally, the preferred aluminum-based adjuvant(s), or ratio of multiple aluminum-based adjuvants, such as aluminum phosphate to aluminum hydroxide is selected by optimization of electrostatic attraction between molecules such that the antigen carries an opposite charge as the adjuvant at the desired pH. For example, aluminum phosphate adjuvant (isoelectric point = 4) adsorbs lysozyme, but not albumin at pH 7.4. Should albumin be the target, aluminum hydroxide adjuvant would be selected (iep 11.4). Alternatively, pretreatment of aluminum hydroxide with phosphate lowers its isoelectric point, making it a preferred adjuvant for more basic antigens.

B. Oil-Emulsions

[160] Oil-emulsion compositions suitable for use as adjuvants in the invention include squalene-water emulsions, such as MF59 (5% Squalene, 0.5% TWEEN™ 80, and 0.5% Span 85, formulated into submicron particles using a microfluidizer). See WO90/14837. See also, Podda, Vaccine (2001) 19: 2673-2680; Frey *et al.*, Vaccine (2003) 21:4234-4237. MF59 is used as the adjuvant in the FLUAD™ influenza virus trivalent subunit vaccine.

[161] Particularly preferred adjuvants for use in the compositions are submicron oil-in-water emulsions. Preferred submicron oil-in-water emulsions for use herein are squalene/water emulsions optionally containing varying amounts of MTP-PE, such as a submicron oil-in-water emulsion containing 4-5% w/v squalene, 0.25-1.0% w/v TWEEN™ 80□ (polyoxyelthylenesorbitan monooleate), and/or 0.25-1.0% SPAN 85™ (sorbitan trioleate), and, optionally, N-acetylmuramyl-L-alanyl-D-isogluatminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-huydroxyphosphophoryloxy)-ethylamine (MTP-PE), for example, the submicron oil-in-water emulsion known as "MF59" (International Publication No. WO90/14837; US Patent Nos. 6,299,884 and 6,451,325, and Ott *et al.*, in Vaccine Design: The Subunit and Adjuvant Approach (Powell, M.F. and Newman, M.J. eds.) Plenum Press, New York, 1995, pp. 277-296). MF59 contains 4-5% w/v Squalene (*e.g.* 4.3%), 0.25-0.5% w/v TWEEN™ 80, and 0.5% w/v SPAN 85™ and optionally

contains various amounts of MTP-PE, formulated into submicron particles using a microfluidizer such as Model 110Y microfluidizer (Microfluidics, Newton, MA). For example, MTP-PE may be present in an amount of about 0-500 μg/dose, more preferably 0-250 μg/dose and most preferably, 0-100 μg/dose. As used herein, the term "MF59-0" refers to the above submicron oil-in-water emulsion lacking MTP-PE, while the term MF59-MTP denotes a formulation that contains MTP-PE. For instance, "MF59-100" contains 100 μg MTP-PE per dose, and so on. MF69, another submicron oil-in-water emulsion for use herein, contains 4.3% w/v squalene, 0.25% w/v TWEEN™ 80, and 0.75% w/v SPAN 85™ and optionally MTP-PE. Yet another submicron oil-in-water emulsion is MF75, also known as SAF, containing 10% squalene, 0.4% TWEEN™ 80, 5% pluronic-blocked polymer L121, and thr-MDP, also microfluidized into a submicron emulsion. MF75-MTP denotes an MF75 formulation that includes MTP, such as from 100-400 μg MTP-PE per dose.

[162] Submicron oil-in-water emulsions, methods of making the same and immunostimulating agents, such as muramyl peptides, for use in the compositions, are described in detail in WO90/14837 and U.S. Patents 6,299,884 and 6,45 1,325.

[163] Complete Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IFA) may also be used as adjuvants in the invention.

C. Saponin Formulations

[164] Saponin formulations, may also be used as adjuvants in the invention. Saponins are a heterologous group of sterol glycosides and triterpenoid glycosides that are found in the bark, leaves, stems, roots and even flowers of a wide range of plant species. Saponins isolated from the bark of the Quillaia saponaria Molina tree have been widely studied as adjuvants. Saponins can also be commercially obtained from Smilax ornata (sarsaprilla), Gypsophilla paniculata (brides veil), and Saponaria officianalis (soap root). Saponin adjuvant formulations include purified formulations, such as QS21, as well as lipid formulations, such as ISCOMs.

[165] Saponin compositions have been purified using High Performance Thin Layer Chromatography (HP-TLC) and Reversed Phase High Performance Liquid Chromatography (RP-HPLC). Specific purified fractions using these techniques have been identified, including QS7, QS17, QS18, QS21, QH-A, QH-B and QH-C. Preferably, the saponin is QS21. A method of production of QS21 is disclosed in U.S. Patent 5,057,540. Saponin formulations may also comprise a sterol, such as cholesterol (see WO96/33739).

[166] Combinations of saponins and cholesterols can be used to form unique particles called Immunostimulating Complexes (ISCOMs). ISCOMs typically also include a phospholipid such as phosphatidylethanolamine or phosphatidylcholine. Any known saponin can be used in ISCOMs. Preferably, the ISCOM includes one or more of Quil A, QHA and QHC. ISCOMs are further described in EP0109942, WO96/11711 and WO96/33739. Optionally, the ISCOMS may be devoid of (an) additional detergent(s). See WO00/07621.

[167] A review of the development of saponin based adjuvants can be found in Barr, *et al.*, Advanced Drug Delivery Reviews (1998) 32:247-271. *See also* Sjolander, *et al.*, Advanced Drug Delivery Reviews (1998) 32:321-338.

D. Virosomes and Virus Like Particles (VLPs)

[168] Virosomes and Virus Like Particles (VLPs) can also be used as adjuvants in the invention. These structures generally contain one or more proteins from a virus optionally combined or formulated with a phospholipid. They are generally non-pathogenic, non-replicating and generally do not contain any of the native viral genome. The viral proteins may be recombinantly produced or isolated from whole viruses. These viral proteins suitable for use in virosomes or VLPs include proteins derived from influenza virus (such as HA or NA), Hepatitis B virus (such as core or capsid proteins), Hepatitis E virus, measles virus, Sindbis virus, Rotavirus, Foot-and-Mouth Disease virus, Retrovirus, Norwalk virus, human Papilloma virus, HIV, RNA-phages, Qß-phage (such as coat

proteins), GA-phage, fr-phage, AP205 phage, and Ty (such as retrotransposon Ty protein p1). VLPs are discussed further in WO03/024480, WO03/024481, and Niikura *et al.*, Virology (2002) 293:273-280; Lenz *et al.*, Journal of Immunology (2001) 5246-5355; Pinto, *et al.*, Journal of Infectious Diseases (2003) 188:327-338; and Gerber *et al.*, Journal of Virology (2001) 75(10):4752-4760. Virosomes are discussed further in, for example, Gluck *et al.*, Vaccine (2002) 20:B10 –B16. Immunopotentiating reconstituted influenza virosomes (IRIV) are used as the subunit antigen delivery system in the intranasal trivalent INFLEXAL™ product {Mischler & Metcalfe (2002) Vaccine 20 Suppl 5:B17-23} and the INFLUVAC PLUS™ product.

E. Bacterial or Microbial Derivatives

[169] Adjuvants suitable for use in the invention include bacterial or microbial derivatives such as:

(1) Non-toxic derivatives of enterobacterial lipopolysaccharide (LPS)

[170] Such derivatives include Monophosphoryl lipid A (MPL) and 3-O-deacylated MPL (3dMPL). 3dMPL is a mixture of 3 De-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains. A preferred "small particle" form of 3 De-O-acylated monophosphoryl lipid A is disclosed in EP 0 689 454. Such "small particles" of 3dMPL are small enough to be sterile filtered through a 0.22 micron membrane (see EP 0 689 454). Other non-toxic LPS derivatives include monophosphoryl lipid A mimics, such as aminoalkyl glucosaminide phosphate derivatives *e.g.* RC 529. See Johnson *et al.* (1999) Bioorg Med Chem Lett 9:2273-2278.

(2) Lipid A Derivatives

[171] Lipid A derivatives include derivatives of lipid A from Escherichia coli such as OM-174. OM-174 is described for example in Meraldi *et al.*, Vaccine (2003) 21:2485-2491; and Pajak, *et al.*, Vaccine (2003) 21:836-842.

(3) Immunostimulatory oligonucleotides

[172] Immunostimulatory oligonucleotides suitable for use as adjuvants in the invention include nucleotide sequences containing a CpG motif (a sequence containing an unmethylated cytosine followed by guanosine and linked by a phosphate bond). Bacterial double stranded RNA or oligonucleotides containing palindromic or poly(dG) sequences have also been shown to be immunostimulatory.

[173] The CpGs can include nucleotide modifications/analogs such as phosphorothioate modifications and can be double-stranded or single-stranded. Optionally, the guanosine may be replaced with an analog such as 2'-deoxy-7-deazaguanosine. See Kandimalla, *et al.*, Nucleic Acids Research (2003) 31(9): 2393-2400; WO02/26757 and WO99/62923 for examples of possible analog substitutions. The adjuvant effect of CpG oligonucleotides is further discussed in Krieg, Nature Medicine (2003) 9(7): 831-835; McCluskie, *et al.*, FEMS Immunology and Medical Microbiology (2002) 32:179-185; WO98/40100; US Patent No. 6,207,646; US Patent No. 6,239,116 and US Patent No. 6,429,199.

[174] The CpG sequence may be directed to TLR9, such as the motif GTCGTT or TTCGTT. See Kandimalla, *et al.*, Biochemical Society Transactions (2003) 31 (part 3): 654-658. The CpG sequence may be specific for inducing a Th1 immune response, such as a CpG-A ODN, or it may be more specific for inducing a B cell response, such a CpG-B ODN. CpG-A and CpG-B ODNs are discussed in Blackwell, *et al.*, J. Immunol. (2003) 170(8):4061-4068; Krieg, TRENDS in Immunology (2002) 23(2): 64-65 and WO01/95935. Preferably, the CpG is a CpG-A ODN.

[175] Preferably, the CpG oligonucleotide is constructed so that the 5' end is accessible for receptor recognition. Optionally, two CpG oligonucleotide sequences may be attached at their 3' ends to form "immunomers". See, for example, Kandimalla, *et al.*, BBRC (2003) 306:948-953; Kandimalla, *et al.*, Biochemical Society Transactions (2003) 31(part 3):664-658; Bhagat *et al.*, BBRC (2003) 300:853-861 and WO03/035836.

(4) ADP-ribosylating toxins and detoxified derivatives thereof.

[176] Bacterial ADP-ribosylating toxins and detoxified derivatives thereof may be used as adjuvants in the invention. Preferably, the protein is derived from *E. coli* (*i.e.*, *E. coli* heat labile enterotoxin "LT), cholera ("CT"), or pertussis ("PT"). The use of detoxified ADP-ribosylating toxins as mucosal adjuvants is described in WO95/17211 and as parenteral adjuvants in WO98/42375. Preferably, the adjuvant is a detoxified LT mutant such as LT-K63, LT-R72, and LTR192G. The use of ADP-ribosylating toxins and detoxified derivatives thereof, particularly LT-K63 and LT-R72, as adjuvants can be found in the following references: Beignon, *et al.*, Infection and Immunity (2002) 70(6):3012-3019; Pizza, *et al.*, Vaccine (2001) 19:2534-2541; Pizza, *et al.*, Int. J. Med. Microbiol (2000) 290(4-5):455-461; Scharton-Kersten *et al.*, Infection and Immunity (2000) 68(9):5306-5313; Ryan *et al.*, Infection and Immunity (1999) 67(12):6270-6280; Partidos *et al.*, Immunol. Lett. (1999) 67(3):209-216; Peppoloni *et al.*, Vaccines (2003) 2(2):285-293; and Pine *et al.*, (2002) J. Control Release (2002) 85(1-3):263-270. Numerical reference for amino acid substitutions is preferably based on the alignments of the A and B subunits of ADP-ribosylating toxins set forth in Domenighini *et al.*, Mol. Microbiol (1995) 15(6):1165-1167.

F. Bioadhesives and Mucoadhesives

[177] Bioadhesives and mucoadhesives may also be used as adjuvants in the invention. Suitable bioadhesives include esterified hyaluronic acid microspheres (Singh *et al.* (2001) J. Cont. Rele. 70:267-276) or mucoadhesives such as cross-linked derivatives of polyacrylic acid, polyvinyl alcohol, polyvinyl pyrollidone, polysaccharides and carboxymethylcellulose. Chitosan and derivatives thereof may also be used as adjuvants in the invention. *See* WO99/27960.

G. Microparticles

[178] Microparticles may also be used as adjuvants in the invention. Microparticles (*i.e.* a particle of ~100nm to ~150μm in diameter, more preferably ~200nm to ~30μm in diameter, and most preferably ~500nm to ~10μm in diameter) formed from materials that are biodegradable and non toxic (*e.g.* a poly(α-hydroxy acid), a polyhydroxybutyric acid, a polyorthoester, a polyanhydride, a polycaprolactone, etc.), with poly(lactide co glycolide) are preferred, optionally treated to have a negatively-charged surface (*e.g.* with SDS) or a positively-charged surface (*e.g.* with a cationic detergent, such as CTAB).

H. Liposomes

[179] Examples of liposome formulations suitable for use as adjuvants are described in US Patent No. 6,090,406, US Patent No. 5,916,588, and EP 0 626 169.

I. Polyoxyethylene ether and Polyoxyethylene Ester Formulations

[180] Adjuvants suitable for use in the invention include polyoxyethylene ethers and polyoxyethylene esters. WO99/52549. Such formulations further include polyoxyethylene sorbitan ester surfactants in combination with an octoxynol (WO01/21207) as well as polyoxyethylene alkyl ethers or ester surfactants in combination with at least one additional non-ionic surfactant such as an octoxynol (WO01/21152).

[181] Preferred polyoxyethylene ethers are selected from the following group: polyoxyethylene-9-lauryl ether (laureth 9), polyoxyethylene-9-steoryl ether, polyoxytheylene-8-steoryl ether, polyoxyethylene-4-lauryl ether, polyoxyethylene-35-lauryl ether, and polyoxyethylene-23-lauryl ether.

J. Polyphosphazene (PCPP)

[182] PCPP formulations are described, for example, in Andrianov *et al.*, "Preparation of hydrogel microspheres by coacervation of aqueous polyphophazene solutions",

Biomaterials (1998) 19(1-3):109-115 and Payne *et al.*, "Protein Release from Polyphosphazene Matrices", Adv. Drug. Delivery Review (1998) 31(3):185-196.

K. Muramyl peptides

[183] Examples of muramyl peptides suitable for use as adjuvants in the invention include N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-l-alanyl-d-isoglutamine (nor-MDP), and N acetylmuramyl-l-alanyl-d-isoglutaminyl-l-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine MTP-PE).

L. Imidazoquinoline Compounds.

[184] Examples of imidazoquinoline compounds suitable for use adjuvants in the invention include Imiquimod and its analogues, described further in Stanley, Clin Exp Dermatol (2002) 27(7):571-577; Jones, Curr Opin Investig Drugs (2003) 4(2):214-218; and U.S. Patents 4,689,338, 5,389,640, 5,268,376, 4,929,624, 5,266,575, 5,352,784, 5,494,916, 5,482,936, 5,346,905, 5,395,937, 5,238,944, and 5,525,612.

M. Thiosemicarbazone Compounds.

[185] Examples of thiosemicarbazone compounds, as well as methods of formulating, manufacturing, and screening for compounds all suitable for use as adjuvants in the invention include those described in WO04/60308. The thiosemicarbazones are particularly effective in the stimulation of human peripheral blood mononuclear cells for the production of cytokines, such as TNF- α.

N. Tryptanthrin Compounds.

[186] Examples of tryptanthrin compounds, as well as methods of formulating, manufacturing, and screening for compounds all suitable for use as adjuvants in the invention include those described in WO04/64759. The tryptanthrin compounds are particularly effective in the stimulation of human peripheral blood mononuclear cells for the production of cytokines, such as TNF- α.

[187] The invention may also comprise combinations of aspects of one or more of the adjuvants identified above. For example, the following adjuvant compositions may be used in the invention:

(1) a saponin and an oil-in-water emulsion (WO99/11241);

(2) a saponin (*e.g.*, QS21) + a non-toxic LPS derivative (*e.g.* 3dMPL) (see WO94/00153);

(3) a saponin (*e.g.*, QS21) + a non-toxic LPS derivative (*e.g.* 3dMPL) + a cholesterol;

(4) a saponin (*e.g.*, QS21) + 3dMPL + IL 12 (optionally + a sterol) (WO98/57659);

(5) combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions (See European patent applications 0835318, 0735898 and 0761231);

(6) SAF, containing 10% Squalane, 0.4% Tween 80, 5% pluronic-block polymer L121, and thr-MDP, either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion.

(7) RIBI™ adjuvant system (RAS), (Ribi Immunochem) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (DETOX™); and

(8) one or more mineral salts (such as an aluminum salt) + a non-toxic derivative of LPS (such as 3dPML).

(9) one or more mineral salts (such as an aluminum salt) + an immunostimulatory oligonucleotide (such as a nucleotide sequence including a CpG motif).

O. Human Immunomodulators

[188] Human immunomodulators suitable for use as adjuvants in the invention include cytokines, such as interleukins (e.g. IL-l, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, etc.), interferons (e.g. interferon-γ), macrophage colony stimulating factor, and tumor necrosis factor.

[189] Aluminum salts and MF59 are preferred adjuvants for use with injectable influenza vaccines.

[190] Bacterial toxins and bioadhesives are preferred adjuvants for use with mucosally-delivered vaccines, such as nasal vaccines.

Therapeutic methods

[191] The invention provides the compositions described above for use in therapy. The invention provides the compositions described above for inducing or increasing an immune response to S. pyogenes. The invention provides methods for inducing or increasing an immune response to 6*. pyogenes using the compositions described above. The immune response is preferably protective and can include antibodies and/or cell- mediated immunity (including systemic and mucosal immunity). Immune responses include booster responses.

[192] Teenagers and children, including toddles and infants, can receive a vaccine for prophylactic use; therapeutic vaccines typically are administered to teenagers or adults. A vaccine intended for children may also be administered to adults e.g., to assess safety, dosage, immunogenicity, etc.

[193] Diseases caused by *Streptococcus pyogenes* which can be prevented or treated according to the invention include, but are not limited to, pharyngitis (such as streptococcal sore throat), scarlet fever, impetigo, erysipelas, cellulitis, septicemia, toxic shock syndrome, necrotizing fasciitis, and sequelae such as rheumatic fever and acute glomerulonephritis. The compositions may also be effective against other streptococcal bacteria, *e.g.*, GBS.

Tests to determine the efficacy of the immune response

[194] One way of assessing efficacy of therapeutic treatment involves monitoring GAS infection after administration of the composition of the invention. One way of assessing efficacy of prophylactic treatment involves monitoring immune responses against the mutant SLO proteins in the compositions of the invention after administration of the composition.

[195] Another way of assessing the immunogenicity of the component proteins of the immunogenic compositions of the present invention is to express mutant SLO proteins recombinantly and to screen patient sera or mucosal secretions by immunoblot. A positive reaction between the protein and the patient serum indicates that the patient has previously mounted an immune response to the protein in question; *i.e.*, the protein is an immunogen. This method may also be used to identify immunodominant proteins and/or epitopes.

[196] Another way of checking efficacy of therapeutic treatment involves monitoring GAS infection after administration of the compositions of the invention. One way of checking efficacy of prophylactic treatment involves monitoring immune responses both systemically (such as monitoring the level of IgG1 and IgG2a production) and mucosally (such as monitoring the level of IgA production) against SLO after administration of the composition. Typically, serum specific antibody responses are determined post-immunization but pre-challenge whereas mucosal specific antibody body responses are determined post-immunization and post-challenge.

[197] The vaccine compositions of the present invention can be evaluated in *in vitro* and *in vivo* animal models prior to host, *e.g.*, human, administration. Particularly useful mouse models include those in which intraperitoneal immunization is followed by either intraperitoneal challenge or intranasal challenge.

[198] The efficacy of immunogenic compositions of the invention can also be determined *in vivo* by immunizing animal models, (*e.g.*, guinea pigs or mice) with the immunogenic compositions and ascertaining the level of protection obtained after challenge with GAS.

[199] *In vivo* efficacy models include but are not limited to: (i) a murine infection model using human GAS serotypes; (ii) a murine disease model which is a murine model using a mouse-adapted GAS strain, such as the M23 strain which is particularly virulent in mice, and (iii) a primate model using human GAS isolates.

[200] The immune response may be one or both of a TH1 immune response and a TH2 response. The immune response may be an improved or an enhanced or an altered immune response. The immune response may be one or both of a systemic and a mucosal immune response. Preferably the immune response is an enhanced system and/or mucosal response.

[201] An enhanced systemic and/or mucosal immunity is reflected in an enhanced TH1 and/or TH2 immune response. Preferably, the enhanced immune response includes an increase in the production of IgG1 and/or IgG2a and/or IgA.

[202] Preferably the mucosal immune response is a TH2 immune response. Preferably, the mucosal immune response includes an increase in the production of IgA.

[203] Activated TH2 cells enhance antibody production and are therefore of value in responding to extracellular infections. Activated TH2 cells may secrete one or more of IL-4, IL-5, IL-6, and IL-10. A TH2 immune response may result in the production of IgG1, IgE, IgA and memory B cells for future protection.

[204] A TH2 immune response may include one or more of an increase in one or more of the cytokines associated with a TH2 immune response (such as IL-4, IL-5, IL-6 and IL-10), or an increase in the production of IgG1, IgE, IgA and memory B cells. Preferably, the enhanced TH2 immune response will include an increase in IgG1 production.

[205] A TH1 immune response may include one or more of an increase in CTLs, an increase in one or more of the cytokines associated with a TH1 immune response (such as IL-2, IFNγ, and TNFβ), an increase in activated macrophages, an increase in NK activity, or an increase in the production of IgG2a. Preferably, the enhanced TH1 immune response will include an increase in IgG2a production.

[206] Immunogenic compositions of the invention, in particular, immunogenic composition comprising one or more mutant SLO proteins of the present invention may be used either alone or in combination with other GAS antigens optionally with an immunoregulatory agent capable of eliciting a Th1 and/or Th2 response.

[207] The invention also comprises an immunogenic composition comprising one or more immunoregulatory agent, such as a mineral salt, such as an aluminium salt and an oligonucleotide containing a CpG motif. Most preferably, the immunogenic composition includes both an aluminium salt and an oligonucleotide containing a CpG motif. Alternatively, the immunogenic composition includes an ADP ribosylating toxin, such as a detoxified ADP ribosylating toxin and an oligonucleotide containing a CpG motif. Preferably, one or more of the immunoregulatory agents include an adjuvant. The adjuvant may be selected from one or more of the group consisting of a TH1 adjuvant and TH2 adjuvant.

[208] The compositions of the invention will preferably elicit both a cell mediated immune response as well as a humoral immune response in order to effectively address a GAS infection. This immune response will preferably induce long lasting (*e.g.*, neutralizing) antibodies and a cell mediated immunity that can quickly respond upon exposure to one or more GAS antigens.

[209] In one particularly preferred embodiment, the immunogenic composition comprises one or more mutant SLO protein(s) which elicit(s) a neutralizing antibody response and one or more mutant SLO protein(s) which elicit(s) a cell mediated immune response. In this way, the neutralizing antibody response prevents or inhibits an initial GAS infection while the cell-mediated immune response capable of eliciting an enhanced Th1 cellular response prevents further spreading of the GAS infection.

[210] Compositions of the invention will generally be administered directly to a patient. The compositions of the present invention may be administered, either alone or as part of a composition, via a variety of different routes. Certain routes may be favored for certain compositions, as resulting in the generation of a more effective immune response, preferably a CMI response, or as being less likely to induce side effects, or as being easier for administration.

[211] Delivery methods include parenteral injection (*e.g.*, subcutaneous, intraperitoneal, intravenous, intramuscular, or interstitial injection) and rectal, oral (*e.g.*, tablet, spray), vaginal, topical, transdermal (*e.g.*, see WO 99/27961), transcutaneous (*e.g.*, see WO02/074244 and WO02/064162), intranasal (*e.g.*, see WO03/028760), ocular, aural, and pulmonary or other mucosal administration.

[212] By way of example, the compositions of the present invention may be administered via a systemic route or a mucosal route or a transdermal route or it may be administered directly into a specific tissue. As used herein, the term "systemic administration" includes but is not limited to any parenteral routes of administration. In particular, parenteral administration includes but is not limited to subcutaneous, intraperitoneal, intravenous, intraarterial, intramuscular, or intrasternal injection, intravenous, intraarterial, or kidney dialytic infusion techniques. Preferably, the systemic, parenteral administration is intramuscular injection. As used herein, the term "mucosal administration" includes but is not limited to oral, intranasal, intravaginal, intrarectal, intratracheal, intestinal and ophthalmic administration.

[213] Dosage treatment can be a single dose schedule or a multiple dose schedule. Multiple doses may be used in a primary immunization schedule and/or in a booster immunization schedule. In a multiple dose schedule the various doses may be given by the same or different routes *e.g.*, a parenteral prime and mucosal boost, a mucosal prime and parenteral boost, etc.

[214] The compositions of the invention may be prepared in various forms. For example, a composition can be prepared as an injectable, either as a liquid solution or a suspension. Solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared (*e.g.*, a lyophilized composition). A composition can be prepared for oral administration, such as a tablet or capsule, as a spray, or as a syrup (optionally flavored). A composition can be prepared for pulmonary administration, *e.g.*, as an inhaler, using a fine powder or a spray. A composition can be prepared as a suppository or pessary. A composition can be prepared for nasal, aural or ocular administration *e.g.*, as drops. A composition can be in kit form, designed such that a combined composition is reconstituted just prior to administration to a patient. Such kits may comprise one or more mutant SLO or other antigens in liquid form and one or more lyophilized antigens.

[215] Immunogenic compositions used as vaccines comprise an immunologically effective amount of mutant SLO or other antigens (or nucleic acid molecules encoding the antigens), as well as any other components, as needed, such as antibiotics. An "immunologically effective amount" is an amount which, when administered to an individual, either in a single dose or as part of a series, increases a measurable immune response or prevents or reduces a clinical symptom.

[216] The immunogenic compositions of the present invention may be administered in combination with an antibiotic treatment regime. In one embodiment, the antibiotic is administered prior to administration of the antigen of the invention or the composition comprising the one or more mutant SLO proteins of the invention.

[217] In another embodiment, the antibiotic is administered subsequent to the administration of a mutant SLO protein of the invention. Examples of antibiotics suitable for use in the treatment of a GAS infection include but are not limited to penicillin or a derivative thereof or clindamycin, cephalosporins, glycopeptides (*e.g.*, vancomycin), and cycloserine.

[218] The amount of active agent in a composition varies, however, depending upon the health and physical condition of the individual to be treated, age, the taxonomic group of individual to be treated (*e.g.*, non-human primate, primate, etc.), the capacity of the individual's immune system to synthesize antibodies, the degree of protection desired, the formulation of the vaccine, the treating doctor's assessment of the medical situation, and other relevant factors. The amount will fall in a relatively broad range which can be determined through routine trials.

Kits

[219] The invention also provides kits comprising one or more containers of compositions of the invention. Compositions can be in liquid form or can be lyophilized, as can individual antigens. Suitable containers for the compositions include, for example, bottles, vials, syringes, and test tubes. Containers can be formed from a variety of materials, including glass or plastic. A container may have a sterile access port (for example, the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle).

[220] The kit can further comprise a second container comprising a pharmaceutically-acceptable buffer, such as phosphate-buffered saline, Ringer's solution, or dextrose solution. It can also contain other materials useful to the end-user, including other buffers, diluents, filters, needles, and syringes. The kit can also comprise a second or third container with another active agent, for example an antibiotic.

[221] The kit can also comprise a package insert containing written instructions for methods of inducing immunity against S. pyogenes or for treating 5. pyogenes infections. The package insert can be an unapproved draft package insert or can be a package insert approved by the Food and Drug Administration (FDA) or other regulatory body.

[222] The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples, which are provided for purposes of illustration only and are not intended to limit the scope of the invention.

EXAMPLE 1

Cloning of wild-type and mutant SLO proteins

[223] Genes encoding wild-type and mutant SLO proteins were amplified by PCR using the primers from the SF370 genome shown in Table 1.

[224] The PCR products were digested with Nhel-Xhol and ligated with pet24b+ (Novagen) vector cut with the same enzymes. E. coli DH5α electrocompetent cells were transformed with the ligation reactions. LBPTK medium was added and, after incubation for Ih at 37°C, with agitation at 250 rpm, bacteria were plated onto LBPTK plates containing 50 μg/ml kanamycin. Positive colonies were identified by colony PCR.

[225] Plasmids from positive colonies were prepared from an overnight culture in LBPTK medium containing 50 μg/ml kanamycin and analyzed by DNA sequencing, which confirmed the expected insert gene under the T7 polymerase promoter. The final DNA and protein sequences of the cloned genes are shown in the sequence listing. See Table 2.

Table 1.

gene	primers
SLO wild-type *tag-less*	25F NheI, GTGCGTGCTAGCGAATCGAACAAACAAAACACTGC (SEQ ID NO:35) 25rev = GCATTCGATCCTCGAGCTACTTATAAGTAATCGAACCATATG (SEQ ID NO:36)
SLO P427L *tag-less*	External primers: 25F NheI, GTGCGTGCTAGCGAATCGAACAAACAAAACACTGC (SEQ ID NO:35) 25rev, GCATTCGATCCTCGAGCTACTTATAAGTAATCGAACCATATG (SEQ ID NO:36) Internal primers: PL427_for, GCTACCTTCAGTAGAAAAAACCTAGCTTATCCTATTTCATACACC (SEQ ID NO:37) PL427_rev, GGTGTATGAAATAGGATAAGCTAGGTTTTTTCTACTGAAGGTAGC (SEQ ID NO:38)
SLO Wild Type *His-tagged*	25F NheI, GTGCGTGCTAGCGAATCGAACAAACAAAACACTGC (SEQ ID NO:35) 25revhis, GCATTCGATCCTCGAGCTTATAAGTAATCGAACCATATGGG (SEQ ID NO:39)
SLO W535F *His-tagged*	External primers: 25F NheI, GTGCGTGCTAGCGAATCGAACAAACAAAACACTGC (SEQ ID NO:35) 25revhis, GCATTCGATCCTCGAGCTTATAAGTAATCGAACCATATGGG (SEQ ID NO:39) Internal primers: WF535_for, GAGTGCACTGGCTTAGCTTTCGAATGGTGGCGAAAAGTGATC(SEQ ID NO:40) WF535_rev, GATCACTTTTCGCCACCATTCGAAAGCTAAGCCAGTGCACTC (SEQ ID NO:41)
SLO W535F-D482N *His-tagged*	External primers: 25F NheI, GTGCGTGCTAGCGAATCGAACAAACAAAACACTGC (SEQ ID NO:35) 25revhis, GCATTCGATCCTCGAGCTTATAAGTAATCGAACCATATGGG (SEQ ID NO:39) Internal primers: WF535_for, GAGTGCACTGGCTTAGCTTTCGAATGGTGGCGAAAAGTGATC (SEQ ID NO:40) WF535_rev, GATCACTTTTCGCCACCATTCGAAAGCTAAGCCAGTGCACTC (SEQ ID NO:41) and DN482_for, GTTGCTCAATATGAAATCCTTTGGAATGAAATCAATTATGATGACAAAGGAAAAG (SEQ ID NO:42) DN482_rev, CTTTTCCTTTGTCATCATAATTGATTTCATTCCAAAGGATTTCATATTGAGCAAC (SEQ ID NO:43)
SLO C530G *His-tagged*	External primers: 25F NheI, GTGCGTGCTAGCGAATCGAACAAACAAAACACTGC (SEQ ID NO:35) 25revhis, GCATTCGATCCTCGAGCTTATAAGTAATCGAACCATATGGG (SEQ ID NO:39) Internal primers: CG530_for, CCGTATCATGGCTAGAGAGGGCACTGGCTTAGCTTGGGAATG (SEQ ID NO:44) CG530_rev, CATTCCCAAGCTAAGCCAGTGCCCTCTCTAGCCATGATACGG (SEQ ID NO:45)

SLO P427L *His-tagged*	External primers: 25F NheI, GTGCGTGCTAGCGAATCGAACAAACAAAACACTGC (SEQ ID NO:35) 25 revhis, GCATTCGATCCTCGAGCTTATAAGTAATCGAACCATATGGG (SEQ ID NO:39) Internal primers: PL427_for, GCTACCTTCAGTAGAAAAAACCTAGCTTATCCTATTTCATACACC (SEQ ID NO:37) PL427_rev, GGTGTATGAAATAGGATAAGCTAGGTTTTTTCTACTGAAGGTAGC (SEQ ID NO:38)
SLO P427L-W535F-C535G *tag-less*	External primers: 25_F, GTGCGTGCTAGCGAATCGAACAAACAAAAC (SEQ ID NO:46) 25_stopR, GCGTCTCGAGTCACTTATAAGTAATCGAACCATA (SEQ ID NO:47) Internal primers: W-C_for, CCGTATCATGGCTAGAGAGGGCACTGGCTTAGCTTTCGAATG (SEQ ID NO:48) W-C_rev, CATTCGAAAGCTAAGCCAGTGCCCTCTCTAGCCATGATACGG (SEQ ID NO:49)
SLO P427L-W535F *tag-less*	External primers: 25_F, GTGCGTGCTAGCGAATCGAACAAACAAAAC (SEQ ID NO:46) 25_stopR, GCGTCTCGAGTCACTTATAAGTAATCGAACCATA (SEQ ID NO:47) Internal primers: WF535_for, GAGTGCACTGGCTTAGCTTTCGAATGGTGGCGAAAAGTGATC (SEQ ID NO:40) WF535_rev, GATCACTTTTCGCCACCATTCGAAAGCTAAGCCAGTGCACTC (SEQ ID NO:41)
SLO P427L-C530G *tag-less*	External primers: 25_F, GTGCGTGCTAGCGAATCGAACAAACAAAAC (SEQ ID NO:46) 25_stopR, GCGTCTCGAGTCACTTATAAGTAATCGAACCATA(SEQ ID NO:47) Internal primers: CG530_for, CCGTATCATGGCTAGAGAGGGCACTGGCTTAGCTTGGGAATG (SEQ ID NO:44) CG530_rev, CATTCCCAAGCTAAGCCAGTGCCCTCTCTAGCCATGATACGG (SEQ ID NO:45)
SLO ΔA248 *his-tagged*	External primers: 25F NheI, GTGCGTGCTAGCGAATCGAACAAACAAAACACTGC (SEQ ID NO:35) 25 revhis, GCATTCGATCCTCGAGCTTATAAGTAATCGAACCATATGGG (SEQ ID NO:39) Internal primers: Δ248for, CTGGTGGTAATACGCTTCCTAGAACACAATATACTGAATCAATGG (SEQ ID NO:50) Δ248rev, CCATTGATTCAGTATATTGTGTTCTAGGAAGCGTATTACCACCAG (SEQ ID NO:51)

[226] Table 2.

	sequence identifier			
SLO gene	amino acid		nucleotide	
	tag-less	*His-tagged*	*tag-less*	*His-tagged*
wild-type	1-12	13	28	14
P427L	20	15	29	57
C530G	22	16	31	58
W535F	21	18	30	52
ΔA248	23	17		59
W535F + D482N	24	19		53
P427L + C530G	26	54	33	
P427L + W535F	25	55	32	
P427L + C530G + W535F	27	56	34	

[227] *E. coli* BL21(DE3) (Novagen) competent cells were transformed with the correct construct. LBPTK medium was added and, after incubation for 1h at 37°C, with agitation at 250 rpm, bacteria were plated onto LBPTK plates containing 50 μg/ml kanamycin. BL21(DE3) pet24b+ SLO wild-type *tag-less* cells were grown at 25°C and induced with 1 mM IPTG. Clone expression was verified by SDS PAGE (*tag-less*, **FIGS. 8A** and **8B**; His-tagged, **FIG. 9**).

EXAMPLE 2

Purification of His-tagged proteins

[228] *E. coli* pellets were suspended in lysis buffer and mixed for 30-40 minutes at room temperature. Lysates were centrifuged at 30-40000 x *g* for 20-25 minutes and supernatants were loaded onto wash buffer A equilibrated columns (Poly-Prep with 1 ml of Ni-Activated Chelating Sepharose Fast Flow resin). The loaded resin was washed three times with wash buffer A and three times with wash buffer B. Proteins were eluted

with elution buffer in Eppendorf tubes containing 2mM final of DTT. Total elution proteins are quantified with Bradford reagent and then analyzed by SDS-polyacrylamide gel electrophoresis (**FIGS. 8** and **9**).

Buffers

lysis buffer:

10 ml B-PER™ (Bacterial-Protein Extraction Reagent, Pierce cat. 78266)

$MgCl_2$ final concentration of 0.1 mM

DNAsi I (Sigma cat. D-4263) 100 units

lysozyme (Sigma cat. L-7651) final concentration of 1 mg/ml

wash buffer A: 50 mM NaH_2PO_4, 300 mM NaCl, pH 8,0

wash buffer B: 20 mM imidazole, 50 mM NaH_2PO_4, 300 mM NaCl, pH 8.0

elution buffer: 250 mM imidazole, 50 mM NaH_2PO_4, 300 mM NaCl, pH 8.0

EXAMPLE 3

Purification of tag-less proteins

Lysate preparation

[229] About 80-110 g of bacterial culture pellet were suspended in 200-280 ml B-PER™ reagent (Pierce) supplemented with 6 tablets of COMPLETE® protease inhibitor, 10 ml 0,2M EDTA pH 7.5 (5 mM final concentration), 10 ml of a 100 mg/ml lysozyme solution, 8 ml of a 10000 K units/ml DNAse I solution and 1 ml of 50 mM $MgCl_2$ solution. Bacterial lysis was achieved by shaking the bacterial suspension for 60 minutes until a homogeneous suspension was obtained.

[230] Following centrifugation for 60 minutes at 13000 rpm (25400 x *g*), the supernatant was filtered using a 0.22 µm filter and is diluted with H_2O until a 1.8-1.9 mS conductivity was obtained. The pH was adjusted to 8.0. Protein concentration was determined by the Bradford method.

Anionic exchange chromatography

[231] The supernatant derived from the lysate treated as described above was loaded on an HP 50/10 Q Sepharose column (~200 ml), previously equilibrated with 30 mM TRIS, pH 8.0. The flow-through was collected. Fractions containing the GAS25 protein were pooled and dialyzed against 10 mM Na phosphate, pH 6.8. Protein concentration was determined by the Bradford method.

Buffer A: 30mM TRIS, pH 8.0
Buffer B: 30mM TRIS, 1M NaCl, pH 8.0
Equilibrium and Loading: 0% B
Gradient: 0-25% B in 5 CV – 25% B 2 CV
Wash: 100%B 2 CV + 3 CV
Flux: 20 ml/min
Fraction volume: 14 ml

Hydroxylapatite chromatography

[232] The previously obtained pool was loaded on a CHT20 column previously equilibrated with 10mM Na-phosphate, pH 6.8. The flow through was collected.

Buffer A: 10mM Na-phosphate, pH 6.8
Buffer B: 500mM Na phosphate, pH 6.8
Wash: 8 CV
Wash: 30%B 6 CV
Gradient: 30-100%B (10 CV)
Wash: 100%B
Flux: 5 ml/min.
Fraction volume: 5 ml

[233] Fraction aliquots were loaded on 12% Criterion gels under reducing and non-reducing conditions. Fractions containing GAS25 protein were pooled and protein concentration was determined by Bradford method.

Gel filtration chromatography

[234] The collected pool was concentrated using an Amicon filter in order to get a volume < 10 ml. The concentrated material was loaded on a HiLoad Superdex 200 26/60 equilibrated with at least 3-4 column volumes of PBS.

Buffer: PBS
Elution: Isocratic
Flux: 2.5 ml/min.
Fraction volume: 5 ml

[235] Fractions containing GAS25 protein were pooled and protein concentration was determined by Bradford. An additional estimation of protein concentration was performed by UV measurement considering Abs 0.1% (=1 g/l) 1.119. Protein purity is analyzed by polyacrylamide gel electrophoresis (**FIG. 11**).

EXAMPLE 4

Hemolytic assays

Protocol for quantitative hemolytic assay

[236] Serial dilutions of toxin were prepared in 96-well plates with U-shaped bottoms using PBS + 0.5% BSA. One ml of sheep blood was washed three times in PBS (with centrifugation at 3000 x g), and blood cells were suspended in 5 ml of PBS. An equal volume of suspension was added to 50 µl of each toxin dilution and incubated at 37°C for 30 min. Triton (2%) in water was used to give 100% hemolysis, and PBS + 0.5% BSA was used as negative control. Plates were then centrifuged for 5 min at 1,000 x g, and the

supernatant was transferred carefully to 96-well flat-bottomed plates. The absorbance was read at 540 nm.

Comparison of E. coli extracts containing wild-type SLO and SLO mutant P427L

[237] The gene encoding SLO P427L was amplified using PCR from the SF370 M1 genome and cloned into the vector pET21b+, which allowed expression in *E. coli* BL21DE3 of the His-tagged protein. Soluble extracts of *E. coli* expressing similar amounts of the wild-type and mutated streptolysin O proteins (see **FIG. 5**) were used to perform a hemolytic assay to compare the cytolytic properties of the two antigens. The result of the assay is shown in **FIG. 2**, which demonstrates that the mutated protein is at least 100 times less toxic than wild-type.

Comparison of purified wild-type SLO and SLO mutant P427L

[238] The SLO P427L mutant was purified according to purification standard procedures for His-tagged recombinant proteins (**FIG. 3**). Different concentrations of the purified wt and mutated proteins were used to repeat the hemolytic assay, which confirmed the decreased cytolytic activity (**FIG. 4**).

Hemolytic activity of E. coli extracts containing His-tagged and tag-less wild-type SLO and SLO mutant P427L

[239] We compared the hemolytic activity of *E. coli* lysates transformed with wild-type recombinant SLO (rSLO) without a His tag (BL21 DE3, Novagen No. 71382- pET24) and P427L mutant rSLO without a His tag (BL21 DE3, Novagen No. 71382- pET24). *E. coli* BL21 DE3 (Novagen, No. 71382) transformed with pET24 without insert was used as a negative control. The positive control was a hypotonic solution containing Triton 2% in water. The negative control was the protein dilution buffer (PBS containing 0.5% BSA, pH 7.4).

[240] Hemolysis was determined by measuring absorbance at 540 nm (A_{540nm}) of the supernatants. The titer was calculated as the dilution with 50% of maximum A_{540nm}.

[241] Results are shown in Tables 3 and 4 and in **FIG. 6**. These data demonstrate that, under the same conditions, mutant P427L is 1000 times less hemolytic than wild type SLO.

Table 3.

E. coli	**CFU/ml**
negative control	3.9×10^8
Wild-type rSLO (*tag-less*)	1.2×10^9
P427L rSLO (*tag-less*)	1.03×10^9

Table 4.

	rSLO wild-type *tag-less*	**rSLOP427L *tag-less***
titer (OD=50% hemolysis)	50,000	48
titer Wt/P427L	1042	

Comparison of wild-type SLO and various SLO mutants

[242] Hemolytic activity of wild-type SLO was compared with hemolytic activity of several different SLO mutants. The results are shown in **FIG. 13** and in Table 5, below. One hemolytic unit (HU) is defined as the amount of toxin required to obtained 50% of maximum lysis obtained treating the blood cells with 2% Triton.

Table 5.

Protein	HU/mg	HU/mg-SLO/ mutants
rSLO WT	22760	1
C530G	620	37
W535F	160	146
W535F-D482N	**<< 20**	**>> 1000**
P427L	about 20	about 1000
Δala248	**<< 20**	**>> 1000**
Neg. Control	<< 20	>> 1000

[243] Due to differences in protein purity, the hemolysis units/mg of mutants indicated in bold are overestimated; however, it is clear that (1) mutant W535F is less hemolytic than mutant C530G; (2) mutant P427L is about 1000 times less hemolytic than wild type and about 6-25 times less hemolytic than other two mutants W535F and C530G; and (3) mutant ΔA248 is certainly less hemolytic than wild type).

Effect of cholesterol

[244] Two-fivefold serial dilutions in PBS-BSA 0.5% of *E. coli* lysates or *E. coli* lysate with 200 mg/ml of cholesterol obtained after cells' growing at 30°C and induction with 1mM IPTG at 25°C and OD_{600nm} about 0.4-0.6, were assayed for their haemolytic activity. Fifty microliters of a 2% sheep erythrocyte solution in PBS were treated with an equal volume of protein preparations obtained by lysing bacteria, 3 hours after induction, with lysis buffer (B-PER solution-PIERCE- 1 mM $MgCl_2$, 100K units/ml DNAse (Sigma) and lysozyme (Sigma) for 30-40 minutes. The insoluble fraction was then centrifuged (15 minutes, 21000 x g, 4°C), and the supernatant (*E. coli* lysate) was transferred to a new Eppendorf tube containing DTT at final concentration of 5 mM.

[245] Under this condition, cholesterol did not inhibit either wild-type or mutant SLO until a 100-fold dilution factor was used; thus, there was no effect on the mutant-induced lysis. In contrast, wild-type-induced lysis was greatly reduced. Lysis induced by the negative control was not influenced by cholesterol, which suggests that cholesterol-induced inhibition is specific. See Table 6 and **FIG. 7**.

Table 6

	rSLO wild-type *tag-less*	rSLO P427L *tag-less*
titer (OD=50% hemolysis)	400	40
titre Wt/P427L	10	

EXAMPLE 5

Inhibition of hemolysis

Protocol

[246] Serial two-fold dilutions of sera from mice immunized with wild-type or mutant SLO proteins (without adjuvants or with Alum or MF59™ as adjuvants) were prepared in 96-well plates with U-shaped bottoms using PBS + 0.5% BSA. Sera of mice immunized with PBS or with adjuvant alone, as appropriate, were used as negative controls. An equal volume of a 50-100 ng/ml (3.5-7 HU) toxin solution in PBS + 0.5% BSA was added, and the plates were incubated at room temperature for 20 minutes under agitation (800 rpm). After incubation, 50 ml of this solution were transferred to a new 96-well plate, and an equal volume of a sheep red blood cell suspension (washed 3 x in PBS) was added and incubated at 37°C for 30 min. Plates were then centrifuged for 1 min at 1,000 x g, the supernatant was carefully transferred to 96-well flat-bottomed plates, and the absorbance was read at 540 nm. In the results described below, inhibition titer is expressed as the sera dilution that reduced Triton-induced hemolysis by 50%.

Inhibition of SLO hemolysis by wild-type SLO antisera

[247] Inhibition of SLO hemolysis by anti-wild-type SLO antisera is shown in **FIG. 14**, **FIG. 15**, **FIG. 16**, and Tables 7-9. Anti-SLO sera titers are included between 1/7,000 and 1/14,000 (arithmetic mean, 1/12,167 ± 2,714. Negative control sera (Freund's adjuvant) titers are included between 1/375 and 1/4,000 (arithmetic mean, 1/1,854 ± 1,384).

Table 7 (shown graphically in **FIG. 15**).

arithmetic mean of tested sera - % hemolysis		
dilution factor/sera	anti-SLO sera	negative control sera
125		9
250		10
500		19
1,000	2	38
2,000	2	69
4,000	2	84
8,000	19	93
16,000	78	97
32,000	99	
64,000	97	
128,000	100	

Table 8

anti-SLO sera (Freund's adjuvant)		negative control sera (Freund's adjuvant)	
serum	50% hemolysis inhib.	serum	50% hemolysis inhib.
A	14,000	1	4,000
B	7,000	2	1,500
C	12,000	3	375
D	12,000	4	3,000
E	14,000	5	1,500
F	14,000	6	750

Table 9 (shown graphically in **FIG. 16**)

ng/ml SLO	% hemolysis
1.6	4
3.1	3
6.3	6
12.5	30
25	94
50	100
100	100
200	100

Titration of hemolytic activity of wild-type SLO, chemically detoxified wild-type SLO and SLO mutants

[248] Titration of hemolytic activity of wild-type SLO, chemically detoxified wild-type SLO, and SLO mutants (P427L; P427L + W535F) is shown in **FIGS. 17-19** and in Table 10.

Table 10 (shown graphically in **FIG. 18**).

protein	HU/mg	HU/mg-SLO/mutants
SLO wild-type *tag-less*	728,307	1
SLO P427L *tag-less*	711	1,024
SLO P427L + W535F *tag-less*	<22 (stim. 10)	>33.000
SLO wild-type *tag-less*	45,511	
SLO wild-type *tag-less*, detoxified	<<89	>>511

Inhibition of SLO hemolysis by antiserum against mutant SLO proteins

[249] Inhibition of SLO hemolysis by antisera against mutant SLO proteins is shown in **FIGS. 20-22** and Tables 11-13. Using 50 ng/ml (3.5 HU) of toxin, the sera dilution required to obtain 50% reduction of SLO hemolytic activity for SLO mutant W535-P427L is

1/17,860 using Alum adjuvant and 1/7991 using MF59™ adjuvant. Negative control (adjuvant alone) titers are 1/1,000 (Alum) and 1/125 (MF59™).

Table 11 (shown graphically in **FIG. 20**).

50 ng/ml (3.5 HU) of wild-type SLO	
adjuvant	specific inhibition/non-specific inhibition
alum	18
MF™59	64

Table 12 (shown graphically in **FIG. 21**)

100 ng/ml (37 HU) of wild-type SLO	
adjuvant	specific inhibition/non-specific inhibition
alum	>227
MF™59	>117

Table 13 (shown graphically in **FIG. 22**)

ng/ml SLO	% hemolysis
1.6	3.5
3.1	5.8
6.3	13
12.5	42
25	86
50	100
100	100
200	100

[250] A comparison of the sera dilutions required to obtain 50% reduction of SLO hemolytic activity for wild-type and for SLO mutant W535-P427L, both using Alum adjuvant, is

shown in **FIG. 25**. Using 100 ng/ml (7 HU) of toxin, the serum dilution required to obtain 50% reduction of SLO hemolytic activity for SLO mutant W535-P427L is 1/12000, compared with a serum dilution of 1/8750 +/- 1500. The negative control (adjuvant alone) dilution is about 1/50.

EXAMPLE 6

In vivo protection experiments

[251] The purified SLO P427L protein, together with Freund's adjuvant, was administered intraperitoneally to 40 mice. The mice were then challenged intranasally with the 3348 M1 GAS strain. Table 14 reports the data obtained in 3 separate experiments, showing that 100% protection was consistently achieved in all experiments.

Table 14. Infection survival rate of mice

	% surviving mice		
antigen	**Experiment 1**	**Experiment 2**	**Experiment 3**
GAS25 Pro247Leu	100	100	100
E. coli contaminants (negative control)	10	10	10
homologous M1 protein (positive control)	100	90	90

[252] Groups of 10-20 mice were immunized with 20 μg of the recombinant protein at days 0, 21 and 35. Mice of negative control groups were immunized either with GST alone or with *E. coli* contaminants, depending on the version of the GAS recombinant protein used. Two weeks after the third immunization, blood samples were taken. A few days afterwards, immunized mice were challenged intranasally with 10^8 cfu (50 μl) of the M1 3348 GAS strains. Mice survival was monitored for a 10-14 day period. Immune sera obtained from the different groups were tested for immunogenicity on the entire SLO recombinant protein (western blot analysis).The results are shown in Tables 15 and 16.

Table 15

Protein	# mice	% survival	% negative control survival
GAS25_Pro247Leu His	10	90	30
GAS25_Pro247Leu His	10	100	20
GAS25_Pro247Leu His	10	80	30
GAS25_WT	20	95	15
GAS25_WT	10	100	40

Table 16

Protein	# mice	% survival	% negative control survival
rSLO WT *his-tagged*	20	100	45
C530G *his-tagged*	20	100	45
W535F *his-tagged*	20	100	45
W535F-D482N *his-tagged*	20	100	45
P427L *his-tagged*	20	95	45
Δala248 *his-tagged*	20	100	45

Protection against intranasal challenge with GAS M1 strain by SLO mutant P427L-W535F

[253] Thirty mice were immunized intraperitoneally with the SLO mutant P427L-W535F, with either Alum or MF59 as adjuvants, and challenged intranasally with a GAS M1 strain. The results are shown in **FIG. 26**. Seventy-seven percent of the mice immunized with the SLO mutant P427L-W535F and Alum were protected against intranasal challenge with a GAS M1 strain, as compared with 3% of the negative control mice (immunized with adjuvant only). Ninety percent of the mice immunized with the SLO mutant P427L-

W535F and MF59 were protected against intranasal challenge with a GAS M1 strain, as compared with 10% of the negative control mice (immunized with adjuvant only). These protection levels are comparable with those obtained by immunizing mice with wild-type SLO.

EXAMPLE 7

Protocols

[254] *Intravenous injection of SLO.* A solution of either wild-type or mutant SLO in PBS is diluted in a solution of PBS + 2 mM DTT, then 100 µl is injected into the tail vein of a mouse. Mice are observed for 2-3 days. Injection of wild-type SLO typically results in death within a few minutes.

[255] *In vivo lethality inhibition assay.* For lethality inhibition mediated by immune sera, 10 µg/mouse of wild-type SLO (a solution of 100 µg/ml in PBS, 2 mM DTT) are incubated for 20 minutes with rotation "end over end" at room temperature with either anti-SLO serum or control serum (obtained from mice immunized with adjuvant alone). After incubation, the samples are inoculated in the mice by intravenous injection into the tail vein. Mice are observed for 2-3 days.

[256] The results for wild-type SLO and mutant SLO P427L-W535F are shown in Table 17.

Table 17.

wild-type SLO		P427L-W535F	
µg/mouse	dead/treated	µg/mouse	dead/treated
		100	0/4
50	4/4	50	0/4
10	8/8	10	0/8
2	0/4		
0.4	0/4		
0.04	0/4		

[257] Acute *in vivo* acute toxicity was assessed using a dose of 10 μg/mouse of wild-type SLO as a positive control and injection of Freund's adjuvant alone as a negative control. Ten μg/mouse of wild-type SLO was incubated with either wild-type SLO antiserum or with control serum and inoculated into mice as described above. The results are shown in Table 18.

Table 18.

wild-type SLO (10 μg/mouse)		
sera	serum dilution	dead/treated
none		8/8
wild-type SLO	1/5	0/4
wild-type SLO	1/10	0/4
wild-type SLO	1/20	4/4
wild-type SLO	1/50	4/4
wild-type SLO	1/100	4/4
negative control	1/5	4/4

[258] The results of another set of experiments performed as described above are shown in Tables 19 and 20. *In vivo* acute toxicity was assessed using either 5 or 10 μg/mouse of wild-type SLO. In particular, 10 μg/mouse of wild type SLO were preincubated either with sera from mice immunized with GAS25 P427L-W535F or only PBS (no serum). In addition, 5 μg/mouse of wild type SLO were preincubated either with sera from mice immunized with GAS25 P427L-W535F or sera from mice immunized with PBS plus adjuvant (Alum), as negative control serum.

[259] The results demonstrate that lethal doses of wild-type SLO are neutralized by anti-SLO P427L-W535F sera but not by negative control sera at the same dilution.

Table 19.

wild-type SLO (10 µg/mouse)		
Sera	serum dilution	dead/treated
none	---	4/4
anti-SLO P427L-W535F, alum adjuvant	1/5	0/4

Table 20.

wild-type SLO (5 µg/mouse)		
Sera	serum dilution	dead/treated
anti-SLO P427L-W535F, alum adjuvant	1/5	0/4
negative control (alum alone)	1/5	4/4

EXAMPLE 8

Immunization with SLO P427L-W535F protects mice against intravenous injection of wild-type SLO

[260] Five-week old mice were immunized intraperitoneally three times (day 0, day 21, and day 35) with either wild-type SLO or with the SLO mutant P427L-W535F using alum as an adjuvant (20 µg protein in 2 mg/ml aluminium hydroxide). Mice immunized with adjuvant alone were used as a negative control. On day 55 mice were injected intravenously with different concentrations of a solution of wild-type SLO in PBS, 2 mM DTT and monitored for at least 72 hours. The results are shown in Table 21.

Table 21

	Dose of wild-type tagless SLO injected into mouse tail vein			
	2.5 μg/mouse	5 μg/mouse	10 μg/mouse	20 μg/mouse
	survival (no. of mice treated)	survival (no. of mice treated)	survival (no. of mice treated)	survival (no. of mice treated)
adjuvant (alum)	100% (4)	0% (12)	not tested	not tested
wild-type SLO tagless	not tested	100% (8)	100% (4)	100% (4)
SLO P427L-W535F tagless	not tested	100% (8)	100% (4)	100% (4)

[261] Five μg/mouse of wild-type SLO is lethal for mice immunized with adjuvant alone; these mice died within a few minutes after SLO injection. However, even 20 μg/mouse of the same wild-type SLO preparation did not kill mice immunized with either wild-type SLO or with the P427L-W535F SLO mutant.

CLAIMS

1. A purified mutant streptolysin 0 (SLO) protein, wherein the amino acids at positions P427 and W535 according to SEQ ID NO: 1 are substituted by another amino acid, and wherein the hemolytic activity of the mutant SLO protein is reduced by at least 50% relative to wild-type SLO.

2. The purified mutant SLO protein of claim 1, wherein the amino acid at position P427 according to SEQ ID NO:1 is substituted by leucine, glutamic acid or lysine.

3. The purified mutant SLO protein of claim 1 or claim 2, wherein the amino acid at position W535 according to SEQ ID NO: 1 is substituted by phenylalanine.

4. The purified mutant SLO protein of any one of claims 1-3, wherein the amino acid at position C530 according to SEQ ID NO: 1 is substituted by glycine.

5. The purified mutant SLO protein of any one of claims 1-4, wherein the amino acid at position A248 according to SEQ ID NO: 1 is deleted.

6. The purified mutant SLO protein of claim 4, wherein the amino acid at position P425 according to SEQ ID NO:1 is substituted by leucine, the amino acid at position W535 according to SEQ ID NO:1 is substituted by phenylalanine and the amino acid at position C530 according to SEQ ID NO: 1 is substituted by glycine.

7. The purified mutant SLO protein of claim 1 which comprises an amino acid sequence selected from the group consisting of SEQ ID NO:25 and SEQ ID NO:27.

8. The purified mutant SLO protein of claim 1 which is coupled to a carrier protein.

9. The purified mutant SLO protein of claim 8 wherein the carrier protein is selected from the group consisting of a bacterial toxin, a bacterial toxoid, a N. meningitidis outer membrane protein, a heat shock protein, a pertussis protein, H. influenzae protein D, a cytokine, a lymphokine, a hormone, a growth factor, C. difficile toxin A, C. difficile toxin B, and an iron-uptake protein.

10. A nucleic acid molecule encoding the mutant SLO protein of any one of claims 1-7.

11. The nucleic acid molecule of claim 10 wherein the mutant SLO protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO:25 and SEQ ID NO:27.

12. The nucleic acid molecule of claim 10 which comprises a nucleotide sequence selected from the group of coding sequences shown in SEQ ID NO:32 and, SEQ ID NO:34.

13. A vaccine composition comprising:

(a) an active agent selected from:

(i) purified mutant streptolysin O (SLO) protein as defined in any one of claims 1-7; and

(ii) a nucleic acid molecule which encodes the purified mutant streptolysin 0 (SLO) protein; and

(b) a pharmaceutically acceptable carrier.

14. The vaccine composition of claim 13 further comprising one or more active agents selected from the group consisting of:

(iii) a GAS antigen;

(iv) a nucleic acid molecule which encodes a GAS antigen;

(v) an active agent which is usefil in a pediatric vaccine, such as a polypeptide antigen of a pathogen selected from the group consisting of N. meningitidis, S. pneumoniae, Bordetella pertussis, Moraxella catarrhalis, Clostridium tetani, Chorinebacterim diphteriae, respiratory syncytial virus, polio virus, measles virus, mumps virus, rubella virus, and rotavirus polypeptide antigens, or a nucleic acid molecule which encodes the polypeptide antigen; and

(vi) an active agent which is useful in a vaccine for elderly or immunocompromised individuals, such as a polypeptide antigen of a pathogen selected from the group consisting of Enterococcus faecalis, Staphylococcus aureaus, Staphylococcus epidermis, Pseudomonas aeruginosa, Legionella pneumophila, Listeria monocytogenes, influenza virus, and parainfluenza virus polypeptide antigens, or a nucleic acid molecule which encodes the polypeptide antigen.

15. A method of making a vaccine for the prevention or treatment of infection by Streptococcus pyogenes comprising combining:

(a) an active agent selected from:

(i) a purified mutant streptolysin O (SLO) protein as defined in any one of claims 1-7; and

(ii) a nucleic acid molecule which encodes the purified mutant streptolysin 0 (SLO) protein; and

(b) a pharmaceutically acceptable carrier.

16. The method of claim 15 wherein the active agent is the purified mutant SLO protein and the mutant SLO protein is made by a method comprising:

(a) culturing a host cell comprising an expression construct which encodes the mutant SLO protein; and

(b) recovering the mutant SLO protein.

17. The method of claim 15 further comprising combining with the pharmaceutically acceptable carrier one or more active agents selected from the group consisting of:

(iii) a GAS antigen; and

(iv) a nucleic acid molecule which encodes a GAS antigen.

18. Use of an active agent in the manufacture of a medicament for treating or preventing infection by Streptococcus pyogenes, wherein the active agent is selected from the group consisting of:

(i) a purified mutant streptolysin 0 (SLO) protein as defined in any one of claims 1-7; and

(ii) a nucleic acid molecule which encodes the purified mutant streptolysin 0 (SLO) protein.

19. A purified mutant SLO protein of any one of claims 1 to 9 or a nucleic acid molecule of any one of claims 10 to 12 for use as a vaccine.

20. A purified mutant SLO protein of any one of claims 1 to 9, a nucleic acid molecule of any one of claims 10 to 12, or a vaccine composition of claim 13 or claim 14 for use in therapy.

21. A purified mutant SLO protein of any one of claims 1 to 9, a nucleic acid molecule of any one of claims 10 to 12, or a vaccine composition of claim 13 or claim 14 for treating or preventing infection by Streptococcus pyogenes.

SEQUENCE LISTING

<110> Bensi, Giuliano
Margarit Y Ros, Immaculada
Chiarot, Emiliano
Grandi, Guido
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145                 150                 155                 160
Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr Tyr Pro Ala
                165                 170                 175
Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys Pro Asp Ala
            180                 185                 190
Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp Leu Pro Gly
        195                 200                 205
Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr Tyr Ala Asn
    210                 215                 220
Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His Asp Asn Tyr
225                 230                 235                 240
Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr Glu Ser Met
                245                 250                 255
Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val Asn Ser Lys
            260                 265                 270
Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile Ser Lys Gly
        275                 280                 285
Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe Tyr Thr Val
    290                 295                 300
Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp Lys Ser Val
305                 310                 315                 320
Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu Ala Pro Pro
                325                 330                 335
Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe Val Lys Leu
            340                 345                 350
Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe Ser Ala Ala
        355                 360                 365
Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser Asp Ile Leu
    370                 375                 380
Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp Ala Ala Glu
385                 390                 395                 400
His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg Asn Val Ile
                405                 410                 415
Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr Pro Ile Ser
            420                 425                 430
Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly Val Asn Asn
        435                 440                 445
Arg Ser Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr Ser Gly Lys
    450                 455                 460
Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln Tyr Glu Ile Leu
465                 470                 475                 480
Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu Val Ile Thr Lys
                485                 490                 495
Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser Pro Phe Ser Thr
            500                 505                 510
Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg Ile Met Ala Arg
        515                 520                 525
Glu Cys Thr Gly Leu Ala Trp Glu Trp Trp Arg Lys Val Ile Asp Glu
    530                 535                 540
Arg Asp Val Lys Leu Ser Lys Glu Ile Asn Val Asn Ile Ser Gly Ser
```

```
545                 550                 555                 560
Thr Leu Ser Pro Tyr Gly Ser Ile Thr Tyr Lys
                565                 570
```

<210> 3
<211> 571
<212> PRT
<213> S. pyogenes

<400> 3

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Met Ser Asn Lys Lys Thr Phe Lys Lys Tyr Ser Arg Val Ala Gly Leu
 1               5                  10                  15
Leu Thr Ala Ala Leu Ile Ile Gly Asn Leu Val Thr Ala Asn Ala Glu
            20                  25                  30
Ser Asn Lys Gln Asn Thr Ala Ser Thr Glu Thr Thr Thr Thr Asn Glu
        35                  40                  45
Gln Pro Lys Pro Glu Ser Ser Glu Leu Thr Thr Glu Lys Ala Gly Gln
    50                  55                  60
Lys Met Asp Asp Met Leu Asn Ser Asn Asp Met Ile Lys Leu Ala Pro
65                  70                  75                  80
Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys Lys Ser Glu
                85                  90                  95
Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile Asn Asp Lys
            100                 105                 110
Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala Lys Asn Gly
        115                 120                 125
Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys Lys Ala Asp
    130                 135                 140
Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn Thr Thr Pro
145                 150                 155                 160
Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr Tyr Pro Ala
                165                 170                 175
Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys Pro Asp Ala
            180                 185                 190
Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp Leu Pro Gly
        195                 200                 205
Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr Tyr Ala Asn
    210                 215                 220
Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His Asp Asn Tyr
225                 230                 235                 240
Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr Glu Ser Met
                245                 250                 255
Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val Asn Ser Lys
            260                 265                 270
Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile Ser Lys Gly
        275                 280                 285
Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe Tyr Thr Val
    290                 295                 300
Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp Lys Ser Val
305                 310                 315                 320
Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu Ala Pro Pro
                325                 330                 335
Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe Val Lys Leu
            340                 345                 350
Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe Ser Ala Ala
        355                 360                 365
Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser Asp Ile Leu
    370                 375                 380
Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp Ala Ala Glu
385                 390                 395                 400
```

```
His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg Asn Val Ile
                405                 410                 415
Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr Pro Ile Ser
            420                 425                 430
Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly Val Asn Asn
        435                 440                 445
Arg Ser Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr Ser Gly Lys
    450                 455                 460
Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln Tyr Glu Ile Leu
465                 470                 475                 480
Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu Val Ile Thr Lys
                485                 490                 495
Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser Pro Phe Ser Thr
            500                 505                 510
Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg Ile Met Ala Arg
        515                 520                 525
Glu Cys Thr Gly Leu Ala Trp Glu Trp Trp Arg Lys Val Ile Asp Glu
    530                 535                 540
Arg Asp Val Lys Leu Ser Lys Glu Ile Asn Val Asn Ile Ser Gly Ser
545                 550                 555                 560
Thr Leu Ser Pro Tyr Gly Ser Ile Thr Tyr Lys
                565                 570
```

<210> 4
<211> 571
<212> PRT
<213> S. pyogenes

<400> 4

```
Met Ser Asn Lys Lys Thr Phe Lys Lys Tyr Ser Arg Val Ala Gly Leu
 1               5                  10                  15
Leu Thr Ala Ala Leu Ile Ile Gly Asn Leu Val Thr Ala Asn Ala Glu
            20                  25                  30
Ser Asn Lys Gln Asn Thr Ala Ser Thr Glu Thr Thr Thr Thr Asn Glu
        35                  40                  45
Gln Pro Lys Pro Glu Ser Ser Glu Leu Thr Thr Glu Lys Ala Gly Gln
    50                  55                  60
Lys Met Asp Asp Met Leu Asn Ser Asn Asp Met Ile Lys Leu Ala Pro
65                  70                  75                  80
Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys Lys Ser Glu
                85                  90                  95
Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile Asn Asp Lys
            100                 105                 110
Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala Lys Asn Gly
        115                 120                 125
Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys Lys Ala Asp
    130                 135                 140
Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn Thr Thr Pro
145                 150                 155                 160
Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr Tyr Pro Ala
                165                 170                 175
Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys Pro Asp Ala
            180                 185                 190
Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp Leu Pro Gly
        195                 200                 205
Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr Tyr Ala Asn
    210                 215                 220
Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His Asp Asn Tyr
225                 230                 235                 240
Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr Glu Ser Met
```

```
                245                 250                 255
Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val Asn Ser Lys
            260                 265                 270
Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile Ser Lys Gly
        275                 280                 285
Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe Tyr Thr Val
    290                 295                 300
Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp Lys Ser Val
305                 310                 315                 320
Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu Ala Pro Pro
                325                 330                 335
Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe Val Lys Leu
            340                 345                 350
Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe Ser Ala Ala
        355                 360                 365
Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser Asp Ile Leu
    370                 375                 380
Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp Ala Ala Glu
385                 390                 395                 400
His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg Asn Val Ile
                405                 410                 415
Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr Pro Ile Ser
            420                 425                 430
Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly Val Asn Asn
        435                 440                 445
Arg Ser Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr Ser Gly Lys
    450                 455                 460
Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln Tyr Glu Ile Leu
465                 470                 475                 480
Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu Val Ile Thr Lys
                485                 490                 495
Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser Pro Phe Ser Thr
            500                 505                 510
Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg Ile Met Ala Arg
        515                 520                 525
Glu Cys Thr Gly Leu Ala Trp Glu Trp Trp Arg Lys Val Ile Asp Glu
    530                 535                 540
Arg Asp Val Lys Leu Ser Lys Glu Ile Asn Val Asn Ile Ser Gly Ser
545                 550                 555                 560
Thr Leu Ser Pro Tyr Gly Ser Ile Thr Tyr Lys
                565                 570
```

<210> 5
<211> 571
<212> PRT
<213> S. pyogenes

<400> 5

```
Met Ser Asn Lys Lys Thr Phe Lys Lys Tyr Ser Arg Val Ala Gly Leu
 1               5                  10                  15
Leu Thr Ala Ala Leu Ile Ile Gly Asn Leu Val Thr Ala Asn Ala Glu
            20                  25                  30
Ser Asn Lys Gln Asn Thr Thr Ser Thr Glu Thr Thr Thr Thr Asn Glu
        35                  40                  45
Gln Pro Lys Pro Glu Ser Ser Glu Leu Thr Ile Glu Lys Ala Gly Gln
    50                  55                  60
Lys Met Asp Asp Met Leu Asn Ser Asn Asp Met Ile Lys Leu Ala Pro
65                  70                  75                  80
Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys Lys Ser Glu
                85                  90                  95
```

```
Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile Asn Asp Lys
            100                 105                 110
Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala Lys Asn Gly
        115                 120                 125
Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys Lys Ala Asp
    130                 135                 140
Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn Thr Thr Pro
145                 150                 155                 160
Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Met Thr Tyr Pro Ala
                165                 170                 175
Ala Leu Gln Leu Ala Asp Lys Gly Phe Thr Glu Asn Lys Pro Asp Ala
            180                 185                 190
Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp Leu Pro Gly
        195                 200                 205
Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr Tyr Ala Asn
    210                 215                 220
Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His Asp Asn Tyr
225                 230                 235                 240
Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr Glu Ser Met
                245                 250                 255
Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val Asn Ser Lys
            260                 265                 270
Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile Ser Lys Gly
        275                 280                 285
Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe Tyr Thr Val
    290                 295                 300
Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp Lys Ser Val
305                 310                 315                 320
Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu Ala Pro Pro
                325                 330                 335
Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe Val Lys Leu
            340                 345                 350
Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe Ser Ala Ala
        355                 360                 365
Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser Asp Ile Leu
    370                 375                 380
Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp Ala Ala Glu
385                 390                 395                 400
His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg Asn Val Ile
                405                 410                 415
Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr Pro Ile Ser
            420                 425                 430
Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly Val Asn Asn
        435                 440                 445
Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr Ser Gly Lys
    450                 455                 460
Ile Asn Leu Ser His Arg Gly Ala Tyr Val Ala Gln Tyr Glu Ile Leu
465                 470                 475                 480
Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu Val Ile Thr Lys
                485                 490                 495
Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser Pro Phe Ser Thr
            500                 505                 510
Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg Ile Met Ala Arg
        515                 520                 525
Glu Cys Thr Gly Leu Ala Trp Glu Trp Trp Arg Lys Val Ile Asp Glu
    530                 535                 540
Arg Asp Val Lys Leu Ser Lys Glu Ile Asn Val Asn Ile Ser Gly Ser
545                 550                 555                 560
Thr Leu Ser Pro Tyr Gly Ser Ile Thr Tyr Lys
                565                 570
```

<210> 6
<211> 571
<212> PRT
<213> S. pyogenes

<400> 6

```
Met Ser Asn Lys Lys Thr Phe Lys Lys Tyr Ser Arg Val Ala Gly Leu
 1               5                  10                  15
Leu Thr Ala Ala Leu Ile Ile Gly Asn Leu Val Thr Ala Asn Ala Glu
            20                  25                  30
Ser Asn Lys Gln Asn Thr Ala Ser Thr Glu Thr Thr Thr Thr Asn Glu
        35                  40                  45
Gln Pro Lys Pro Glu Ser Ser Glu Leu Thr Ile Glu Lys Ala Gly Gln
    50                  55                  60
Lys Met Asp Asp Met Leu Asn Ser Asn Asp Met Ile Lys Leu Ala Pro
65                  70                  75                  80
Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys Lys Ser Glu
                85                  90                  95
Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile Asn Asp Lys
            100                 105                 110
Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala Lys Asn Gly
        115                 120                 125
Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys Lys Ala Asp
    130                 135                 140
Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn Thr Thr Pro
145                 150                 155                 160
Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Met Thr Tyr Pro Ala
                165                 170                 175
Ala Leu Gln Leu Ala Asp Lys Gly Phe Thr Glu Asn Lys Pro Asp Ala
            180                 185                 190
Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp Leu Pro Gly
        195                 200                 205
Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr Tyr Ala Asn
    210                 215                 220
Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His Asp Asn Tyr
225                 230                 235                 240
Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr Glu Ser Met
                245                 250                 255
Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val Asn Ser Lys
            260                 265                 270
Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile Ser Lys Gly
        275                 280                 285
Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe Tyr Thr Val
    290                 295                 300
Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp Lys Ser Val
305                 310                 315                 320
Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu Ala Pro Pro
                325                 330                 335
Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe Val Lys Leu
            340                 345                 350
Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe Ser Ala Ala
        355                 360                 365
Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser Asp Ile Leu
    370                 375                 380
Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp Ala Ala Glu
385                 390                 395                 400
His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg Asn Val Ile
                405                 410                 415
Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr Pro Ile Ser
            420                 425                 430
```

```
Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly Val Asn Asn
        435                 440                 445
Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr Ser Gly Lys
    450                 455                 460
Ile Asn Leu Ser His Arg Gly Ala Tyr Val Ala Gln Tyr Glu Ile Leu
465                 470                 475                 480
Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu Val Ile Thr Lys
                485                 490                 495
Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser Pro Phe Ser Thr
            500                 505                 510
Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg Ile Met Ala Arg
        515                 520                 525
Glu Cys Thr Gly Leu Ala Trp Glu Trp Trp Arg Lys Val Ile Asp Glu
    530                 535                 540
Arg Asp Val Lys Leu Ser Lys Glu Ile Asn Val Asn Ile Ser Gly Ser
545                 550                 555                 560
Thr Leu Ser Pro Tyr Gly Ser Ile Thr Tyr Lys
                565                 570
```

<210> 7
<211> 571
<212> PRT
<213> S. pyogenes

```
<400> 7
Met Ser Asn Lys Lys Thr Phe Lys Lys Tyr Ser Arg Val Ala Gly Leu
 1               5                  10                  15
Leu Thr Val Ala Leu Ile Ile Gly Asn Leu Val Thr Ala Asn Ala Glu
            20                  25                  30
Ser Asn Lys Gln Asn Thr Ala Ser Thr Glu Thr Thr Thr Thr Asn Glu
        35                  40                  45
Gln Pro Lys Pro Glu Ser Ser Glu Leu Thr Ile Glu Lys Ala Gly Gln
    50                  55                  60
Lys Met Asp Asp Met Leu Asn Ser Asn Asp Met Ile Lys Leu Ala Pro
65                  70                  75                  80
Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys Lys Ser Glu
                85                  90                  95
Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile Asn Asp Lys
            100                 105                 110
Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala Lys Asn Gly
        115                 120                 125
Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys Lys Ala Asp
    130                 135                 140
Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn Thr Thr Pro
145                 150                 155                 160
Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr Tyr Pro Ala
                165                 170                 175
Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys Pro Asp Ala
            180                 185                 190
Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp Leu Pro Gly
        195                 200                 205
Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr Tyr Ala Asn
    210                 215                 220
Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His Asp Asn Tyr
225                 230                 235                 240
Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr Glu Ser Met
                245                 250                 255
Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val Asn Ser Lys
            260                 265                 270
Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile Ser Lys Gly
```

```
        275                 280                 285
Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe Tyr Thr Val
    290                 295                 300
Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp Lys Ser Val
305                 310                 315                 320
Thr Phe Lys Asp Leu Gln Arg Lys Gly Val Ser Asn Glu Ala Pro Pro
                325                 330                 335
Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe Val Lys Leu
            340                 345                 350
Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe Ser Ala Ala
        355                 360                 365
Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser Asp Ile Leu
    370                 375                 380
Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp Ala Ala Glu
385                 390                 395                 400
His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg Asn Val Ile
                405                 410                 415
Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr Pro Ile Ser
            420                 425                 430
Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly Val Asn Asn
        435                 440                 445
Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr Ser Gly Lys
    450                 455                 460
Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln Tyr Glu Ile Leu
465                 470                 475                 480
Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu Val Ile Thr Lys
                485                 490                 495
Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser Pro Phe Ser Thr
            500                 505                 510
Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg Ile Met Ala Arg
        515                 520                 525
Glu Cys Thr Gly Leu Ala Trp Glu Trp Trp Arg Lys Val Ile Asp Glu
    530                 535                 540
Arg Asp Val Lys Leu Ser Lys Glu Ile Asn Val Asn Ile Ser Gly Ser
545                 550                 555                 560
Thr Leu Ser Pro Tyr Gly Ser Ile Thr Tyr Lys
                565                 570
```

<210> 8
<211> 571
<212> PRT
<213> S. pyogenes

<400> 8

```
Met Ser Asn Lys Lys Thr Phe Lys Lys Tyr Ser Arg Val Ala Gly Leu
 1               5                  10                  15
Leu Thr Ala Ala Leu Ile Ile Gly Asn Leu Val Thr Ala Asn Ala Glu
            20                  25                  30
Ser Asn Lys Gln Asn Thr Ala Ser Thr Glu Thr Thr Thr Thr Asn Glu
        35                  40                  45
Gln Pro Lys Pro Glu Ser Ser Glu Leu Thr Thr Glu Lys Ala Gly Gln
    50                  55                  60
Lys Thr Asp Asp Met Leu Asn Ser Asn Asp Met Ile Lys Leu Ala Pro
65                  70                  75                  80
Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys Lys Ser Glu
                85                  90                  95
Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile Asn Asp Lys
            100                 105                 110
Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala Lys Asn Gly
        115                 120                 125
```

```
Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys Lys Ala Asp
    130                 135                 140
Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn Thr Thr Pro
145                 150                 155                 160
Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr Tyr Pro Ala
                165                 170                 175
Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys Pro Asp Ala
            180                 185                 190
Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp Leu Pro Gly
        195                 200                 205
Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr Tyr Ala Asn
    210                 215                 220
Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His Asp Asn Tyr
225                 230                 235                 240
Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr Glu Ser Met
                245                 250                 255
Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val Asn Ser Lys
            260                 265                 270
Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile Ser Lys Gly
        275                 280                 285
Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe Tyr Thr Val
    290                 295                 300
Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp Lys Ser Val
305                 310                 315                 320
Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu Ala Pro Pro
                325                 330                 335
Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe Val Lys Leu
            340                 345                 350
Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe Ser Ala Ala
        355                 360                 365
Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser Asp Ile Leu
    370                 375                 380
Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp Ala Ala Glu
385                 390                 395                 400
His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg Asn Val Ile
                405                 410                 415
Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr Pro Ile Ser
            420                 425                 430
Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly Val Asn Asn
        435                 440                 445
Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr Ser Gly Lys
    450                 455                 460
Ile Asn Leu Ser His Arg Gly Ala Tyr Val Ala Gln Tyr Glu Ile Leu
465                 470                 475                 480
Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu Val Ile Thr Lys
                485                 490                 495
Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser Pro Phe Ser Thr
            500                 505                 510
Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg Ile Met Ala Arg
        515                 520                 525
Glu Cys Thr Gly Leu Ala Trp Glu Trp Trp Arg Lys Val Ile Asp Glu
    530                 535                 540
Arg Asp Val Lys Leu Ser Lys Glu Ile Asn Val Asn Ile Ser Gly Ser
545                 550                 555                 560
Thr Leu Ser Pro Tyr Gly Ser Ile Thr Tyr Lys
                565                 570
```

<210> 9
<211> 571
<212> PRT

<213> S. pyogenes

<400> 9

```
Met Ser Asn Lys Lys Thr Phe Lys Lys Tyr Ser Arg Val Ala Gly Leu
 1               5                  10                  15
Leu Thr Ala Ala Leu Ile Ile Gly Asn Leu Val Thr Ala Asn Ala Glu
            20                  25                  30
Ser Asn Lys Gln Asn Thr Ala Ser Thr Glu Thr Thr Thr Thr Asn Glu
        35                  40                  45
Gln Pro Lys Pro Glu Ser Ser Glu Leu Thr Thr Glu Lys Ala Gly Gln
    50                  55                  60
Lys Thr Asp Asp Met Leu Asn Ser Asn Asp Met Ile Lys Leu Ala Pro
65                  70                  75                  80
Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys Lys Ser Glu
                85                  90                  95
Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile Asn Asp Lys
            100                 105                 110
Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala Lys Asn Gly
        115                 120                 125
Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys Lys Ala Asp
    130                 135                 140
Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn Thr Thr Pro
145                 150                 155                 160
Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr Tyr Pro Ala
                165                 170                 175
Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys Pro Asp Ala
            180                 185                 190
Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp Leu Pro Gly
        195                 200                 205
Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr Tyr Ala Asn
    210                 215                 220
Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His Asp Asn Tyr
225                 230                 235                 240
Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr Glu Ser Met
                245                 250                 255
Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val Asn Ser Lys
            260                 265                 270
Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile Ser Lys Gly
        275                 280                 285
Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe Tyr Thr Val
    290                 295                 300
Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp Lys Ser Val
305                 310                 315                 320
Thr Phe Lys Asp Leu Gln Arg Lys Gly Val Ser Asn Glu Ala Pro Pro
                325                 330                 335
Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe Val Lys Leu
            340                 345                 350
Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe Ser Ala Ala
        355                 360                 365
Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser Asp Ile Leu
    370                 375                 380
Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp Ala Ala Glu
385                 390                 395                 400
His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg Asn Val Ile
                405                 410                 415
Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr Pro Ile Ser
            420                 425                 430
Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly Val Asn Asn
        435                 440                 445
Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr Ser Gly Lys
    450                 455                 460
```

```
Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln Tyr Glu Ile Leu
465                 470                 475                 480
Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu Val Ile Thr Lys
                485                 490                 495
Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser Pro Phe Ser Thr
            500                 505                 510
Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg Ile Met Ala Arg
        515                 520                 525
Glu Cys Thr Gly Leu Ala Trp Glu Trp Trp Arg Lys Val Ile Asp Glu
    530                 535                 540
Arg Asp Val Lys Leu Ser Lys Glu Ile Asn Val Asn Ile Ser Gly Ser
545                 550                 555                 560
Thr Leu Ser Pro Tyr Gly Ser Ile Thr Tyr Lys
                565                 570
```

<210> 10
<211> 571
<212> PRT
<213> S. pyogenes

```
<400> 10
Met Ser Asn Lys Lys Thr Phe Lys Lys Tyr Ser Arg Val Ala Gly Leu
 1               5                  10                  15
Leu Thr Ala Ala Leu Ile Ile Gly Asn Leu Val Thr Ala Asn Ala Glu
            20                  25                  30
Ser Asn Lys Gln Asn Thr Ala Ser Thr Glu Thr Thr Thr Thr Asn Glu
        35                  40                  45
Gln Pro Lys Pro Glu Ser Ser Glu Leu Thr Thr Glu Lys Ala Gly Gln
    50                  55                  60
Lys Thr Asp Asp Met Leu Asn Ser Asn Asp Met Ile Lys Leu Ala Pro
65                  70                  75                  80
Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys Lys Ser Glu
                85                  90                  95
Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile Asn Asp Lys
            100                 105                 110
Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala Lys Asn Gly
        115                 120                 125
Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys Lys Ala Asp
    130                 135                 140
Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn Thr Thr Pro
145                 150                 155                 160
Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr Tyr Pro Ala
                165                 170                 175
Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys Pro Asp Ala
            180                 185                 190
Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp Leu Pro Gly
        195                 200                 205
Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr Tyr Ala Asn
    210                 215                 220
Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His Asp Asn Tyr
225                 230                 235                 240
Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr Glu Ser Met
                245                 250                 255
Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val Asn Ser Lys
            260                 265                 270
Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile Ser Lys Gly
        275                 280                 285
Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe Tyr Thr Val
    290                 295                 300
Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp Lys Ser Val
```

```
305                 310                 315                 320
Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu Ala Pro Pro
                325                 330                 335
Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe Val Lys Leu
            340                 345                 350
Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe Ser Ala Ala
        355                 360                 365
Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser Asp Ile Leu
    370                 375                 380
Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp Ala Ala Glu
385                 390                 395                 400
His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg Asn Val Ile
                405                 410                 415
Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr Pro Ile Ser
            420                 425                 430
Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly Val Asn Asn
        435                 440                 445
Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr Ser Gly Lys
    450                 455                 460
Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln Tyr Glu Ile Leu
465                 470                 475                 480
Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu Val Ile Thr Lys
                485                 490                 495
Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser Pro Phe Ser Thr
            500                 505                 510
Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg Ile Met Ala Arg
        515                 520                 525
Glu Cys Thr Gly Leu Ala Trp Glu Trp Trp Arg Lys Val Ile Asp Glu
    530                 535                 540
Arg Asp Val Lys Leu Ser Lys Glu Ile Asn Val Asn Ile Ser Gly Ser
545                 550                 555                 560
Thr Leu Ser Pro Tyr Gly Ser Ile Thr Tyr Lys
                565                 570
```

<210> 11
<211> 571
<212> PRT
<213> S. pyogenes

```
<400> 11
Met Ser Asn Lys Lys Thr Phe Lys Lys Tyr Ser Arg Val Ala Gly Leu
 1                5                  10                  15
Leu Thr Ala Ala Leu Ile Ile Gly Asn Leu Val Thr Ala Asn Ala Glu
            20                  25                  30
Ser Asn Lys Gln Asn Thr Ala Ser Thr Glu Thr Thr Thr Thr Asn Glu
        35                  40                  45
Gln Pro Lys Pro Glu Ser Ser Glu Leu Thr Thr Glu Lys Ala Gly Gln
    50                  55                  60
Lys Thr Asp Asp Met Leu Asn Ser Asn Asp Met Ile Lys Leu Ala Pro
65                  70                  75                  80
Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys Lys Ser Glu
                85                  90                  95
Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile Asn Asp Lys
            100                 105                 110
Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala Lys Asn Gly
        115                 120                 125
Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys Lys Ala Asp
    130                 135                 140
Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn Thr Thr Pro
145                 150                 155                 160
```

```
Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr Tyr Pro Ala
                165                 170                 175
Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys Pro Asp Ala
            180                 185                 190
Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp Leu Pro Gly
        195                 200                 205
Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr Tyr Ala Asn
    210                 215                 220
Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His Asp Asn Tyr
225                 230                 235                 240
Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr Glu Ser Met
                245                 250                 255
Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val Asn Ser Lys
            260                 265                 270
Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile Ser Lys Gly
        275                 280                 285
Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe Tyr Thr Val
    290                 295                 300
Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp Lys Ser Val
305                 310                 315                 320
Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu Ala Pro Pro
                325                 330                 335
Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe Val Lys Leu
            340                 345                 350
Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe Ser Ala Ala
        355                 360                 365
Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser Asp Ile Leu
    370                 375                 380
Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp Ala Ala Glu
385                 390                 395                 400
His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg Asn Val Ile
                405                 410                 415
Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr Pro Ile Ser
            420                 425                 430
Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly Val Asn Asn
        435                 440                 445
Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr Ser Gly Lys
    450                 455                 460
Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln Tyr Glu Ile Leu
465                 470                 475                 480
Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu Val Ile Thr Lys
                485                 490                 495
Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser Pro Phe Ser Thr
            500                 505                 510
Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg Ile Met Ala Arg
        515                 520                 525
Glu Cys Thr Gly Leu Ala Trp Glu Trp Trp Arg Lys Val Ile Asp Glu
    530                 535                 540
Arg Asp Val Lys Leu Ser Lys Glu Ile Asn Val Asn Ile Ser Gly Ser
545                 550                 555                 560
Thr Leu Ser Pro Tyr Gly Ser Ile Thr Tyr Lys
                565                 570
```

<210> 12
<211> 571
<212> PRT
<213> S. pyogenes

<400> 12
Met Ser Asn Lys Lys Thr Phe Lys Lys Tyr Ser Arg Val Ala Gly Leu

```
 1               5                  10                  15
Leu Thr Ala Ala Leu Ile Ile Gly Asn Leu Val Thr Ala Asn Ala Glu
            20                  25                  30
Ser Asn Lys Gln Asn Thr Ala Ser Thr Glu Thr Thr Thr Thr Asn Glu
        35                  40                  45
Gln Pro Lys Pro Glu Ser Ser Glu Leu Thr Thr Glu Lys Ala Gly Gln
    50                  55                  60
Lys Thr Asp Asp Met Leu Asn Ser Asn Asp Met Ile Lys Leu Ala Pro
65                  70                  75                  80
Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys Lys Ser Glu
                85                  90                  95
Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile Asn Asp Lys
            100                 105                 110
Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala Lys Asn Gly
        115                 120                 125
Glu Thr Ile Glu Asn Phe Ala Pro Lys Glu Gly Val Lys Lys Ala Asp
    130                 135                 140
Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn Thr Thr Pro
145                 150                 155                 160
Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr Tyr Pro Ala
                165                 170                 175
Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys Pro Asp Ala
            180                 185                 190
Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp Leu Pro Gly
        195                 200                 205
Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr Tyr Ala Asn
    210                 215                 220
Val Ser Thr Ala Ile Asp Asn Leu Ile Asn Gln Trp His Asp Asn Tyr
225                 230                 235                 240
Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr Glu Ser Met
                245                 250                 255
Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val Asn Ser Lys
            260                 265                 270
Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile Ser Lys Gly
        275                 280                 285
Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe Tyr Thr Val
    290                 295                 300
Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Ala Lys Ser Val
305                 310                 315                 320
Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu Ala Pro Pro
                325                 330                 335
Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe Val Lys Leu
            340                 345                 350
Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe Ser Ala Ala
        355                 360                 365
Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser Asp Ile Leu
    370                 375                 380
Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp Ala Ala Glu
385                 390                 395                 400
His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg Asn Val Ile
                405                 410                 415
Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr Pro Ile Ser
            420                 425                 430
Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly Val Asn Asn
        435                 440                 445
Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr Ser Gly Lys
    450                 455                 460
Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln Tyr Glu Ile Leu
465                 470                 475                 480
Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu Val Ile Thr Lys
                485                 490                 495
```

```
Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser Pro Phe Ser Thr
            500                 505                 510
Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg Ile Met Ala Arg
        515                 520                 525
Glu Cys Thr Gly Leu Ala Trp Glu Trp Trp Arg Lys Val Ile Asp Glu
    530                 535                 540
Arg Asp Val Lys Leu Ser Lys Glu Ile Asn Val Asn Ile Ser Gly Ser
545                 550                 555                 560
Thr Leu Ser Pro Tyr Gly Ser Ile Thr Tyr Lys
                565                 570
```

<210> 13
<211> 551
<212> PRT
<213> S. pyogenes

```
<400> 13
Met Ala Ser Glu Ser Asn Lys Gln Asn Thr Ala Ser Thr Glu Thr Thr
 1               5                  10                  15
Thr Thr Asn Glu Gln Pro Lys Pro Glu Ser Ser Glu Leu Thr Thr Glu
            20                  25                  30
Lys Ala Gly Gln Lys Thr Asp Asp Met Leu Asn Ser Asn Asp Met Ile
        35                  40                  45
Lys Leu Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu
    50                  55                  60
Lys Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu
65                  70                  75                  80
Ile Asn Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu
                85                  90                  95
Ala Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val
            100                 105                 110
Lys Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile
        115                 120                 125
Asn Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg
    130                 135                 140
Thr Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn
145                 150                 155                 160
Lys Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile
                165                 170                 175
Asp Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro
            180                 185                 190
Thr Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp
        195                 200                 205
His Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr
    210                 215                 220
Thr Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn
225                 230                 235                 240
Val Asn Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser
                245                 250                 255
Ile Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile
            260                 265                 270
Phe Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe
        275                 280                 285
Asp Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn
    290                 295                 300
Glu Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val
305                 310                 315                 320
Phe Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala
                325                 330                 335
Phe Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr
```

```
            340                 345                 350
Ser Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly
        355                 360                 365
Asp Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile
    370                 375                 380
Arg Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala
385                 390                 395                 400
Tyr Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala
                405                 410                 415
Gly Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr
            420                 425                 430
Thr Ser Gly Lys Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln
        435                 440                 445
Tyr Glu Ile Leu Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu
    450                 455                 460
Val Ile Thr Lys Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser
465                 470                 475                 480
Pro Phe Ser Thr Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg
                485                 490                 495
Ile Met Ala Arg Glu Cys Thr Gly Leu Ala Trp Glu Trp Trp Arg Lys
            500                 505                 510
Val Ile Asp Glu Arg Asp Val Lys Leu Ser Lys Glu Ile Asn Val Asn
        515                 520                 525
Ile Ser Gly Ser Thr Leu Ser Pro Tyr Gly Ser Ile Thr Tyr Lys Leu
    530                 535                 540
Glu His His His His His His
545                 550
```

<210> 14
<211> 1656
<212> DNA
<213> S. pyogenes

<400> 14

```
atggctagcg aatcgaacaa acaaaacact gctagtacag aaaccacaac gacaaatgag 60
caaccaaagc cagaaagtag tgagctaact actgaaaaag caggtcagaa aacggatgat 120
atgcttaact ctaacgatat gattaagctt gctcccaaag aaatgccact agaatctgca 180
gaaaaagaag aaaaaaagtc agaagacaaa aaaaagagcg aagaagatca cactgaagaa 240
atcaatgaca agatttattc actaaattat aatgagcttg aagtacttgc taaaaatggt 300
gaaaccattg aaaattttgt tcctaaagaa ggcgttaaga aagctgataa atttattgtc 360
attgaaagaa agaaaaaaaa tatcaacact acaccagtcg atatttccat tattgactct 420
gtcactgata ggacctatcc agcagccctt cagctggcta ataaaggttt taccgaaaac 480
aaaccagacg cggtagtcac caagcgaaac ccacaaaaaa tccatattga tttaccaggt 540
atgggagaca aagcaacggt tgaggtcaat gaccctacct atgccaatgt ttcaacagct 600
attgataatc ttgttaacca atggcatgat aattattctg gtggtaatac gcttcctgcc 660
agaacacaat atactgaatc aatggtatat tctaagtcac agattgaagc agctctaaat 720
gttaatagca aaatcttaga tggtacttta ggcattgatt tcaagtcgat ttcaaaaggt 780
gaaaagaagg tgatgattgc agcatacaag caaatttttt acaccgtatc agcaaacctt 840
cctaataatc ctgcggatgt gtttgataaa tcggtgacct ttaaagagtt gcaacgaaaa 900
ggtgtcagca atgaagctcc gccactcttt gtgagtaacg tagcctatgg tcgaactgtt 960
tttgtcaaac tagaaacaag ttctaaaagt aatgatgttg aagcggcctt tagtgcagct 1020
ctaaaaggaa cagatgttaa aactaatgga aaatattctg atatcttaga aaatagctca 1080
tttacagctg tcgttttagg aggagatgct gcagagcaca ataaggtagt cacaaaagac 1140
tttgatgtta ttagaaacgt tatcaaagac aatgctacct tcagtagaaa aaacccagct 1200
tatcctattt catacaccag tgttttcctt aaaaataata aaattgcggg tgtcaataac 1260
agaactgaat acgttgaaac aacatctacc gagtacacta gtggaaaaat taacctgtct 1320
catcaaggcg cgtatgttgc tcaatatgaa atcctttggg atgaaatcaa ttatgatgac 1380
aaaggaaaag aagtgattac aaaacgacgt tgggacaaca actggtatag taagacatca 1440
ccatttagca cagttatccc actaggagct aattcacgaa atatccgtat catggctaga 1500
gagtgcactg gcttagcttg ggaatggtgg cgaaaagtga tcgacgaaag agatgtgaaa 1560
```

ctgtctaaag aaatcaatgt caatatctca ggatcaacct tgagcccata tggttcgatt 1620
acttataagc tcgagcacca ccaccaccac cactga 1656

<210> 15
<211> 551
<212> PRT
<213> S. pyogenes

<400> 15

```
Met Ala Ser Glu Ser Asn Lys Gln Asn Thr Ala Ser Thr Glu Thr Thr
 1               5                  10                  15
Thr Thr Asn Glu Gln Pro Lys Pro Glu Ser Ser Glu Leu Thr Thr Glu
            20                  25                  30
Lys Ala Gly Gln Lys Thr Asp Asp Met Leu Asn Ser Asn Asp Met Ile
        35                  40                  45
Lys Leu Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu
    50                  55                  60
Lys Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu
65                  70                  75                  80
Ile Asn Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu
                85                  90                  95
Ala Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val
            100                 105                 110
Lys Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile
        115                 120                 125
Asn Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg
    130                 135                 140
Thr Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn
145                 150                 155                 160
Lys Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile
                165                 170                 175
Asp Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro
            180                 185                 190
Thr Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp
        195                 200                 205
His Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr
    210                 215                 220
Thr Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn
225                 230                 235                 240
Val Asn Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser
                245                 250                 255
Ile Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile
            260                 265                 270
Phe Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe
        275                 280                 285
Asp Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn
    290                 295                 300
Glu Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val
305                 310                 315                 320
Phe Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala
                325                 330                 335
Phe Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr
            340                 345                 350
Ser Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly
        355                 360                 365
Asp Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile
    370                 375                 380
Arg Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Leu Ala
385                 390                 395                 400
Tyr Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala
```

```
                405                 410                 415
Gly Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr
            420                 425                 430
Thr Ser Gly Lys Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln
        435                 440                 445
Tyr Glu Ile Leu Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu
    450                 455                 460
Val Ile Thr Lys Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser
465                 470                 475                 480
Pro Phe Ser Thr Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg
                485                 490                 495
Ile Met Ala Arg Glu Cys Thr Gly Leu Ala Trp Glu Trp Trp Arg Lys
            500                 505                 510
Val Ile Asp Glu Arg Asp Val Lys Leu Ser Lys Glu Ile Asn Val Asn
        515                 520                 525
Ile Ser Gly Ser Thr Leu Ser Pro Tyr Gly Ser Ile Thr Tyr Lys Leu
    530                 535                 540
Glu His His His His His His
545                 550
```

<210> 16
<211> 551
<212> PRT
<213> S. pyogenes

<400> 16

```
Met Ala Ser Glu Ser Asn Lys Gln Asn Thr Ala Ser Thr Glu Thr Thr
 1               5                  10                  15
Thr Thr Asn Glu Gln Pro Lys Pro Glu Ser Ser Glu Leu Thr Thr Glu
            20                  25                  30
Lys Ala Gly Gln Lys Thr Asp Asp Met Leu Asn Ser Asn Asp Met Ile
        35                  40                  45
Lys Leu Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu
    50                  55                  60
Lys Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu
65                  70                  75                  80
Ile Asn Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu
                85                  90                  95
Ala Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val
            100                 105                 110
Lys Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile
        115                 120                 125
Asn Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg
    130                 135                 140
Thr Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn
145                 150                 155                 160
Lys Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile
                165                 170                 175
Asp Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro
            180                 185                 190
Thr Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp
        195                 200                 205
His Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr
    210                 215                 220
Thr Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn
225                 230                 235                 240
Val Asn Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser
                245                 250                 255
Ile Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile
            260                 265                 270
Phe Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe
```

```
        275                 280                 285
Asp Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn
    290                 295                 300
Glu Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val
305                 310                 315                 320
Phe Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala
                325                 330                 335
Phe Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr
            340                 345                 350
Ser Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly
        355                 360                 365
Asp Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile
    370                 375                 380
Arg Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala
385                 390                 395                 400
Tyr Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala
                405                 410                 415
Gly Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr
            420                 425                 430
Thr Ser Gly Lys Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln
        435                 440                 445
Tyr Glu Ile Leu Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu
    450                 455                 460
Val Ile Thr Lys Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser
465                 470                 475                 480
Pro Phe Ser Thr Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg
                485                 490                 495
Ile Met Ala Arg Glu Gly Thr Gly Leu Ala Trp Glu Trp Trp Arg Lys
            500                 505                 510
Val Ile Asp Glu Arg Asp Val Lys Leu Ser Lys Glu Ile Asn Val Asn
        515                 520                 525
Ile Ser Gly Ser Thr Leu Ser Pro Tyr Gly Ser Ile Thr Tyr Lys Leu
    530                 535                 540
Glu His His His His His His
545                 550
```

<210> 17
<211> 550
<212> PRT
<213> S. pyogenes

<400> 17

```
Met Ala Ser Glu Ser Asn Lys Gln Asn Thr Ala Ser Thr Glu Thr Thr
 1               5                  10                  15
Thr Thr Asn Glu Gln Pro Lys Pro Glu Ser Ser Glu Leu Thr Thr Glu
            20                  25                  30
Lys Ala Gly Gln Lys Thr Asp Asp Met Leu Asn Ser Asn Asp Met Ile
        35                  40                  45
Lys Leu Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu
    50                  55                  60
Lys Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu
65                  70                  75                  80
Ile Asn Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu
                85                  90                  95
Ala Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val
            100                 105                 110
Lys Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile
        115                 120                 125
Asn Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg
    130                 135                 140
Thr Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn
```

```
145                 150                 155                 160
Lys Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile
                165                 170                 175
Asp Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro
            180                 185                 190
Thr Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp
        195                 200                 205
His Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Arg Thr Gln Tyr Thr
    210                 215                 220
Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val
225                 230                 235                 240
Asn Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile
                245                 250                 255
Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe
            260                 265                 270
Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp
        275                 280                 285
Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu
    290                 295                 300
Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe
305                 310                 315                 320
Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe
                325                 330                 335
Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser
            340                 345                 350
Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp
        355                 360                 365
Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg
    370                 375                 380
Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr
385                 390                 395                 400
Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly
                405                 410                 415
Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr
            420                 425                 430
Ser Gly Lys Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln Tyr
        435                 440                 445
Glu Ile Leu Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu Val
    450                 455                 460
Ile Thr Lys Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser Pro
465                 470                 475                 480
Phe Ser Thr Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg Ile
                485                 490                 495
Met Ala Arg Glu Cys Thr Gly Leu Ala Trp Glu Trp Trp Arg Lys Val
            500                 505                 510
Ile Asp Glu Arg Asp Val Lys Leu Ser Lys Glu Ile Asn Val Asn Ile
        515                 520                 525
Ser Gly Ser Thr Leu Ser Pro Tyr Gly Ser Ile Thr Tyr Lys Leu Glu
    530                 535                 540
His His His His His His
545                 550
```

<210> 18
<211> 551
<212> PRT
<213> S. pyogenes

```
<400> 18
Met Ala Ser Glu Ser Asn Lys Gln Asn Thr Ala Ser Thr Glu Thr Thr
 1               5                  10                  15
Thr Thr Asn Glu Gln Pro Lys Pro Glu Ser Ser Glu Leu Thr Thr Glu
```

```
            20                  25                  30
Lys Ala Gly Gln Lys Thr Asp Asp Met Leu Asn Ser Asn Asp Met Ile
        35                  40                  45
Lys Leu Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu
    50                  55                  60
Lys Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu
65                  70                  75                  80
Ile Asn Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu
                85                  90                  95
Ala Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val
            100                 105                 110
Lys Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile
        115                 120                 125
Asn Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg
    130                 135                 140
Thr Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn
145                 150                 155                 160
Lys Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile
                165                 170                 175
Asp Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro
            180                 185                 190
Thr Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp
        195                 200                 205
His Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr
    210                 215                 220
Thr Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn
225                 230                 235                 240
Val Asn Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser
                245                 250                 255
Ile Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile
            260                 265                 270
Phe Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe
        275                 280                 285
Asp Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn
    290                 295                 300
Glu Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val
305                 310                 315                 320
Phe Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala
                325                 330                 335
Phe Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr
            340                 345                 350
Ser Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly
        355                 360                 365
Asp Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile
    370                 375                 380
Arg Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala
385                 390                 395                 400
Tyr Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala
                405                 410                 415
Gly Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr
            420                 425                 430
Thr Ser Gly Lys Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln
        435                 440                 445
Tyr Glu Ile Leu Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu
    450                 455                 460
Val Ile Thr Lys Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser
465                 470                 475                 480
Pro Phe Ser Thr Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg
                485                 490                 495
Ile Met Ala Arg Glu Cys Thr Gly Leu Ala Phe Glu Trp Trp Arg Lys
            500                 505                 510
```

```
Val Ile Asp Glu Arg Asp Val Lys Leu Ser Lys Glu Ile Asn Val Asn
        515                 520                 525
Ile Ser Gly Ser Thr Leu Ser Pro Tyr Gly Ser Ile Thr Tyr Lys Leu
    530                 535                 540
Glu His His His His His His
545                 550
```

<210> 19
<211> 551
<212> PRT
<213> S. pyogenes

<400> 19

```
Met Ala Ser Glu Ser Asn Lys Gln Asn Thr Ala Ser Thr Glu Thr Thr
 1               5                  10                  15
Thr Thr Asn Glu Gln Pro Lys Pro Glu Ser Ser Glu Leu Thr Thr Glu
            20                  25                  30
Lys Ala Gly Gln Lys Thr Asp Asp Met Leu Asn Ser Asn Asp Met Ile
        35                  40                  45
Lys Leu Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu
    50                  55                  60
Lys Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu
65                  70                  75                  80
Ile Asn Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu
                85                  90                  95
Ala Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val
            100                 105                 110
Lys Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile
        115                 120                 125
Asn Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg
    130                 135                 140
Thr Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn
145                 150                 155                 160
Lys Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile
                165                 170                 175
Asp Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro
            180                 185                 190
Thr Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp
        195                 200                 205
His Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr
    210                 215                 220
Thr Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn
225                 230                 235                 240
Val Asn Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser
                245                 250                 255
Ile Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile
            260                 265                 270
Phe Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe
        275                 280                 285
Asp Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn
    290                 295                 300
Glu Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val
305                 310                 315                 320
Phe Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala
                325                 330                 335
Phe Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr
            340                 345                 350
Ser Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly
        355                 360                 365
Asp Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile
    370                 375                 380
```

```
Arg Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala
385                 390                 395                 400
Tyr Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala
                405                 410                 415
Gly Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr
            420                 425                 430
Thr Ser Gly Lys Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln
        435                 440                 445
Tyr Glu Ile Leu Trp Asn Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu
    450                 455                 460
Val Ile Thr Lys Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser
465                 470                 475                 480
Pro Phe Ser Thr Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg
                485                 490                 495
Ile Met Ala Arg Glu Cys Thr Gly Leu Ala Phe Glu Trp Trp Arg Lys
            500                 505                 510
Val Ile Asp Glu Arg Asp Val Lys Leu Ser Lys Glu Ile Asn Val Asn
        515                 520                 525
Ile Ser Gly Ser Thr Leu Ser Pro Tyr Gly Ser Ile Thr Tyr Lys Leu
    530                 535                 540
Glu His His His His His His
545                 550
```

<210> 20
<211> 543
<212> PRT
<213> S. pyogenes

<400> 20

```
Met Ala Ser Glu Ser Asn Lys Gln Asn Thr Ala Ser Thr Glu Thr Thr
 1               5                  10                  15
Thr Thr Asn Glu Gln Pro Lys Pro Glu Ser Ser Glu Leu Thr Thr Glu
            20                  25                  30
Lys Ala Gly Gln Lys Thr Asp Asp Met Leu Asn Ser Asn Asp Met Ile
        35                  40                  45
Lys Leu Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu
    50                  55                  60
Lys Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu
65                  70                  75                  80
Ile Asn Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu
                85                  90                  95
Ala Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val
            100                 105                 110
Lys Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile
        115                 120                 125
Asn Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg
    130                 135                 140
Thr Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn
145                 150                 155                 160
Lys Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile
                165                 170                 175
Asp Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro
            180                 185                 190
Thr Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp
        195                 200                 205
His Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr
    210                 215                 220
Thr Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn
225                 230                 235                 240
Val Asn Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser
                245                 250                 255
```

```
Ile Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile
            260                 265                 270
Phe Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe
        275                 280                 285
Asp Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn
    290                 295                 300
Glu Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val
305                 310                 315                 320
Phe Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala
                325                 330                 335
Phe Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr
            340                 345                 350
Ser Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly
        355                 360                 365
Asp Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile
    370                 375                 380
Arg Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Leu Ala
385                 390                 395                 400
Tyr Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala
                405                 410                 415
Gly Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr
            420                 425                 430
Thr Ser Gly Lys Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln
        435                 440                 445
Tyr Glu Ile Leu Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu
    450                 455                 460
Val Ile Thr Lys Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser
465                 470                 475                 480
Pro Phe Ser Thr Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg
                485                 490                 495
Ile Met Ala Arg Glu Cys Thr Gly Leu Ala Trp Glu Trp Trp Arg Lys
            500                 505                 510
Val Ile Asp Glu Arg Asp Val Lys Leu Ser Lys Glu Ile Asn Val Asn
        515                 520                 525
Ile Ser Gly Ser Thr Leu Ser Pro Tyr Gly Ser Ile Thr Tyr Lys
    530                 535                 540
```

<210> 21
<211> 571
<212> PRT
<213> S. pyogenes

```
<400> 21
Met Ser Asn Lys Lys Thr Phe Lys Lys Tyr Ser Arg Val Ala Gly Leu
 1               5                  10                  15
Leu Thr Ala Ala Leu Ile Ile Gly Asn Leu Val Thr Ala Asn Ala Glu
            20                  25                  30
Ser Asn Lys Gln Asn Thr Ala Ser Thr Glu Thr Thr Thr Thr Asn Glu
        35                  40                  45
Gln Pro Lys Pro Glu Ser Ser Glu Leu Thr Thr Glu Lys Ala Gly Gln
    50                  55                  60
Lys Thr Asp Asp Met Leu Asn Ser Asn Asp Met Ile Lys Leu Ala Pro
65                  70                  75                  80
Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys Lys Ser Glu
                85                  90                  95
Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile Asn Asp Lys
            100                 105                 110
Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala Lys Asn Gly
        115                 120                 125
Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys Lys Ala Asp
    130                 135                 140
```

```
Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn Thr Thr Pro
145                 150                 155                 160
Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr Tyr Pro Ala
                165                 170                 175
Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys Pro Asp Ala
            180                 185                 190
Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp Leu Pro Gly
        195                 200                 205
Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr Tyr Ala Asn
    210                 215                 220
Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His Asp Asn Tyr
225                 230                 235                 240
Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr Glu Ser Met
                245                 250                 255
Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val Asn Ser Lys
            260                 265                 270
Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile Ser Lys Gly
        275                 280                 285
Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe Tyr Thr Val
    290                 295                 300
Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp Lys Ser Val
305                 310                 315                 320
Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu Ala Pro Pro
                325                 330                 335
Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe Val Lys Leu
            340                 345                 350
Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe Ser Ala Ala
        355                 360                 365
Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser Asp Ile Leu
    370                 375                 380
Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp Ala Ala Glu
385                 390                 395                 400
His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg Asn Val Ile
                405                 410                 415
Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr Pro Ile Ser
            420                 425                 430
Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly Val Asn Asn
        435                 440                 445
Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr Ser Gly Lys
    450                 455                 460
Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln Tyr Glu Ile Leu
465                 470                 475                 480
Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu Val Ile Thr Lys
                485                 490                 495
Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser Pro Phe Ser Thr
            500                 505                 510
Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg Ile Met Ala Arg
        515                 520                 525
Glu Cys Thr Gly Leu Ala Phe Glu Trp Trp Arg Lys Val Ile Asp Glu
    530                 535                 540
Arg Asp Val Lys Leu Ser Lys Glu Ile Asn Val Asn Ile Ser Gly Ser
545                 550                 555                 560
Thr Leu Ser Pro Tyr Gly Ser Ile Thr Tyr Lys
                565                 570
```

<210> 22
<211> 571
<212> PRT
<213> S. pyogenes

<400> 22

```
Met Ser Asn Lys Lys Thr Phe Lys Lys Tyr Ser Arg Val Ala Gly Leu
 1               5                  10                  15
Leu Thr Ala Ala Leu Ile Ile Gly Asn Leu Val Thr Ala Asn Ala Glu
            20                  25                  30
Ser Asn Lys Gln Asn Thr Ala Ser Thr Glu Thr Thr Thr Thr Asn Glu
        35                  40                  45
Gln Pro Lys Pro Glu Ser Ser Glu Leu Thr Thr Glu Lys Ala Gly Gln
    50                  55                  60
Lys Thr Asp Asp Met Leu Asn Ser Asn Asp Met Ile Lys Leu Ala Pro
65                  70                  75                  80
Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys Lys Ser Glu
                85                  90                  95
Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile Asn Asp Lys
            100                 105                 110
Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala Lys Asn Gly
        115                 120                 125
Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys Lys Ala Asp
    130                 135                 140
Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn Thr Thr Pro
145                 150                 155                 160
Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr Tyr Pro Ala
                165                 170                 175
Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys Pro Asp Ala
            180                 185                 190
Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp Leu Pro Gly
        195                 200                 205
Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr Tyr Ala Asn
    210                 215                 220
Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His Asp Asn Tyr
225                 230                 235                 240
Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr Glu Ser Met
                245                 250                 255
Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val Asn Ser Lys
            260                 265                 270
Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile Ser Lys Gly
        275                 280                 285
Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe Tyr Thr Val
    290                 295                 300
Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp Lys Ser Val
305                 310                 315                 320
Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu Ala Pro Pro
                325                 330                 335
Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe Val Lys Leu
            340                 345                 350
Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe Ser Ala Ala
        355                 360                 365
Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser Asp Ile Leu
    370                 375                 380
Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp Ala Ala Glu
385                 390                 395                 400
His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg Asn Val Ile
                405                 410                 415
Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr Pro Ile Ser
            420                 425                 430
Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly Val Asn Asn
        435                 440                 445
Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr Ser Gly Lys
    450                 455                 460
Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln Tyr Glu Ile Leu
465                 470                 475                 480
Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu Val Ile Thr Lys
```

```
                485                 490                 495
Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser Pro Phe Ser Thr
            500                 505                 510
Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg Ile Met Ala Arg
        515                 520                 525
Glu Gly Thr Gly Leu Ala Trp Glu Trp Trp Arg Lys Val Ile Asp Glu
    530                 535                 540
Arg Asp Val Lys Leu Ser Lys Glu Ile Asn Val Asn Ile Ser Gly Ser
545                 550                 555                 560
Thr Leu Ser Pro Tyr Gly Ser Ile Thr Tyr Lys
                565                 570
```

<210> 23
<211> 570
<212> PRT
<213> S. pyogenes

<400> 23

```
Met Ser Asn Lys Lys Thr Phe Lys Lys Tyr Ser Arg Val Ala Gly Leu
 1               5                  10                  15
Leu Thr Ala Ala Leu Ile Ile Gly Asn Leu Val Thr Ala Asn Ala Glu
            20                  25                  30
Ser Asn Lys Gln Asn Thr Ala Ser Thr Glu Thr Thr Thr Thr Asn Glu
        35                  40                  45
Gln Pro Lys Pro Glu Ser Ser Glu Leu Thr Thr Glu Lys Ala Gly Gln
    50                  55                  60
Lys Thr Asp Asp Met Leu Asn Ser Asn Asp Met Ile Lys Leu Ala Pro
65                  70                  75                  80
Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys Lys Ser Glu
                85                  90                  95
Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile Asn Asp Lys
            100                 105                 110
Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala Lys Asn Gly
        115                 120                 125
Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys Lys Ala Asp
    130                 135                 140
Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn Thr Thr Pro
145                 150                 155                 160
Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr Tyr Pro Ala
                165                 170                 175
Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys Pro Asp Ala
            180                 185                 190
Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp Leu Pro Gly
        195                 200                 205
Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr Tyr Ala Asn
    210                 215                 220
Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His Asp Asn Tyr
225                 230                 235                 240
Ser Gly Gly Asn Thr Leu Pro Arg Thr Gln Tyr Thr Glu Ser Met Val
                245                 250                 255
Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val Asn Ser Lys Ile
            260                 265                 270
Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile Ser Lys Gly Glu
        275                 280                 285
Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe Tyr Thr Val Ser
    290                 295                 300
Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp Lys Ser Val Thr
305                 310                 315                 320
Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu Ala Pro Pro Leu
                325                 330                 335
Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe Val Lys Leu Glu
```

```
            340                 345                 350
Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe Ser Ala Ala Leu
        355                 360                 365
Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser Asp Ile Leu Glu
    370                 375                 380
Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp Ala Ala Glu His
385                 390                 395                 400
Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg Asn Val Ile Lys
                405                 410                 415
Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr Pro Ile Ser Tyr
            420                 425                 430
Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly Val Asn Asn Arg
        435                 440                 445
Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr Ser Gly Lys Ile
    450                 455                 460
Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln Tyr Glu Ile Leu Trp
465                 470                 475                 480
Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu Val Ile Thr Lys Arg
                485                 490                 495
Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser Pro Phe Ser Thr Val
            500                 505                 510
Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg Ile Met Ala Arg Glu
        515                 520                 525
Cys Thr Gly Leu Ala Trp Glu Trp Trp Arg Lys Val Ile Asp Glu Arg
    530                 535                 540
Asp Val Lys Leu Ser Lys Glu Ile Asn Val Asn Ile Ser Gly Ser Thr
545                 550                 555                 560
Leu Ser Pro Tyr Gly Ser Ile Thr Tyr Lys
                565                 570
```

<210> 24
<211> 571
<212> PRT
<213> S. pyogenes

<400> 24

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Met Ser Asn Lys Lys Thr Phe Lys Lys Tyr Ser Arg Val Ala Gly Leu
 1               5                  10                  15
Leu Thr Ala Ala Leu Ile Ile Gly Asn Leu Val Thr Ala Asn Ala Glu
            20                  25                  30
Ser Asn Lys Gln Asn Thr Ala Ser Thr Glu Thr Thr Thr Thr Asn Glu
        35                  40                  45
Gln Pro Lys Pro Glu Ser Ser Glu Leu Thr Thr Glu Lys Ala Gly Gln
    50                  55                  60
Lys Thr Asp Asp Met Leu Asn Ser Asn Asp Met Ile Lys Leu Ala Pro
65                  70                  75                  80
Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu Lys Lys Ser Glu
                85                  90                  95
Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu Ile Asn Asp Lys
            100                 105                 110
Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu Ala Lys Asn Gly
        115                 120                 125
Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val Lys Lys Ala Asp
    130                 135                 140
Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile Asn Thr Thr Pro
145                 150                 155                 160
Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg Thr Tyr Pro Ala
                165                 170                 175
Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn Lys Pro Asp Ala
            180                 185                 190
Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile Asp Leu Pro Gly
```

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        195                 200                 205
Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro Thr Tyr Ala Asn
    210                 215                 220
Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp His Asp Asn Tyr
225                 230                 235                 240
Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr Thr Glu Ser Met
                245                 250                 255
Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn Val Asn Ser Lys
            260                 265                 270
Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser Ile Ser Lys Gly
        275                 280                 285
Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile Phe Tyr Thr Val
    290                 295                 300
Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe Asp Lys Ser Val
305                 310                 315                 320
Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn Glu Ala Pro Pro
                325                 330                 335
Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val Phe Val Lys Leu
            340                 345                 350
Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala Phe Ser Ala Ala
        355                 360                 365
Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr Ser Asp Ile Leu
    370                 375                 380
Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly Asp Ala Ala Glu
385                 390                 395                 400
His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile Arg Asn Val Ile
                405                 410                 415
Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Pro Ala Tyr Pro Ile Ser
            420                 425                 430
Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala Gly Val Asn Asn
        435                 440                 445
Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr Thr Ser Gly Lys
    450                 455                 460
Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln Tyr Glu Ile Leu
465                 470                 475                 480
Trp Asn Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu Val Ile Thr Lys
                485                 490                 495
Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser Pro Phe Ser Thr
            500                 505                 510
Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg Ile Met Ala Arg
        515                 520                 525
Glu Cys Thr Gly Leu Ala Phe Glu Trp Trp Arg Lys Val Ile Asp Glu
    530                 535                 540
Arg Asp Val Lys Leu Ser Lys Glu Ile Asn Val Asn Ile Ser Gly Ser
545                 550                 555                 560
Thr Leu Ser Pro Tyr Gly Ser Ile Thr Tyr Lys
                565                 570
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<210> 25
<211> 543
<212> PRT
<213> S. pyogenes

<400> 25

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Met Ala Ser Glu Ser Asn Lys Gln Asn Thr Ala Ser Thr Glu Thr Thr
 1               5                  10                  15
Thr Thr Asn Glu Gln Pro Lys Pro Glu Ser Ser Glu Leu Thr Thr Glu
            20                  25                  30
Lys Ala Gly Gln Lys Thr Asp Asp Met Leu Asn Ser Asn Asp Met Ile
        35                  40                  45
Lys Leu Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu
```

```
    50                  55                  60
Lys Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu
65                  70                  75                  80
Ile Asn Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu
                85                  90                  95
Ala Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val
            100                 105                 110
Lys Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile
        115                 120                 125
Asn Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg
    130                 135                 140
Thr Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn
145                 150                 155                 160
Lys Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile
                165                 170                 175
Asp Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro
            180                 185                 190
Thr Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp
        195                 200                 205
His Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr
    210                 215                 220
Thr Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn
225                 230                 235                 240
Val Asn Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser
                245                 250                 255
Ile Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile
            260                 265                 270
Phe Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe
        275                 280                 285
Asp Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn
    290                 295                 300
Glu Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val
305                 310                 315                 320
Phe Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala
                325                 330                 335
Phe Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr
            340                 345                 350
Ser Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly
        355                 360                 365
Asp Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile
    370                 375                 380
Arg Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Leu Ala
385                 390                 395                 400
Tyr Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala
                405                 410                 415
Gly Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr
            420                 425                 430
Thr Ser Gly Lys Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln
        435                 440                 445
Tyr Glu Ile Leu Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu
    450                 455                 460
Val Ile Thr Lys Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser
465                 470                 475                 480
Pro Phe Ser Thr Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg
                485                 490                 495
Ile Met Ala Arg Glu Cys Thr Gly Leu Ala Phe Glu Trp Trp Arg Lys
            500                 505                 510
Val Ile Asp Glu Arg Asp Val Lys Leu Ser Lys Glu Ile Asn Val Asn
        515                 520                 525
Ile Ser Gly Ser Thr Leu Ser Pro Tyr Gly Ser Ile Thr Tyr Lys
    530                 535                 540
```

<210> 26
<211> 543
<212> PRT
<213> S. pyogenes

<400> 26

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Met Ala Ser Glu Ser Asn Lys Gln Asn Thr Ala Ser Thr Glu Thr Thr
 1               5                  10                  15
Thr Thr Asn Glu Gln Pro Lys Pro Glu Ser Ser Glu Leu Thr Thr Glu
            20                  25                  30
Lys Ala Gly Gln Lys Thr Asp Asp Met Leu Asn Ser Asn Asp Met Ile
        35                  40                  45
Lys Leu Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu
    50                  55                  60
Lys Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu
65                  70                  75                  80
Ile Asn Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu
                85                  90                  95
Ala Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val
            100                 105                 110
Lys Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile
        115                 120                 125
Asn Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg
    130                 135                 140
Thr Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn
145                 150                 155                 160
Lys Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile
                165                 170                 175
Asp Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro
            180                 185                 190
Thr Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp
        195                 200                 205
His Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr
    210                 215                 220
Thr Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn
225                 230                 235                 240
Val Asn Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser
                245                 250                 255
Ile Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile
            260                 265                 270
Phe Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe
        275                 280                 285
Asp Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn
    290                 295                 300
Glu Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val
305                 310                 315                 320
Phe Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala
                325                 330                 335
Phe Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr
            340                 345                 350
Ser Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly
        355                 360                 365
Asp Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile
    370                 375                 380
Arg Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Leu Ala
385                 390                 395                 400
Tyr Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala
                405                 410                 415
Gly Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr
            420                 425                 430
```

```
Thr Ser Gly Lys Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln
        435                 440                 445
Tyr Glu Ile Leu Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu
    450                 455                 460
Val Ile Thr Lys Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser
465                 470                 475                 480
Pro Phe Ser Thr Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg
                485                 490                 495
Ile Met Ala Arg Glu Gly Thr Gly Leu Ala Trp Glu Trp Trp Arg Lys
            500                 505                 510
Val Ile Asp Glu Arg Asp Val Lys Leu Ser Lys Glu Ile Asn Val Asn
        515                 520                 525
Ile Ser Gly Ser Thr Leu Ser Pro Tyr Gly Ser Ile Thr Tyr Lys
    530                 535                 540

<210> 27
<211> 543
<212> PRT
<213> S. pyogenes

<400> 27
Met Ala Ser Glu Ser Asn Lys Gln Asn Thr Ala Ser Thr Glu Thr Thr
 1                5                  10                  15
Thr Thr Asn Glu Gln Pro Lys Pro Glu Ser Ser Glu Leu Thr Thr Glu
            20                  25                  30
Lys Ala Gly Gln Lys Thr Asp Asp Met Leu Asn Ser Asn Asp Met Ile
        35                  40                  45
Lys Leu Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu
    50                  55                  60
Lys Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu
65                  70                  75                  80
Ile Asn Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu
                85                  90                  95
Ala Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val
            100                 105                 110
Lys Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile
        115                 120                 125
Asn Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg
    130                 135                 140
Thr Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn
145                 150                 155                 160
Lys Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile
                165                 170                 175
Asp Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro
            180                 185                 190
Thr Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp
        195                 200                 205
His Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr
    210                 215                 220
Thr Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn
225                 230                 235                 240
Val Asn Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser
                245                 250                 255
Ile Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile
            260                 265                 270
Phe Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe
        275                 280                 285
Asp Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn
    290                 295                 300
Glu Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val
305                 310                 315                 320
```

```
Phe Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala
                325                 330                 335
Phe Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr
            340                 345                 350
Ser Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly
        355                 360                 365
Asp Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile
    370                 375                 380
Arg Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Leu Ala
385                 390                 395                 400
Tyr Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala
                405                 410                 415
Gly Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr
            420                 425                 430
Thr Ser Gly Lys Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln
        435                 440                 445
Tyr Glu Ile Leu Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu
    450                 455                 460
Val Ile Thr Lys Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser
465                 470                 475                 480
Pro Phe Ser Thr Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg
                485                 490                 495
Ile Met Ala Arg Glu Gly Thr Gly Leu Ala Phe Glu Trp Trp Arg Lys
            500                 505                 510
Val Ile Asp Glu Arg Asp Val Lys Leu Ser Lys Glu Ile Asn Val Asn
        515                 520                 525
Ile Ser Gly Ser Thr Leu Ser Pro Tyr Gly Ser Ile Thr Tyr Lys
    530                 535                 540
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<210> 28
<211> 1632
<212> DNA
<213> S. pyogenes

<400> 28

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atggctagcg aatcgaacaa acaaaacact gctagtacag aaaccacaac gacaaatgag 60
caaccaaagc cagaaagtag tgagctaact actgaaaaag caggtcagaa aacggatgat 120
atgcttaact ctaacgatat gattaagctt gctcccaaag aaatgccact agaatctgca 180
gaaaaagaag aaaaaaagtc agaagacaaa aaaaagagcg aagaagatca cactgaagaa 240
atcaatgaca agatttattc actaaattat aatgagcttg aagtacttgc taaaaatggt 300
gaaaccattg aaaattttgt tcctaaagaa ggcgttaaga aagctgataa atttattgtc 360
attgaaagaa agaaaaaaaa tatcaacact acaccagtcg atatttccat tattgactct 420
gtcactgata ggacctatcc agcagccctt cagctggcta ataaaggttt taccgaaaac 480
aaaccagacg cggtagtcac caagcgaaac ccacaaaaaa tccatattga tttaccaggt 540
atgggagaca aagcaacggt tgaggtcaat gaccctacct atgccaatgt ttcaacagct 600
attgataatc ttgttaacca atggcatgat aattattctg gtggtaatac gcttcctgcc 660
agaacacaat atactgaatc aatggtatat tctaagtcac agattgaggc agctctaaat 720
gttaatagca aaatcttaga tggtacttta ggcattgatt tcaagtcgat ttcaaaaggt 780
gaaaagaagg tgatgattgc agcatacaag caaatttttt acaccgtatc agcaaacctt 840
cctaataatc ctgcggatgt gtttgataaa tcggtgacct ttaaagagtt gcaacgaaaa 900
ggtgtcagca atgaagctcc gccactcttt gtgagtaacg tagcctatgg tcgaactgtt 960
tttgtcaaac tagaaacaag ttctaaaagt aatgatgttg aagcggcctt tagtgcagct 1020
ctaaaaggaa cagatgttaa aactaatgga aaatattctg atatcttaga aaatagctca 1080
tttacagctg tcgttttagg aggagatgct gcagagcaca ataaggtagt cacaaaagac 1140
tttgatgtta ttagaaacgt tatcaaagac aatgctacct tcagtagaaa aaacctagct 1200
tatcctattt catacaccag tgttttcctt aaaaataata aaattgcggg tgtcaataac 1260
agaactgaat acgttgaaac aacatctacc gagtacacta gtggaaaaat taacctgtct 1320
catcaaggcg cgtatgttgc tcaatatgaa atcctttggg atgaaatcaa ttatgatgac 1380
aaaggaaaag aagtgattac aaaacgacgt tgggacaaca actggtatag taagacatca 1440
ccatttagca cagttatccc actaggagct aattcacgaa atatccgtat catggctaga 1500
gagtgcactg gcttagcttg ggaatggtgg cgaaaagtga tcgacgaaag agatgtgaaa 1560
```

ctgtctaaag aaatcaatgt caatatctca ggatcaacct tgagcccata tggttcgatt 1620
acttataagt ag 1632

<210> 29
<211> 1632
<212> DNA
<213> S. pyogenes

<400> 29
atggctagcg aatcgaacaa acaaaacact gctagtacag aaaccacaac gacaaatgag 60
caaccaaagc cagaaagtag tgagctaact actgaaaaag caggtcagaa aacggatgat 120
atgcttaact ctaacgatat gattaagctt gctcccaaag aaatgccact agaatctgca 180
gaaaaagaag aaaaaaagtc agaagacaaa aaaaagagcg aagaagatca cactgaagaa 240
atcaatgaca agatttattc actaaattat aatgagcttg aagtacttgc taaaaatggt 300
gaaaccattg aaaattttgt tcctaaagaa ggcgttaaga aagctgataa atttattgtc 360
attgaaagaa agaaaaaaaa tatcaacact acaccagtcg atatttccat tattgactct 420
gtcactgata ggacctatcc agcagccctt cagctggcta ataaaggttt taccgaaaac 480
aaaccagacg cggtagtcac caagcgaaac ccacaaaaaa tccatattga tttaccaggt 540
atgggagaca aagcaacggt tgaggtcaat gaccctacct atgccaatgt ttcaacagct 600
attgataatc ttgttaacca atggcatgat aattattctg gtggtaatac gcttcctgcc 660
agaacacaat atactgaatc aatggtatat tctaagtcac agattgaggc agctctaaat 720
gttaatagca aaatcttaga tggtacttta ggcattgatt tcaagtcgat ttcaaaaggt 780
gaaaagaagg tgatgattgc agcatacaag caaatttttt acaccgtatc agcaaacctt 840
cctaataatc ctgcggatgt gtttgataaa tcggtgacct ttaaagagtt gcaacgaaaa 900
ggtgtcagca atgaagctcc gccactcttt gtgagtaacg tagcctatgg tcgaactgtt 960
tttgtcaaac tagaaacaag ttctaaaagt aatgatgttg aagcggcctt tagtgcagct 1020
ctaaaaggaa cagatgttaa aactaatgga aaatattctg atatcttaga aaatagctca 1080
tttacagctg tcgttttagg aggagatgct gcagagcaca ataaggtagt cacaaaagac 1140
tttgatgtta ttagaaacgt tatcaaagac aatgctacct tcagtagaaa aaacctagct 1200
tatcctattt catacaccag tgttttcctt aaaaataata aaattgcggg tgtcaataac 1260
agaactgaat acgttgaaac aacatctacc gagtacacta gtggaaaaat taacctgtct 1320
catcaaggcg cgtatgttgc tcaatatgaa atcctttggg atgaaatcaa ttatgatgac 1380
aaaggaaaag aagtgattac aaaacgacgt tgggacaaca actggtatag taagacatca 1440
ccatttagca cagttatccc actaggagct aattcacgaa atatccgtat catggctaga 1500
gagggcactg gcttagcttt cgaatggtgg cgaaaagtga tcgacgaaag agatgtgaaa 1560
ctgtctaaag aaatcaatgt caatatctca ggatcaacct tgagcccata tggttcgatt 1620
acttataagt ga 1632

<210> 30
<211> 1632
<212> DNA
<213> S. pyogenes

<400> 30
atggctagcg aatcgaacaa acaaaacact gctagtacag aaaccacaac gacaaatgag 60
caaccaaagc cagaaagtag tgagctaact actgaaaaag caggtcagaa aacggatgat 120
atgcttaact ctaacgatat gattaagctt gctcccaaag aaatgccact agaatctgca 180
gaaaaagaag aaaaaaagtc agaagacaaa aaaaagagcg aagaagatca cactgaagaa 240
atcaatgaca agatttattc actaaattat aatgagcttg aagtacttgc taaaaatggt 300
gaaaccattg aaaattttgt tcctaaagaa ggcgttaaga aagctgataa atttattgtc 360
attgaaagaa agaaaaaaaa tatcaacact acaccagtcg atatttccat tattgactct 420
gtcactgata ggacctatcc agcagccctt cagctggcta ataaaggttt taccgaaaac 480
aaaccagacg cggtagtcac caagcgaaac ccacaaaaaa tccatattga tttaccaggt 540
atgggagaca aagcaacggt tgaggtcaat gaccctacct atgccaatgt ttcaacagct 600
attgataatc ttgttaacca atggcatgat aattattctg gtggtaatac gcttcctgcc 660
agaacacaat atactgaatc aatggtatat tctaagtcac agattgaggc agctctaaat 720
gttaatagca aaatcttaga tggtacttta ggcattgatt tcaagtcgat ttcaaaaggt 780
gaaaagaagg tgatgattgc agcatacaag caaatttttt acaccgtatc agcaaacctt 840
cctaataatc ctgcggatgt gtttgataaa tcggtgacct ttaaagagtt gcaacgaaaa 900
ggtgtcagca atgaagctcc gccactcttt gtgagtaacg tagcctatgg tcgaactgtt 960
tttgtcaaac tagaaacaag ttctaaaagt aatgatgttg aagcggcctt tagtgcagct 1020

ctaaaaggaa cagatgttaa aactaatgga aaatattctg atatcttaga aaatagctca 1080
tttacagctg tcgttttagg aggagatgct gcagagcaca ataaggtagt cacaaaagac 1140
tttgatgtta ttagaaacgt tatcaaagac aatgctacct tcagtagaaa aaacctagct 1200
tatcctattt catacaccag tgttttcctt aaaaataata aaattgcggg tgtcaataac 1260
agaactgaat acgttgaaac aacatctacc gagtacacta gtggaaaaat taacctgtct 1320
catcaaggcg cgtatgttgc tcaatatgaa atcctttggg atgaaatcaa ttatgatgac 1380
aaaggaaaag aagtgattac aaaacgacgt tgggacaaca actggtatag taagacatca 1440
ccatttagca cagttatccc actaggagct aattcacgaa atatccgtat catggctaga 1500
gagggcactg gcttagcttt cgaatggtgg cgaaaagtga tcgacgaaag agatgtgaaa 1560
ctgtctaaag aaatcaatgt caatatctca ggatcaacct tgagcccata tggttcgatt 1620
acttataagt ga 1632

<210> 31
<211> 1632
<212> DNA
<213> S. pyogenes

<400> 31
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caaccaaagc cagaaagtag tgagctaact actgaaaaag caggtcagaa aacggatgat 120
atgcttaact ctaacgatat gattaagctt gctcccaaag aaatgccact agaatctgca 180
gaaaaagaag aaaaaaagtc agaagacaaa aaaaagagcg aagaagatca cactgaagaa 240
atcaatgaca agatttattc actaaattat aatgagcttg aagtacttgc taaaaatggt 300
gaaaccattg aaaattttgt tcctaaagaa ggcgttaaga aagctgataa atttattgtc 360
attgaaagaa agaaaaaaaa tatcaacact acaccagtcg atatttccat tattgactct 420
gtcactgata ggacctatcc agcagccctt cagctggcta ataaaggttt taccgaaaac 480
aaaccagacg cggtagtcac caagcgaaac ccacaaaaaa tccatattga tttaccaggt 540
atgggagaca aagcaacggt tgaggtcaat gaccctacct atgccaatgt ttcaacagct 600
attgataatc ttgttaacca atggcatgat aattattctg gtggtaatac gcttcctgcc 660
agaacacaat atactgaatc aatggtatat tctaagtcac agattgaggc agctctaaat 720
gttaatagca aaatcttaga tggtacttta ggcattgatt tcaagtcgat ttcaaaaggt 780
gaaaagaagg tgatgattgc agcatacaag caaatttttt acaccgtatc agcaaacctt 840
cctaataatc ctgcggatgt gtttgataaa tcggtgacct ttaaagagtt gcaacgaaaa 900
ggtgtcagca atgaagctcc gccactcttt gtgagtaacg tagcctatgg tcgaactgtt 960
tttgtcaaac tagaaacaag ttctaaaagt aatgatgttg aagcggcctt tagtgcagct 1020
ctaaaaggaa cagatgttaa aactaatgga aaatattctg atatcttaga aaatagctca 1080
tttacagctg tcgttttagg aggagatgct gcagagcaca ataaggtagt cacaaaagac 1140
tttgatgtta ttagaaacgt tatcaaagac aatgctacct tcagtagaaa aaacctagct 1200
tatcctattt catacaccag tgttttcctt aaaaataata aaattgcggg tgtcaataac 1260
agaactgaat acgttgaaac aacatctacc gagtacacta gtggaaaaat taacctgtct 1320
catcaaggcg cgtatgttgc tcaatatgaa atcctttggg atgaaatcaa ttatgatgac 1380
aaaggaaaag aagtgattac aaaacgacgt tgggacaaca actggtatag taagacatca 1440
ccatttagca cagttatccc actaggagct aattcacgaa atatccgtat catggctaga 1500
gagtgcactg gcttagcttt cgaatggtgg cgaaaagtga tcgacgaaag agatgtgaaa 1560
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acttataagt ag 1632

<210> 32
<211> 1632
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<213> S. pyogenes

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atgcttaact ctaacgatat gattaagctt gctcccaaag aaatgccact agaatctgca 180
gaaaaagaag aaaaaaagtc agaagacaaa aaaaagagcg aagaagatca cactgaagaa 240
atcaatgaca agatttattc actaaattat aatgagcttg aagtacttgc taaaaatggt 300
gaaaccattg aaaattttgt tcctaaagaa ggcgttaaga aagctgataa atttattgtc 360
attgaaagaa agaaaaaaaa tatcaacact acaccagtcg atatttccat tattgactct 420
gtcactgata ggacctatcc agcagccctt cagctggcta ataaaggttt taccgaaaac 480

aaaccagacg cggtagtcac caagcgaaac ccacaaaaaa tccatattga tttaccaggt 540
atgggagaca aagcaacggt tgaggtcaat gaccctacct atgccaatgt ttcaacagct 600
attgataatc ttgttaacca atggcatgat aattattctg gtggtaatac gcttcctgcc 660
agaacacaat atactgaatc aatggtatat tctaagtcac agattgaggc agctctaaat 720
gttaatagca aaatcttaga tggtacttta ggcattgatt tcaagtcgat ttcaaaaggt 780
gaaaagaagg tgatgattgc agcatacaag caaatttttt acaccgtatc agcaaacctt 840
cctaataatc ctgcggatgt gtttgataaa tcggtgacct ttaaagagtt gcaacgaaaa 900
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gagggcactg gcttagcttg ggaatggtgg cgaaaagtga tcgacgaaag agatgtgaaa 1560
ctgtctaaag aaatcaatgt caatatctca ggatcaacct tgagcccata tggttcgatt 1620
acttataagt ag 1632

<210> 33
<211> 1632
<212> DNA
<213> S. pyogenes

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atgcttaact ctaacgatat gattaagctt gctcccaaag aaatgccact agaatctgca 180
gaaaaagaag aaaaaaagtc agaagacaaa aaaaagagcg aagaagatca cactgaagaa 240
atcaatgaca agatttattc actaaattat aatgagcttg aagtacttgc taaaaatggt 300
gaaaccattg aaaatttttgt tcctaaagaa ggcgttaaga aagctgataa atttattgtc 360
attgaaagaa agaaaaaaaa tatcaacact acaccagtcg atatttccat tattgactct 420
gtcactgata ggacctatcc agcagccctt cagctggcta ataaaggttt taccgaaaac 480
aaaccagacg cggtagtcac caagcgaaac ccacaaaaaa tccatattga tttaccaggt 540
atgggagaca aagcaacggt tgaggtcaat gaccctacct atgccaatgt ttcaacagct 600
attgataatc ttgttaacca atggcatgat aattattctg gtggtaatac gcttcctgcc 660
agaacacaat atactgaatc aatggtatat tctaagtcac agattgaggc agctctaaat 720
gttaatagca aaatcttaga tggtacttta ggcattgatt tcaagtcgat ttcaaaaggt 780
gaaaagaagg tgatgattgc agcatacaag caaatttttt acaccgtatc agcaaacctt 840
cctaataatc ctgcggatgt gtttgataaa tcggtgacct ttaaagagtt gcaacgaaaa 900
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ccatttagca cagttatccc actaggagct aattcacgaa atatccgtat catggctaga 1500
gagggcactg gcttagcttt cgaatggtgg cgaaaagtga tcgacgaaag agatgtgaaa 1560
ctgtctaaag aaatcaatgt caatatctca ggatcaacct tgagcccata tggttcgatt 1620
acttataagt ga 1632

<210> 34
<211> 35
<212> DNA
<213> S. pyogenes

<400> 34
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<210> 35
<211> 42
<212> DNA
<213> S. pyogenes

<400> 35
gcattcgatc ctcgagctac ttataagtaa tcgaaccata tg 42

<210> 36
<211> 45
<212> DNA
<213> S. pyogenes

<400> 36
gctaccttca gtagaaaaaa cctagcttat cctatttcat acacc 45

<210> 37
<211> 45
<212> DNA
<213> S. pyogenes

<400> 37
ggtgtatgaa ataggataag ctaggttttt tctactgaag gtagc 45

<210> 38
<211> 41
<212> DNA
<213> S. pyogenes

<400> 38
gcattcgatc ctcgagctta taagtaatcg aaccatatgg g 41

<210> 39
<211> 42
<212> DNA
<213> S. pyogenes

<400> 39
gagtgcactg gcttagcttt cgaatggtgg cgaaaagtga tc 42

<210> 40
<211> 42
<212> DNA
<213> S. pyogenes

<400> 40
gatcactttt cgccaccatt cgaaagctaa gccagtgcac tc 42

<210> 41
<211> 55
<212> DNA
<213> S. pyogenes

<400> 41
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<210> 42
<211> 55

<212> DNA
<213> S. pyogenes

<400> 42
cttttccttt gtcatcataa ttgatttcat tccaaaggat ttcatattga gcaac 55

<210> 43
<211> 42
<212> DNA
<213> S. pyogenes

<400> 43
ccgtatcatg gctagagagg gcactggctt agcttgggaa tg 42

<210> 44
<211> 42
<212> DNA
<213> S. pyogenes

<400> 44
cattcccaag ctaagccagt gccctctcta gccatgatac gg 42

<210> 45
<211> 30
<212> DNA
<213> S. pyogenes

<400> 45
gtgcgtgcta gcgaatcgaa caaacaaaac 30

<210> 46
<211> 34
<212> DNA
<213> S. pyogenes

<400> 46
gcgtctcgag tcacttataa gtaatcgaac cata 34

<210> 47
<211> 42
<212> DNA
<213> S. pyogenes

<400> 47
ccgtatcatg gctagagagg gcactggctt agctttcgaa tg 42

<210> 48
<211> 42
<212> DNA
<213> S. pyogenes

<400> 48
cattcgaaag ctaagccagt gccctctcta gccatgatac gg 42

<210> 49
<211> 45
<212> DNA
<213> S. pyogenes

<400> 49
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<210> 50
<211> 45
<212> DNA
<213> S. pyogenes

<400> 50
ccattgattc agtatattgt gttctaggaa gcgtattacc accag 45

<210> 51
<211> 1656
<212> DNA
<213> S. pyogenes

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atgcttaact ctaacgatat gattaagctt gctcccaaag aaatgccact agaatctgca 180
gaaaaagaag aaaaaaagtc agaagacaaa aaaaagagcg aagaagatca cactgaagaa 240
atcaatgaca agatttattc actaaattat aatgagcttg aagtacttgc taaaaatggt 300
gaaaccattg aaaattttgt tcctaaagaa ggcgttaaga aagctgataa atttattgtc 360
attgaaagaa agaaaaaaaa tatcaacact acaccagtcg atatttccat tattgactct 420
gtcactgata ggacctatcc agcagcccct cagctggcta ataaaggttt taccgaaaac 480
aaaccagacg cggtagtcac caagcgaaac ccacaaaaaa tccatattga tttaccaggt 540
atgggagaca aagcaacggt tgaggtcaat gaccctacct atgccaatgt ttcaacagct 600
attgataatc ttgttaacca atggcatgat aattattctg gtggtaatac gcttcctgcc 660
agaacacaat atactgaatc aatggtatat tctaagtcac agattgaagc agctctaaat 720
gttaatagca aaatcttaga tggtacttta ggcattgatt tcaagtcgat ttcaaaaggt 780
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ggtgtcagca atgaagctcc gccactcttt gtgagtaacg tagcctatgg tcgaactgtt 960
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gagtgcactg gcttagcttt cgaatggtgg cgaaaagtga tcgacgaaag agatgtgaaa 1560
ctgtctaaag aaatcaatgt caatatctca ggatcaacct tgagcccata tggttcgatt 1620
acttataagc tcgagcacca ccaccaccac cactga 1656

<210> 52
<211> 1656
<212> DNA
<213> S. pyogenes

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caaccaaagc cagaaagtag tgagctaact actgaaaaag caggtcagaa aacggatgat 120
atgcttaact ctaacgatat gattaagctt gctcccaaag aaatgccact agaatctgca 180
gaaaaagaag aaaaaaagtc agaagacaaa aaaaagagcg aagaagatca cactgaagaa 240
atcaatgaca agatttattc actaaattat aatgagcttg aagtacttgc taaaaatggt 300
gaaaccattg aaaattttgt tcctaaagaa ggcgttaaga aagctgataa atttattgtc 360
attgaaagaa agaaaaaaaa tatcaacact acaccagtcg atatttccat tattgactct 420
gtcactgata ggacctatcc agcagcccct cagctggcta ataaaggttt taccgaaaac 480
aaaccagacg cggtagtcac caagcgaaac ccacaaaaaa tccatattga tttaccaggt 540
atgggagaca aagcaacggt tgaggtcaat gaccctacct atgccaatgt ttcaacagct 600
attgataatc ttgttaacca atggcatgat aattattctg gtggtaatac gcttcctgcc 660

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agaacacaat atactgaatc aatggtatat tctaagtcac agattgaagc agctctaaat 720
gttaatagca aaatcttaga tggtacttta ggcattgatt tcaagtcgat ttcaaaaggt 780
gaaaagaagg tgatgattgc agcatacaag caaatttttt acaccgtatc agcaaacctt 840
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gagtgcactg gcttagcttt cgaatggtgg cgaaaagtga tcgacgaaag agatgtgaaa 1560
ctgtctaaag aaatcaatgt caatatctca ggatcaacct tgagcccata tggttcgatt 1620
acttataagc tcgagcacca ccaccaccac cactga                          1656

<210> 53
<211> 551
<212> PRT
<213> S. pyogenes

<400> 53
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            20                  25                  30
Lys Ala Gly Gln Lys Thr Asp Asp Met Leu Asn Ser Asn Asp Met Ile
        35                  40                  45
Lys Leu Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu
    50                  55                  60
Lys Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu
65                  70                  75                  80
Ile Asn Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu
                85                  90                  95
Ala Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val
            100                 105                 110
Lys Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile
        115                 120                 125
Asn Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg
    130                 135                 140
Thr Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn
145                 150                 155                 160
Lys Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile
                165                 170                 175
Asp Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro
            180                 185                 190
Thr Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp
        195                 200                 205
His Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr
    210                 215                 220
Thr Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn
225                 230                 235                 240
Val Asn Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser
                245                 250                 255
Ile Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile
            260                 265                 270
Phe Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe
        275                 280                 285
Arg Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn
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    290                 295                 300
Glu Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val
305                 310                 315                 320
Phe Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala
                325                 330                 335
Phe Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr
            340                 345                 350
Ser Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly
        355                 360                 365
Asp Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile
    370                 375                 380
Arg Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Leu Ala
385                 390                 395                 400
Tyr Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala
                405                 410                 415
Gly Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr
            420                 425                 430
Thr Ser Gly Lys Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln
        435                 440                 445
Tyr Glu Ile Leu Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu
    450                 455                 460
Val Ile Thr Lys Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser
465                 470                 475                 480
Pro Phe Ser Thr Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg
                485                 490                 495
Ile Met Ala Arg Glu Gly Thr Gly Leu Ala Trp Glu Trp Trp Arg Lys
            500                 505                 510
Val Ile Asp Glu Arg Asp Val Lys Leu Ser Lys Glu Ile Asn Val Asn
        515                 520                 525
Ile Ser Gly Ser Thr Leu Ser Pro Tyr Gly Ser Ile Thr Tyr Lys Leu
    530                 535                 540
Glu His His His His His His
545                 550
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<210> 54
<211> 551
<212> PRT
<213> S. pyogenes

<400> 54

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Met Ala Ser Glu Ser Asn Lys Gln Asn Thr Ala Ser Thr Glu Thr Thr
 1                5                  10                  15
Thr Thr Asn Glu Gln Pro Lys Pro Glu Ser Ser Glu Leu Thr Thr Glu
            20                  25                  30
Lys Ala Gly Gln Lys Thr Asp Asp Met Leu Asn Ser Asn Asp Met Ile
        35                  40                  45
Lys Leu Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu
    50                  55                  60
Lys Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu
65                  70                  75                  80
Ile Asn Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu
                85                  90                  95
Ala Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val
            100                 105                 110
Lys Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile
        115                 120                 125
Asn Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg
    130                 135                 140
Thr Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn
145                 150                 155                 160
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Lys Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile
                165                 170                 175
Asp Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro
            180                 185                 190
Thr Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp
        195                 200                 205
His Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr
    210                 215                 220
Thr Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn
225                 230                 235                 240
Val Asn Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser
                245                 250                 255
Ile Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile
            260                 265                 270
Phe Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe
        275                 280                 285
Asp Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn
    290                 295                 300
Glu Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val
305                 310                 315                 320
Phe Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala
                325                 330                 335
Phe Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr
            340                 345                 350
Ser Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly
        355                 360                 365
Asp Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile
    370                 375                 380
Arg Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Leu Ala
385                 390                 395                 400
Tyr Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala
                405                 410                 415
Gly Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr
            420                 425                 430
Thr Ser Gly Lys Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln
        435                 440                 445
Tyr Glu Ile Leu Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu
    450                 455                 460
Val Ile Thr Lys Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser
465                 470                 475                 480
Pro Phe Ser Thr Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg
                485                 490                 495
Ile Met Ala Arg Glu Cys Thr Gly Leu Ala Phe Glu Trp Trp Arg Lys
            500                 505                 510
Val Ile Asp Glu Arg Asp Val Lys Leu Ser Lys Glu Ile Asn Val Asn
        515                 520                 525
Ile Ser Gly Ser Thr Leu Ser Pro Tyr Gly Ser Ile Thr Tyr Lys Leu
    530                 535                 540
Glu His His His His His His
545                 550
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<210> 55
<211> 551
<212> PRT
<213> S. pyogenes

<400> 55

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Met Ala Ser Glu Ser Asn Lys Gln Asn Thr Ala Ser Thr Glu Thr Thr
 1               5                  10                  15
Thr Thr Asn Glu Gln Pro Lys Pro Glu Ser Ser Glu Leu Thr Thr Glu
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            20                  25                  30
Lys Ala Gly Gln Lys Thr Asp Asp Met Leu Asn Ser Asn Asp Met Ile
        35                  40                  45
Lys Leu Ala Pro Lys Glu Met Pro Leu Glu Ser Ala Glu Lys Glu Glu
    50                  55                  60
Lys Lys Ser Glu Asp Lys Lys Lys Ser Glu Glu Asp His Thr Glu Glu
65                  70                  75                  80
Ile Asn Asp Lys Ile Tyr Ser Leu Asn Tyr Asn Glu Leu Glu Val Leu
                85                  90                  95
Ala Lys Asn Gly Glu Thr Ile Glu Asn Phe Val Pro Lys Glu Gly Val
            100                 105                 110
Lys Lys Ala Asp Lys Phe Ile Val Ile Glu Arg Lys Lys Lys Asn Ile
        115                 120                 125
Asn Thr Thr Pro Val Asp Ile Ser Ile Ile Asp Ser Val Thr Asp Arg
    130                 135                 140
Thr Tyr Pro Ala Ala Leu Gln Leu Ala Asn Lys Gly Phe Thr Glu Asn
145                 150                 155                 160
Lys Pro Asp Ala Val Val Thr Lys Arg Asn Pro Gln Lys Ile His Ile
                165                 170                 175
Asp Leu Pro Gly Met Gly Asp Lys Ala Thr Val Glu Val Asn Asp Pro
            180                 185                 190
Thr Tyr Ala Asn Val Ser Thr Ala Ile Asp Asn Leu Val Asn Gln Trp
        195                 200                 205
His Asp Asn Tyr Ser Gly Gly Asn Thr Leu Pro Ala Arg Thr Gln Tyr
    210                 215                 220
Thr Glu Ser Met Val Tyr Ser Lys Ser Gln Ile Glu Ala Ala Leu Asn
225                 230                 235                 240
Val Asn Ser Lys Ile Leu Asp Gly Thr Leu Gly Ile Asp Phe Lys Ser
                245                 250                 255
Ile Ser Lys Gly Glu Lys Lys Val Met Ile Ala Ala Tyr Lys Gln Ile
            260                 265                 270
Phe Tyr Thr Val Ser Ala Asn Leu Pro Asn Asn Pro Ala Asp Val Phe
        275                 280                 285
Asp Lys Ser Val Thr Phe Lys Glu Leu Gln Arg Lys Gly Val Ser Asn
    290                 295                 300
Glu Ala Pro Pro Leu Phe Val Ser Asn Val Ala Tyr Gly Arg Thr Val
305                 310                 315                 320
Phe Val Lys Leu Glu Thr Ser Ser Lys Ser Asn Asp Val Glu Ala Ala
                325                 330                 335
Phe Ser Ala Ala Leu Lys Gly Thr Asp Val Lys Thr Asn Gly Lys Tyr
            340                 345                 350
Ser Asp Ile Leu Glu Asn Ser Ser Phe Thr Ala Val Val Leu Gly Gly
        355                 360                 365
Asp Ala Ala Glu His Asn Lys Val Val Thr Lys Asp Phe Asp Val Ile
    370                 375                 380
Arg Asn Val Ile Lys Asp Asn Ala Thr Phe Ser Arg Lys Asn Leu Ala
385                 390                 395                 400
Tyr Pro Ile Ser Tyr Thr Ser Val Phe Leu Lys Asn Asn Lys Ile Ala
                405                 410                 415
Gly Val Asn Asn Arg Thr Glu Tyr Val Glu Thr Thr Ser Thr Glu Tyr
            420                 425                 430
Thr Ser Gly Lys Ile Asn Leu Ser His Gln Gly Ala Tyr Val Ala Gln
        435                 440                 445
Tyr Glu Ile Leu Trp Asp Glu Ile Asn Tyr Asp Asp Lys Gly Lys Glu
    450                 455                 460
Val Ile Thr Lys Arg Arg Trp Asp Asn Asn Trp Tyr Ser Lys Thr Ser
465                 470                 475                 480
Pro Phe Ser Thr Val Ile Pro Leu Gly Ala Asn Ser Arg Asn Ile Arg
                485                 490                 495
Ile Met Ala Arg Glu Gly Thr Gly Leu Ala Phe Glu Trp Trp Arg Lys
            500                 505                 510
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Val Ile Asp Glu Arg Asp Val Lys Leu Ser Lys Glu Ile Asn Val Asn
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Ile Ser Gly Ser Thr Leu Ser Pro Tyr Gly Ser Ile Thr Tyr Lys Leu
    530                 535                 540
Glu His His His His His His
545                 550
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<212> DNA
<213> S. pyogenes

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atgcttaact ctaacgatat gattaagctt gctcccaaag aaatgccact agaatctgca 180
gaaaaagaag aaaaaaagtc agaagacaaa aaaaagagcg aagaagatca cactgaagaa 240
atcaatgaca agatttattc actaaattat aatgagcttg aagtacttgc taaaaatggt 300
gaaaccattg aaaatttttgt tcctaaagaa ggcgttaaga aagctgataa atttattgtc 360
attgaaagaa agaaaaaaaa tatcaacact acaccagtcg atatttccat tattgactct 420
gtcactgata ggacctatcc agcagccctt cagctggcta ataaaggttt taccgaaaac 480
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attgataatc ttgttaacca atggcatgat aattattctg gtggtaatac gcttcctgcc 660
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gttaatagca aaatcttaga tggtacttta ggcattgatt tcaagtcgat ttcaaaaggt 780
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<210> 57
<211> 1656
<212> DNA
<213> S. pyogenes

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atgcttaact ctaacgatat gattaagctt gctcccaaag aaatgccact agaatctgca 180
gaaaaagaag aaaaaaagtc agaagacaaa aaaaagagcg aagaagatca cactgaagaa 240
atcaatgaca agatttattc actaaattat aatgagcttg aagtacttgc taaaaatggt 300
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attgaaagaa agaaaaaaaa tatcaacact acaccagtcg atatttccat tattgactct 420
gtcactgata ggacctatcc agcagccctt cagctggcta ataaaggttt taccgaaaac 480
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<212> DNA
<213> S. pyogenes

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atgcttaact ctaacgatat gattaagctt gctcccaaag aaatgccact agaatctgca 180
gaaaaagaag aaaaaaagtc agaagacaaa aaaaagagcg aagaagatca cactgaagaa 240
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attgaaagaa agaaaaaaaa tatcaacact acaccagtcg atatttccat tattgactct 420
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<211> 1653
<212> DNA
<213> S. pyogenes

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atgcttaact ctaacgatat gattaagctt gctcccaaag aaatgccact agaatctgca 180

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tataagctcg agcaccacca ccaccaccac tga                              1653
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