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(54) Title: A POLYPEPTIDE COMPRISING THE AMINO ACID OF AN N–TERMINAL CHOLINE BINDING PROTEIN A TRUNCATE, VACCINE DERIVED THEREFROM AND USES THEREOF

(57) Abstract

This invention provides an isolated polypeptide comprising an amino acid sequence of an N–terminal choline binding protein A truncate in which the amino acid sequence is set forth in any of SEQ ID NOS: 1,3–7, or 9–11, including fragments, mutants, variants, analogs, or derivatives, thereof. Also, this invention provides isolated polypeptides comprising an amino acid sequence of a N–terminal choline binding protein A truncate, wherein the amino acid is set forth in SEQ ID NO 24, wherein the polypeptide retains its native tertiary structure and methods of preparation. This invention provides an isolated polypeptide comprising an amino acid sequence of an N–terminal choline binding protein A truncate, wherein the polypeptide has lectin activity and does not bind to choline. This invention provides an isolated immunogenic polypeptide comprising an amino acid sequence of an N–terminal choline binding protein A truncate. This invention provides an isolated nucleic acid encoding a polypeptide comprising an amino acid sequence of N–terminal choline binding protein A truncate. Lastly, this invention provides pharmaceutical compositions, vaccines, and diagnostic and therapeutic methods of use.

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A POLYPEPTIDE COMPRISING THE AMINO ACID OF AN N-TERMINAL CHOLINE BINDING PROTEIN A TRUNCATE, VACCINE DERIVED THEREFROM AND USES THEREOF

FIELD OF THE INVENTION

The present invention relates generally to a polypeptide of a N-terminal choline binding protein A truncate. The invention also relates to vaccines which provide protection or elicit protective antibodies to bacterial infection, specifically pneumococcus, and to antibodies and antagonists against such polypeptide for use in diagnosis and passive immune therapy. The polypeptide and/or the nucleic acid encoding the polypeptide are also useful as a competitive inhibitor of bacterial adhesin of pneumococcus. Lastly, this invention is directed to therapeutics using the polypeptide.

BACKGROUND OF THE INVENTION

Streptococcus pneumoniae is a gram positive bacteria which is a major cause of invasive infections such as sepsis, meningitis, otitis media and lobar pneumonia (Tuomanen *et al* NEJM 322:1280-1284, 1995). Pneumococci bind avidly to cells of the upper and lower respiratory tract. Like most bacteria, adherence of pneumococci to human cells is achieved by presentation of bacterial surface proteins that bind to eukaryotic carbohydrates in a lectin-like fashion (Cundell, D. & Tuomanen, E. (1994) *Microb Pathog* **17**:361-374). Pneumococci bind to non-inflamed epithelium, a process that can be viewed as asymptomatic carriage. It has been proposed that the conversion to invasive disease involves the local generation of inflammatory factors which, activating the human cell, change the number and type of receptors available on the human cells (Cundell, D. *et al.* (1995) *Nature*, **377**:435-438). Presented with an opportunity in this new setting, pneumococci appear to take advantage and engage one of these unregulated receptors, the platelet activating factor (PAF) receptor (Cundell *et al.* (1995) *Nature*, **377**:435-438. Within minutes of the appearance of the PAF receptor, pneumococci undergo waves of enhanced adherence and invasion. Inhibition of bacterial binding to activated cells, for instance by soluble receptor analogs, blocks the progression to disease

in animal models (Idanpaan-Heikkila, I. *et al.* (1997) *J. Infect. Dis.*, **176**:704-712). Particularly effective in this regard are soluble carbohydrates containing lacto-N-neotetraose with or without an additional sialic acid which prevent pneumococcal attachment to human cells in vitro and prevent colonization in the lung in vivo.

Choline binding proteins: candidate structural adhesin gene:

Pneumococci produce a family of surface proteins capable of binding to the bacterial surface by non-covalent association to the cell wall teichoic acid or lipoteichoic acid. The surface of *Streptococcus pneumoniae* is decorated with a family of CBPs (Choline Binding Proteins) that are non-covalently bound to the phosphorylcholine. CbpA, is an 75 kD surface-exposed choline binding protein that shows a chimeric architecture. There is a unique N-terminal domain a proline rich region followed by a C-terminal domain comprised of 10 repeated region responsible for binding to choline.

CbpA, is an adhesin (ligand) for the glycoconjugate containing receptors present on the surface of eucaryotic cells. Mutants with defects in *cbpA* showed reduced virulence in the infant rat model for nasopharyngeal colonization. This binding is directed to choline determinants which decorate the teichoic acid and is mediated by a signature choline binding domain in each of the members of this family of proteins. The choline binding domain was discovered and fully characterized by Lopez et al. in his studies of the autolytic enzyme (Ronda *et al.* (1987) *Eur. J. Biochem*, **164**:621-624). Other proteins containing this domain include the autolysin of the pneumococcal phage and the protective antigen, pneumococcal surface protein A (PspA) (Ronda, C. *et al.* (1987) *Eur. J. Biochem.*, **164**:621-624 and McDaniel, L.S., *et al.* (1992) *Microb Pathog*, **13**:261-269). CbpA, fails to colonize the nasopharynx domain which is shared with its other family members C terminus) but its activity of binding to human cells arises from its unique N-terminal domain. Since the process of colonization and the progression to disease depend on pneumococcal attachment to human cells as a primary step, interruption of the function of the N terminal domain, either by cross reactive antibody or by competitive inhibition with a peptide mimicking this domain, may be critical to blocking disease.

Choline binding proteins for anti-pneumococcal vaccines are discussed in PCT International Application No. PCT/US97/07198 and such PCT Application is incorporated in its entirety by reference. Current vaccines against S. pneumoniae employ purified carbohydrates of the capsules of the 23 most common serotypes of this bacterium, but such vaccine is only 50% protective (Shapiro et al. NJEM 325:1453, 1991) and is not immunogenic under the age of 2. Further, a therapeutic polypeptide would offer a therapeutic option in cases of infection with multi resistant organisms. Therefore, the invention herein fills a long felt need by providing a protective vaccines.

SUMMARY OF THE INVENTION

The present invention provides an isolated polypeptide comprising an amino acid sequence of a N-terminal choline binding protein A truncate. The polypeptide comprises the amino acid sequence as set forth in SEQ ID NOS 1, 3- 7, or 9-11, including fragments, mutants, variants, analogs, or derivatives, thereof. Also, this invention provides an isolated polypeptide comprising an amino acid sequence of a N-terminal choline binding protein A truncate having the amino acid as set forth in SEQ ID NO 24, wherein the polypeptide exhibits its tertiary structure and methods of preparation such a polypeptide. The isolated polypeptide are suitable for use in immunizing animals and humans against bacterial infection, preferably pneumococci.

In a still further aspect, the present invention extends to an N-terminal choline binding protein A truncate having lectin activity and no choline binding activity. Still further, this invention provides an immunogenic N-terminal choline binding protein A truncate or a fragment thereof.

The present invention also relates to isolated nucleic acids, such as recombinant DNA molecules or cloned genes, or degenerate variants thereof, mutants, analogs, or fragments thereof, which encode the isolated polypeptide or which competitively inhibit the activity of the polypeptide. Preferably, the isolated nucleic acids which includes degenerates, variants, mutants, analogs, or fragments thereof, has a sequence as set forth in SEQ ID

NOS: 12, 14-17, 19-22 or 23. In a further embodiment of the invention, the full DNA sequence of the recombinant DNA molecule or cloned gene so determined may be operatively linked to an expression control sequence which may be introduced into an appropriate host. The invention accordingly extends to unicellular hosts transformed with the cloned gene or recombinant DNA molecule comprising a DNA sequence encoding the present invention, and more particularly, the DNA sequences or fragments thereof determined from the sequences set forth above.

Antibodies against the isolated polypeptide include naturally raised and recombinantly prepared antibodies. These may include both polyclonal and monoclonal antibodies prepared by known genetic techniques, as well as bi-specific (chimeric) antibodies, and antibodies including other functionalities suiting them for diagnostic use conjunctive with their capability of modulating bacterial adherence including but not limited to acting as competitive agents.

It is still a further object of the present invention to provide a method for the treatment of mammals to control the amount or activity of the bacteria or its subunits, so as to treat or avert the adverse consequences of invasive, spontaneous, or idiopathic pathological states. This invention provides pharmaceutical compositions for use in therapeutic methods which comprise or are based upon the isolated polypeptides, their subunits or their binding partners.

Lastly, this invention provides pharmaceutical compositions, vaccines, and diagnostic and therapeutic methods of use thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1. Schematic representation of choline binding protein A (CbpA) and recombinant truncates R1 (from about amino acid 16 to amino acid 321 from the N-terminus of CbpA as set forth in Figure 2) and R2 (from about amino acid 16 to amino acid 444 from the N-terminus of CbpA as

set forth in Figure 2). Domain A is from about amino acid 153 to amino acid 321 from the N-terminus of CbpA amino acid sequence as set forth in Figure 2 ; domain B is from about amino acid 270 to amino acid 326 from the N-terminus of CbpA amino acid sequence as set forth in Figure 2); and C is from about amino acid 327 to amino acid 433 from the N-terminus of CbpA amino acid sequence as set forth in Figure 2.

FIGURES 2A-B

Comparison of homologies of various serotypes of the nucleic acid and amino acid sequence of the N-terminal region of CbpA.

FIGURE 3. Expression and purification of recombinant R1 and R2.

FIGURE 4. Results of passive protection in mice. Immune sera against recombinant R2 protected mice from lethal S. pneumoniae challenge.

FIGURE 5 Titration of anti-R2 antibody on R6x adhering to LNnT-HSA coated plates.

FIGURE 6. Titration of anti-Cbp-A and absorbed anti-CbpA antibodies for activity blocking pneumococcal adherence to LNnT-HSA coated plates.

FIGURE 7. Results of active protection in mice. Immune sera against recombinant R1 protected mice from lethal S. pneumoniae challenge (challenge 560 cfu serotype 6B).

DETAILED DESCRIPTION

The present invention is directed to an isolated polypeptide comprising an amino acid sequence of a N-terminal choline binding protein A truncate. The polypeptides are suitable for use in immunizing animals against pneumococcal infection. These

polypeptide or peptide fragments thereof, when formulated with an appropriate adjuvant, are used in vaccines for protection against pneumococci, and against other bacteria with cross-reactive proteins.

This invention provides an isolated polypeptide comprising an amino acid sequence of a N-terminal choline binding protein A truncate. In one embodiment the polypeptide has the amino acid sequence as set forth in any of SEQ ID NO 1, 3-5, 7, or 9-11 , including fragments, mutants, variants, analogs, or derivatives, thereof. In another embodiment the polypeptide has the amino acid KXXE (SEQ ID NO 6).

This invention provides an isolated polypeptide comprising an amino acid sequence of a N-terminal choline binding protein A truncate as set forth in Figure 2. In one embodiment, the polypeptide has an amino acid sequence which is a conserved region as set forth in Figure 2. For example, conserved regions include but are not limited to amino acid sequence 158 to 210; 158 to 172; 300 to 321; 331 to 339; 355 to 365; 367 to 374; 379 to 389; 409 to 427; and 430 to 447. Figure 2 sets forth homologies of various serotypes of the nucleic acid and amino acid sequence of the N-terminal region of CbpA which are contemplated by this invention.

Further, this invention provides an isolated polypeptide comprising an amino acid sequence of a N-terminal choline binding protein A truncate having the amino acid as set forth in SEQ ID NO 24, wherein the polypeptide exhibits its tertiary structure. In one embodiment the polypeptide is an analog, fragment, mutant, or variant thereof. Variants contemplated are set forth in Figure 2. This invention also provides an isolated polypeptide comprising an amino acid sequence of a N-terminal choline binding protein A truncate having the amino acid from about position 16 to about position 475 of serotype 4 as set forth in Figure 2 or a corresponding amino acid of serotype 4 as shown in Figure 2 , wherein the polypeptide exhibits its tertiary structure. In one embodiment tertiary structure corresponds to that present in the native protein.

Methods of preparation of the polypeptide are for example as follows: cleaving a full length choline binding protein A with hydroxylamine, wherein the hydroxylamine cleaves the choline binding protein A at amino acid Asparagine (N) at position 475 of serotype R6x and serotype 4, or the corresponding amino acid of serotype R6x or serotype 4 in a different serotype as shown in Figure 2, thereby creating the N-terminal choline binding protein A truncate. Alternative methods which create a truncated choline binding protein A or fragment thereof, and retain the native tertiary structure (i.e. that of the full length choline binding protein A) are contemplated and known to those skilled in the art. Because the polypeptide retains its tertiary structure, the isolated polypeptide is suitable for use as an immunogen in immunizing animals and humans against bacterial infection, preferably pneumococci.

The polypeptide comprising the amino acid sequence of choline binding protein A (CbpA) serotype type 4 is as follows:

ENEGATQVPTSSNRANESQAEQGEQPKKLDSERDKARKEVEEYVKKIVGESY
AKSTKKRHTITVALVNELNNIKNEYLNKIVESTSESQLQILMMESRSKVDEAV
SKFEKDSSSSSSSDSSTKPEASDTAKPNKPTEPGEKVAEAKKKVEEAEKKAKD
QKEEDRRNYPTITYKTLELEIAESDVEVKKAELELVKVKANEPRDEQKIKQAE
AEVESKQAEATRLKKIKTDREEAEEEAKRRADAKEQGKPKGRAKRGVPGEL
ATPDKKENDAKSSDSSVGEETLPSPSLKPEKKVAEAEKKVEEAKKKAEDQKE
EDRRNYPTNTYKTLELEIAESDVEVKKAELELVKEEAKEPRNEEKVKQAKAE
VESKKAEATRLEKIKTDRKKAEEEAKRKAAEEDKVKEKPAEQPQPAPAPKAE
KPAPAPKPEN (SEQ ID NO 24).

"Polypeptide R2" means a polypeptide comprising the amino acid sequences from position 16 to position 444 of the N-terminal truncate of choline binding protein A (CbpA) serotype type 4 (see Figure 1) which has the following sequence :

ENEGATQVPTSSNRANESQAEQGEQPKKLDSERDKARKEVEEYVKKIVGESY
AKSTKKRHTITVALVNELNNIKNEYLNKIVESTSESQLQILMMESRSKVDEAV
SKFEKDSSSSSSSDSSTKPEASDTAKPNKPTEPGEKVAEAKKKVEEAEKKAKD
QKEEDRRNYPTITYKTLELEIAESDVEVKKAELELVKVKANEPRDEQKIKQAE

AEVESKQAEATRLKKIKTDREEAEEEAKRRADAKEQGKPKGRAKRGVPGEL
ATPDKKENDAKSSDSSVGEETLPSPSLKPEKKVAEAEKKVEEAKKKAEDQKE
EDRRNYPTNTYKTLELEIAESDVEVKKAELELVKEEAKEPRNEEKVKQAKAE
VESKKAEATRLEKIKTDRKKAEEEAKRKAAEEDKVKEKPA (SEQ ID NO 1).

The DNA sequence which encodes polypeptide R2 of the N-terminal truncate of choline binding protein A (CbpA) serotype type 4:

GAGAACGAGGGAGCTACCCAAGTACCCACTTCTTCTAATAGGGCAAATGA
AAGTCAGGCAGAACAAGGAGAACAACCTAAAAAACTCGATTCAGAACGA
GATAAGGCAAGGAAAGAGGTCGAGGAATATGTAAAAAAAATAGTGGGTG
AGAGCTATGCAAAATCAACTAAAAAGCGACATACAATTACTGTAGCTCTA
GTTAACGAGTTGAACAACATTAAGAACGAGTATTTGAATAAAATAGTTGA
ATCAACCTCAGAAAGCCAACTACAGATACTGATGATGGAGAGTCGATCAA
AAGTAGATGAAGCTGTGTCTAAGTTTGAAAAGGACTCATCTTCTTCGTCAA
GTTCAGACTCTTCCACTAAACCGGAAGCTTCAGATACAGCGAAGCCAAAC
AAGCCGACAGAACCAGGAGAAAAGGTAGCAGAAGCTAAGAAGAAGGTTG
AAGAAGCTGAGAAAAAAGCCAAGGATCAAAAAGAAGAAGATCGTCGTAA
CTACCCAACCATTACTTACAAAACGCTTGAACTTGAAATTGCTGAGTCCG
ATGTGGAAGTTAAAAAAGCGGAGCTTGAACTAGTAAAAGTGAAAGCTAA
CGAACCTCGAGACGAGCAAAAAATTAAGCAAGCAGAAGCGGAAGTTGAG
AGTAAACAAGCTGAGGCTACAAGGTTAAAAAAAATCAAGACAGATCGTG
AAGAAGCAGAAGAAGAAGCTAAACGAAGAGCAGATGCTAAAGAGCAAG
GTAAACCAAAGGGGCGGGCAAAACGAGGAGTTCCTGGAGAGCTAGCAAC
ACCTGATAAAAAAGAAAATGATGCGAAGTCTTCAGATTCTAGCGTAGGTG
AAGAAACTCTTCCAAGCCCATCCCTGAAACCAGAAAAAAAGGTAGCAGA
AGCTGAGAAGAAGGTTGAAGAAGCTAAGAAAAAAGCCGAGGATCAAAAA
GAAGAAGATCGCCGTAACTACCCAACCAATACTTACAAAACGCTTGAACT
TGAAATTGCTGAGTCCGATGTGGAAGTTAAAAAAGCGGAGCTTGAACTAG
TAAAAGAGGAAGCTAAGGAACCTCGAAACGAGGAAAAAGTTAAGCAAGC
AAAAGCGGAAGTTGAGAGTAAAAAAGCTGAGGCTACAAGGTTAGAAAAA

ATCAAGACAGATCGTAAAAAAGCAGAAGAAGAAGCTAAACGAAAAGCAG
CAGAAGAAGATAAAGTTAAAGAAAAACCAGCTG (SEQ ID NO 12).

Amino acid sequence of CbpA of serotype 4:

ENEGATQVPTSSNRANESQAEQGEQPKKLDSERDKARKEVEEYVKKIVGESY
AKSTKKRHTITVALVNELNNIKNEYLNKIVESTSESQLQILMMESRSKVDEAV
SKFEKDSSSSSSSDSSTKPEASDTAKPNKPTEPGEKVAEAKKKVEEAEKKAKD
QKEEDRRNYPTITYKTLELEIAESDVEVKKAELELVKVKANEPRDEQKIKQAE
AEVESKQAEATRLKKIKTDREEAEEEAKRRADAKEQGKPKGRAKRGVPGEL
ATPDKKENDAKSSDSSVGEETLPSPSLKPEKKVAEAEKKVEEAKKKAEDQKE
EDRRNYPTNTYKTLELEIAESDVEVKKAELELVKEEAKEPRNEEKVKQAKAE
VESKKAEATRLEKIKTDRKKAEEEAKRKAAEEDKVKEKPAEQPQPAPAPKAE
KPAPAPKPENPAEQPKAEKPADQQAEEDYARRSEEEYNRLTQQQPPKTEKPA
QPSTPKTGWKQENGMWYFYNTDGSMATGWLQNNGSWYYLNSNGAMATG
WLQNNGSWYYLNANGSMATGWLQNNGSWYYLNANGSMATGWLQYNGS
WYYLNANGSMATGWLQYNGSWYYLNANGDMATGWVKDGDTWYYLEAS
GAMKASQWFKVSDKWYYVNGSGALAVNTTVDGYGVNANGEWVN. (SEQ ID
NO 2)

DNA sequence encoding the amino acid sequence of the CbpA of serotype 4:

GAGAACGAGGGAGCTACCCAAGTACCCACTTCTTCTAATAGGGCAAATGA
AAGTCAGGCAGAACAAGGAGAACAACCTAAAAAACTCGATTCAGAACGA
GATAAGGCAAGGAAAGAGGTCGAGGAATATGTAAAAAAAATAGTGGGTG
AGAGCTATGCAAAATCAACTAAAAAGCGACATACAATTACTGTAGCTCTA
GTTAACGAGTTGAACAACATTAAGAACGAGTATTTGAATAAAATAGTTGA
ATCAACCTCAGAAAGCCAACTACAGATACTGATGATGGAGAGTCGATCAA
AAGTAGATGAAGCTGTGTCTAAGTTTGAAAAGGACTCATCTTCTTCGTCAA
GTTCAGACTCTTCCACTAAACCGGAAGCTTCAGATACAGCGAAGCCAAAC
AAGCCGACAGAACCAGGAGAAAAGGTAGCAGAAGCTAAGAAGAAGGTTG
AAGAAGCTGAGAAAAAAGCCAAGGATCAAAAAGAAGAAGATCGTCGTAA
CTACCCAACCATTACTTACAAAACGCTTGAACTTGAAATTGCTGAGTCCG

ATGTGGAAGTTAAAAAAGCGGAGCTTGAACTAGTAAAAGTGAAAGCTAA
CGAACCTCGAGACGAGCAAAAAATTAAGCAAGCAGAAGCGGAAGTTGAG
AGTAAACAAGCTGAGGCTACAAGGTTAAAAAAAATCAAGACAGATCGTG
AAGAAGCAGAAGAAGAAGCTAAACGAAGAGCAGATGCTAAAGAGCAAG
GTAAACCAAAGGGGCGGGCAAAACGAGGAGTTCCTGGAGAGCTAGCAAC
ACCTGATAAAAAAGAAAATGATGCGAAGTCTTCAGATTCTAGCGTAGGTG
AAGAAACTCTTCCAAGCCCATCCCTGAAACCAGAAAAAAAGGTAGCAGA
AGCTGAGAAGAAGGTTGAAGAAGCTAAGAAAAAAGCCGAGGATCAAAAA
GAAGAAGATCGCCGTAACTACCCAACCAATACTTACAAAACGCTTGAACT
TGAAATTGCTGAGTCCGATGTGGAAGTTAAAAAAGCGGAGgCTTGAACTA
GTAAAAGAGGAAGCTAAGGAACCTCGAAACGAGGAAAAAGTTAAGCAAG
CAAAAGCGGAAGTTGAGAGTAAAAAAGCTGAGGCTACAAGGTTAGAAAA
AATCAAGACAGATCGTAAAAAAGCAGAAGAAGAAGCTAAACGAAAAGCA
GCAGAAGAAGATAAAGTTAAAGAAAAACCAGCTGAACAACCACAACCAG
CGCCGGCTCCAAAAGCAGAAAAACCAGCTCCAGCTCCAAAACCAGAGAA
TCCAGCTGAACAACCAAAAGCAGAAAAACCAGCTGATCAACAAGCTGAA
GAAGACTATGCTCGTAGATCAGAAGAAGAATATAATCGCTTGACTCAACA
GCAACCGCCAAAAACTGAAAAACCAGCACAACCATCTACTCCAAAAACA
GGCTGGAAACAAGAAAACGGTATGTGGTACTTCTACAATACTGATGGTTC
AATGGCGACAGGATGGCTCCAAAACAAtGGCTCAtGGTAcTACcTCAACAG
CAATGGCGCTATGGCGACAGGATGGCTCCAAAACAATGGTTCATGGTACT
ATCTAAACGCTAATGGTTCAATGGCAACAGGATGGCTCCAAAACAATGGT
TCATGGTACTACCTAAACGCTAATGGTTCAATGGCGACAGGATGGCTCCA
ATACAATGGCTCATGGTACTACCTAAACGCTAATGGTTCAATGGCGACAG
GATGGCTCCAATACAATGGCTCATGGTACTACCTAAACGCTAATGGTGAT
ATGGCGACAGGTTGGGTGAAAGATGGAGATACCTGGTACTATCTTGAAGC
ATCAGGTGCTATGAAAGCAAGCCAATGGTTCAAAGTATCAGATAAATGGT
ACTATGTCAATGGCTCAGGTGCCCTTGCAGTCAACACAACTGTAGATGGC
TATGGAGTCAATGCCAATGGTGAATGGGTAAACTAA (SEQ ID NO 13).

"Polypeptide R1" means a polypeptide comprising the amino acid sequences from position 16 to position 321 of the N-terminal truncate/ choline binding protein A (CbpA) serotype type 4 which has the following sequence :

ENEGATQVPTSSNRANESQAEQGEQPKKLDSERDKARKEVEEYVKKIVGESY
AKSTKKRHTITVALVNELNNIKNEYLNKIVESTSESQLQILMMESRSKVDEAV
SKFEKDSSSSSSSDSSTKPEASDTAKPNKPTEPGEKVAEAKKKVEEAEKKAKD
QKEEDRRNYPTITYKTLELEIAESDVEVKKAELELVKVKANEPRDEQKIKQAE
AEVESKQAEATRLKKIKTDREEAEEEAKRRADAKEQGKPKGRAKRGVPGEL
ATPDKKENDAKSSDSSVGEETL (SEQ ID NO 3).

The DNA sequence which encodes polypeptide R1 is:

GAGAACGAGGGAGCTACCCAAGTACCCACTTCTTCTAATAGGGCAAATGA
AAGTCAGGCAGAACAAGGAGAACAACCTAAAAAACTCGATTCAGAACGA
GATAAGGCAAGGAAAGAGGTCGAGGAATATGTAAAAAAAATAGTGGGTG
AGAGCTATGCAAAATCAACTAAAAAGCGACATACAATTACTGTAGCTCTA
GTTAACGAGTTGAACAACATTAAGAACGAGTATTTGAATAAAATAGTTGA
ATCAACCTCAGAAAGCCAACTACAGATACTGATGATGGAGAGTCGATCAA
AAGTAGATGAAGCTGTGTCTAAGTTTGAAAAGGACTCATCTTCTTCGTCAA
GTTCAGACTCTTCCACTAAACCGGAAGCTTCAGATACAGCGAAGCCAAAC
AAGCCGACAGAACCAGGAGAAAAGGTAGCAGAAGCTAAGAAGAAGGTTG
AAGAAGCTGAGAAAAAAGCCAAGGATCAAAAAGAAGAAGATCGTCGTAA
CTACCCAACCATTACTTACAAAACGCTTGAACTTGAAATTGCTGAGTCCG
ATGTGGAAGTTAAAAAAGCGGAGCTTGAACTAGTAAAAGTGAAAGCTAA
CGAACCTCGAGACGAGCAAAAAATTAAGCAAGCAGAAGCGGAAGTTGAG
AGTAAACAAGCTGAGGCTACAAGGTTAAAAAAAATCAAGACAGATCGTG
AAGAAGCAGAAGAAGAAGCTAAACGAAGAGCAGATGCTAAAGAGCAAG
GTAAACCAAAGGGGCGGGCAAAACGAGGAGTTCCTGGAGAGCTAGCAAC
ACCTGATAAAAAAGAAAATGATGCGAAGTCTTCAGATTCTAGCGTAGGTG
AAGAAACTCTTC (SEQ ID NO 14).

"Polypeptide C/R2" means a polypeptide comprising a repeat region C within R2, wherein the repeat region C has the amino acid sequences from position 327 to position 433 of the N-terminal choline binding protein A (CbpA) serotype type 4 which has the following sequence:

KPEKKVAEAEKKVEEAKKKAEDQKEEDRRNYPTNTYKTLELEIAESDVEVK KAELELVKEEAKEPRNEEKVKQAKAEVESKKAEATRLEKIKTDRKKAEEEAK RKA (SEQ ID NO 4).

The DNA sequence of polypeptide C/R2

AAACCAGAAAAAAAGGTAGCAGAAGCTGAGAAGAAGGTTGAAGAAGCTA AGAAAAAAGCCGAGGATCAAAAAGAAGAAGATCGCCGTAACTACCCAAC CAATACTTACAAAACGCTTGAACTTGAAATTGCTGAGTCCGATGTGGAAG TTAAAAAAGCGGAGCTTGAACTAGTAAAAGAGGAAGCTAAGGAACCTCG AAACGAGGAAAAAGTTAAGCAAGCAAAAGCGGAAGTTGAGAGTAAAAAA GCTGAGGCTACAAGGTTAGAAAAAATCAAGACAGATCGTAAAAAAGCAG AAGAAGAAGCTAAACGAAAAGCA (SEQ ID NO 15)

"Polypeptide A/R2" means a polypeptide comprising a repeat region A within R2, wherein the repeat region A has the amino acid sequences from position 153 to position 269 of the N-terminal of choline binding protein A (CbpA) serotype type 4 which has the following sequence:

TEPGEKVAEAKKKVEEAEKKAKDQKEEDRRNYPTITYKTLELEIAESDVEVK KAELELVKVKANEPRDEQKIKQAEAEVESKQAEATRLKKIKTDREEAEEEAK RRADA (SEQ ID NO 5). As shown in Figure 1, region A of polypeptide R2 is the same region A as within R1.

The DNA sequence which encodes the polypeptide A/R2 is:

ACAGAACCAGGAGAAAAGGTAGCAGAAGCTAAGAAGAAGGTTGAAGAA GCTGAGAAAAAAGCCAAGGATCAAAAAGAAGAAGATCGTCGTAACTACC CAACCATTACTTACAAAACGCTTGAACTTGAAATTGCTGAGTCCGATGTG GAAGTTAAAAAAGCGGAGCTTGAACTAGTAAAAGTGAAAGCTAACGAAC

CTCGAGACGAGCAAAAAATTAAGCAAGCAGAAGCGGAAGTTGAGAGTAA
ACAAGCTGAGGCTACAAGGTTAAAAAAAATCAAGACAGATCGTGAAGAA
GCAGAAGAAGAAGCTAAACGAAGAGCAGATGCT (SEQ ID NO 16).

The identity or location of one or more amino acid residues may be changed or modified to include variants such as, for example, deletions containing less than all of the residues specified for the protein, substitutions wherein one or more residues specified are replaced by other residues and additions wherein one or more amino acid residues are added to a terminal or medial portion of the polypeptide (see Figure 2). These molecules include: the incorporation of codons "preferred" for expression by selected non-mammalian hosts; the provision of sites for cleavage by restriction endonuclease enzymes; and the provision of additional initial, terminal or intermediate DNA sequences that facilitate construction of readily expressed vectors. Specifically, examples of the amino acid substitutions of serotype 4, included but not limited to, are as follows: E at position 154 is substituted with K; P at position 155 is substituted with L; G at position 156 is substituted with E; E at position 157 is substituted with K; K at position 181 is substituted with E; D at position 182 is substituted with A; R at position 187 is substituted with Y, H, or L; I at position 194 is substituted with N; E at position 200 is substituted with D; E at position 202 is substituted with D; E at position 209 is substituted with K; K at position 212 is substituted with E; V at position 218 is substituted with L; V at position 220 is substituted with K or E; K at position 221 is substituted with E; N at position 223 is substituted with D or K; P at position 225 is substituted with S, T, or R; D at position 227 is substituted with N; E at position 228 is substituted with K; Q at position 229 is substituted with E, G, or D; K at position 230 is substituted with T; K at position 232 is substituted with N; E at position 235 is substituted with K; A at position 236 is substituted with E; E at position 237 is substituted with K; S at position 240 is substituted with N; K at position 241 is substituted with E; Q at position 242 is substituted with K; K at position 249 is substituted with E; K at position 250 is substituted with N; E at position 257 is substituted with Q or K; A at position 263 is substituted with L; K at position 264 is substituted with E; R at position 265 is substituted with N; R at position 266 is

substituted with I; A at position 267 is substituted with K or V; D at position 258 is substituted with T; A at position 269 is substituted with D; A at position 291 is substituted with T, V, P, G, or X; G at position 294 is substituted with G, A, or E; V at position 295 is substituted with D, or A; P at position 295 is substituted with L or F; L at position 2999 is substituted with P or Q; P at position 328 is substituted with S; E at position 329 is substituted with G; E at position 340 is substituted with A; K at position 343 is substituted with E or D; E at position 347 is substituted with K; D at position 349 is substituted with A; R at position 354 is substituted with H; E at position 366 is substituted with D; E at position 375 is substituted with K; K at position 378 is substituted with E; E at position 390 is substituted with G; P at position 391 is substituted with S; N at position 393 is substituted with D; V at position 397 is substituted with I; and K at position 408 is substituted with Q.

"Polypeptide R2 serotype - R6x" means an polypeptide comprising the amino acid sequences from position 16 to position 444 of the N-terminal truncate of Choline Binding Protein A (CbpA) serotype R6x which has the following sequence:

ENEGSTQAATSSNMAKTEHRKAAKQVVDEYIEKMLREIQLDRRKHTQNVAL
NIKLSAIKTKYLRELNVLEEKSKDELPSEIKAKLDAAFEKFKKDTLKPGEKVA
EAKKKVEEAKKKAEDQKEEDRRNYPTNTYKTLELEIAEFDVKVKEAELELVK
EEAKESRNEGTIKQAKEKVESKKAEATRLENIKTDRKKAEEEAKRKADAKLK
EANVATSDQGKPKGRAKRGVPGELATPDKKENDAKSSDSSVGEETLPSSSLK
SGKKVAEAEKKVEEAEKKAKDQKEEDRRNYPTNTYKTLDLEIAESDVKVKE
AELELVKEEAKEPRDEEKIKQAKAKVESKKAEATRLENIKTDRKKAEEEAKR
KAAEEDKVKEKPA (SEQ ID NO 7)

The DNA sequence which encodes polypeptide R2 serotype R6x:

GAAAACGAAGGAAGTACCCAAGCAGCCACTTCTTCTAATATGGCAAAGAC
AGAACATAGGAAAGCTGCTAAACAAGTCGTCGATGAATATATAGAAAAA
ATGTTGAGGGAGATTCAACTAGATAGAAGAAAACATACCCAAAATGTCGC
CTTAAACATAAAGTTGAGCGCAATTAAAACGAAGTATTTGCGTGAATTAA
ATGTTTTAGAAGAGAAGTCGAAAGATGAGTTGCCGTCAGAAATAAAAGCA

AAGTTAGACGCAGCTTTTGAGAAGTTTAAAAAAGATACATTGAAACCAGG
AGAAAAGGTAGCAGAAGCTAAGAAGAAGGTTGAAGAAGCTAAGAAAAAA
GCCGAGGATCAAAAAGAAGAAGATCGTCGTAACTACCCAACCAATACTTA
CAAAACGCTTGAACTTGAAATTGCTGAGTTCGATGTGAAAGTTAAAGAAG
CGGAGCTTGAACTAGTAAAAGAGGAAGCTAAAGAAtCTCGAAACGAGGGC
ACAATTAAGCAAGCAAAAGAGAAAGTTGAGAGTAAAAAAGCTGAGGCTA
CAAGGTTAGAAAACAtCAAGACAGAtCGTAAAAAAGCAGAAGAAGAAGCT
AAACGAAAAGCAGATGCTAAGTTGAAGGAAGCTAATGTAGCGACTTCAG
AtCAAGGTAAACCAAAGGGGCGGGCAAAACGAGGAGTTCCTGGAGAGCTA
GCAACACCTGATAAAAAAGAAAATGATGCGAAGTCTTCAGATTCTAGCGT
AGGTGAAGAAACTCTTCCAAGCTCATCCCTGAAATCAGGAAAAAAGGTAG
CAGAAGCTGAGAAGAAGGTTGAAGAAGCTGAGAAAAAAGCCAAGGATCA
AAAAGAAGAAGATCGCCGTAACTACCCAACCAATACTTACAAAACGCTTG
ACCTTGAAATTGCTGAGTCCGATGTGAAAGTTAAAGAAGCGGAGCTTGAA
CTAGTAAAAGAGGAAGCTAAGGAACCTCGAGACGAGGAAAAAATTAAGC
AAGCAAAAGCGAAAGTTGAGAGTAAAAAAGCTGAGGCTACAAGGTTAGA
AAACATCAAGACAGATCGTAAAAAAGCAGAAGAAGAAGCTAAACGAAAA
GCAGCAGAAGAAGATAAAGTTAAAGAAAAACCAGCTG (SEQ ID NO 17)

Amino acid sequence of CbpA of serotype R6x:

ENEGSTQAATSSNMAKTEHRKAAKQVVDEYIEKMLREIQLDRRKHTQNVAL
NIKLSAIKTKYLRELNVLEEKSKDELPSEIKAKLDAAFEKFKKDTLKPGEKVA
EAKKKVEEAKKKAEDQKEEDRRNYPTNTYKTLELEIAEFDVKVKEAELELVK
EEAKESRNEGTIKQAKEKVESKKAEATRLENIKTDRKKAEEEAKRKADAKLK
EANVATSDQGKPKGRAKRGVPGELATPDKKENDAKSSDSSVGEETLPSSSLK
SGKKVAEAEKKVEEAEKKAKDQKEEDRRNYPTNTYKTLDLEIAESDVKVKE
AELELVKEEAKEPRDEEKIKQAKAKVESKKAEATRLENIKTDRKKAEEEAKR
KAAEEDKVKEKPAEQPQPAPATQPEKPAPKPEKPAEQPKAEKTDDQQAEEDY
ARRSEEEYNRLTQQQPPKTEKPAQPSTPKTGWKQENGMWYFYNTDGSMAT
GWLQNNGSWYYLNANGAMATGWLQNNGSWYYLNANGSMATGWLQNNG
SWYYLNANGAMATGWLQYNGSWYYLNSNGAMATGWLQYNGSWYYLNA

NGDMATGWLQNNGSWYYLNANGDMATGWLQYNGSWYYLNANGDMATG
WVKDGDTWYYLEASGAMKASQWFKVSDKWYYVNGSGALAVNTTVDGYG
VNANGEWVN (SEQ ID NO 8).

DNA sequence encoding the amino acid sequence of the CbpA of serotype R6x:

GAAAACGAAGGAAGTACCCAAGCAGCCACTTCTTCTAATATGGCAAAGAC
AGAACATAGGAAAGCTGCTAAACAAGTCGTCGATGAATATATAGAAAAA
ATGTTGAGGGAGATTCAACTAGATAGAAGAAAACATACCCAAAATGTCGC
CTTAAACATAAAGTTGAGCGCAATTAAAACGAAGTATTTGCGTGAATTAA
ATGTTTTAGAAGAGAAGTCGAAAGATGAGTTGCCGTCAGAAATAAAAGCA
AAGTTAGACGCAGCTTTTGAGAAGTTTAAAAAAGATACATTGAAACCAGG
AGAAAAGGTAGCAGAAGCTAAGAAGAAGGTTGAAGAAGCTAAGAAAAAA
GCCGAGGATCAAAAAGAAGAAGATCGTCGTAACTACCCAACCAATACTTA
CAAAACGCTTGAACTTGAAATTGCTGAGTTCGATGTGAAAGTTAAAGAAG
CGGAGCTTGAACTAGTAAAAGAGGAAGCTAAAGAAtCTCGAAACGAGGGC
ACAATTAAGCAAGCAAAAGAGAAAGTTGAGAGTAAAAAAGCTGAGGCTA
CAAGGTTAGAAAACAtCAAGACAGAtCGTAAAAAAGCAGAAGAAGAAGCT
AAACGAAAAGCAGATGCTAAGTTGAAGGAAGCTAATGTAGCGACTtCAGA
tCAAGGTAAACCAAAGGGGCGGGCAAAACGAGGAGTTCCTGGAGAGCTAG
CAACACCTGATAAAAAAGAAAATGATGCGAAGTCTTCAGATTCTAGCGTA
GGTGAAGAAACTCTTCCAAGCTCATCCCTGAAATCAGGAAAAAAGGTAGC
AGAAGCTGAGAAGAAGGTTGAAGAAGCTGAGAAAAAAGCCAAGGATCAA
AAAGAAGAAGATCGCCGTAACTACCCAACCAATACTTACAAAACGCTTGA
CCTTGAAATTGCTGAGTCCGATGTGAAAGTTAAAGAAGCGGAGCTTGAAC
TAGTAAAAGAGGAAGCTAAGGAACCTCGAGACGAGGAAAAAATTAAGCA
AGCAAAAGCGAAAGTTGAGAGTAAAAAAGCTGAGGCTACAAGGTTAGAA
AACATCAAGACAGATCGTAAAAAAGCAGAAGAAGAAGCTAAACGAAAAG
CAGCAGAAGAAGATAAAGTTAAAGAAAAACCAGCTGAACAACCACAACC
AGCGCCGGCTACTCAACCAGAAAAACCAGCTCCAAAACCAGAGAAGCCA
GCTGAACAACCAAAAGCAGAAAAAACAGATGATCAACAAGCTGAAGAAG
ACTATGCTCGTAGATCAGAAGAAGAATATAATCGCTTGACTCAACAGCAA

CCGCCAAAAACTGAAAAACCAGCACAACCATCTACTCCAAAAACAGGCT
GGAAACAAGAAAACGGTATGTGGTACTTCTACAATACTGATGGTTCAATG
GCAACAGGATGGCTCCAAAACAACGGTTCATGGTACTATCTAAACGCTAA
TGGTGCTATGGCGACAGGATGGCTCCAAAACAATGGTTCATGGTACTATC
TAAACGCTAATGGTTCAATGGCAACAGGATGGCTCCAAAACAATGGTTCA
TGGTACTACCTAAACGCTAATGGTGCTATGGCGACAGGATGGCTCCAATA
CAATGGTTCATGGTACTACCTAAACAGCAATGGCGCTATGGCGACAGGAT
GGCTCCAATACAATGGCTCATGGTACTACCTCAACGCTAATGGTGATATG
GCGACAGGATGGCTCCAAAACAACGGTTCATGGTACTACCTCAACGCTAA
TGGTGATATGGCGACAGGATGGCTCCAATACAACGGTTCATGGTATTACC
TCAACGCTAATGGTGATATGGCGACAGGTTGGGTGAAAGATGGAGATACC
TGGTACTATCTTGAAGCATCAGGTGCTATGAAAGCAAGCCAATGGTTCAA
AGTATCAGATAAATGGTACTATGTCAATGGCTCAGGTGCCCTTGCAGTCA
ACACAACTGTAGATGGCTATGGAGTCAATGCCAATGGTGAATGGGTAAAC
TAA (SEQ ID NO 18).

Polypeptide R1 Serotype R6x" means an polypeptide comprising the amino acid sequences from position 16 to position 321 of the N-terminal truncate/truncate of choline binding protein A (CbpA) serotype R6x which has the following sequence :

ENEGSTQAATSSNMAKTEHRKAAKQVVDEYIEKMLREIQLDRRKHTQNVAL
NIKLSAIKTKYLRELNVLEEKSKDELPSEIKAKLDAAFEKFKKDTLKPGEKVA
EAKKKVEEAKKKAEDQKEEDRRNYPTNTYKTLELEIAEFDVKVKEAELELVK
EEAKESRNEGTIKQAKEKVESKKAEATRLENIKTDRKKAEEEAKRKADAKLK
EANVATSDQGKPKGRAKRGVPGELATPDKKENDAKSSDSSVGEETL (SEQ ID NO 9).

The DNA sequence which encodes polypeptide R1 is:

GAAAACGAAGGAAGTACCCAAGCAGCCACTTCTTCTAATATGGCAAAGAC
AGAACATAGGAAAGCTGCTAAACAAGTCGTCGATGAATATATAGAAAAA
ATGTTGAGGGAGATTCAACTAGATAGAAGAAAACATACCCAAAATGTCGC
CTTAAACATAAAGTTGAGCGCAATTAAAACGAAGTATTTGCGTGAATTAA

ATGTTTTAGAAGAGAAGTCGAAAGATGAGTTGCCGTCAGAAATAAAAGCA
AAGTTAGACGCAGCTTTTGAGAAGTTTAAAAAAGATACATTGAAACCAGG
AGAAAAGGTAGCAGAAGCTAAGAAGAAGGTTGAAGAAGCTAAGAAAAAA
GCCGAGGATCAAAAAGAAGAAGATCGTCGTAACTACCCAACCAATACTTA
CAAAACGCTTGAACTTGAAATTGCTGAGTTCGATGTGAAAGTTAAAGAAG
CGGAGCTTGAACTAGTAAAAGAGGAAGCTAAAGAATCTCGAAACGAGGG
CACAATTAAGCAAGCAAAAGAGAAAGTTGAGAGTAAAAAAGCTGAGGCT
ACAAGGTTAGAAAACAtCAAGACAGATCGTAAAAAAGCAGAAGAAGAAG
CTAAACGAAAAGCAGATGCTAAGTTGAAGGAAGCTAATGTAGCGACTTCA
GATCAAGGTAAACCAAAGGGGCGGGCAAAACGAGGAGTTCCTGGAGAGC
TAGCAACACCTGATAAAAAAGAAAATGATGCGAAGTCTTCAGATTCTAGC
GTAGGTGAAGAAACTCTTC (SEQ ID NO 19).

"Polypeptide C/R2 serotype R6x" means an polypeptide comprising a repeat region C within R2 (see Figure 2), wherein the repeat region C has the amino acid sequences from position 327 to position 433 of the N-terminal of choline binding protein A (CbpA) serotype R6x which has the following sequence:
KSGKKVAEAEKKVEEAEKKAKDQKEEDRRNYPTNTYKTLDLEIAESDVKVK
EAELELVKEEAKEPRDEEKIKQAKAKVESKKAEATRLENIKTDRKKAEEEAK
RKA (SEQ ID NO 10)

The DNA sequence of polypeptide C/R2 serotype R6x:
AAATCAGGAAAAAAGGTAGCAGAAGCTGAGAAGAAGGTTGAAGAAGCTG
AGAAAAAAGCCAAGGATCAAAAAGAAGAAGATCGCCGTAACTACCCAAC
CAATACTTACAAAACGCTTGACCTTGAAATTGCTGAGTCCGATGTGAAAG
TTAAAGAAGCGGAGCTTGAACTAGTAAAAGAGGAAGCTAAGGAACCTCG
AGACGAGGAAAAAATTAAGCAAGCAAAAGCGAAAGTTGAGAGTAAAAAA
GCTGAGGCTACAAGGTTAGAAAACATCAAGACAGATCGTAAAAAAGCAG
AAGAAGAAGCTAAACGAAAAGCA (SEQ ID NO 20).

Polypeptide A/R2 serotype R6x" means an polypeptide comprising a repeat region A within R2 (see Figure 2), wherein the repeat region A has the amino acid sequences from position 155 to position 265 of the N-terminal of choline binding protein A (CbpA) serotype R6X which has the following sequence:

PGEKVAEAKKKVEEAKKKAEDQKEEDRRNYPTNTYKTLELEIAEFDVKVKE AELELVKEEAKESRNEGTIKQAKEKVESKKAEATRLENIKTDRKKAEEEAKR KADA (SEQ ID NO 11)

The DNA sequence which encodes the polypeptide A/R2 serotype R6x is:

CCAGGAGAAAAGGTAGCAGAAGCTAAGAAGAAGGTTGAAGAAGCTAAGA AAAAAGCCGAGGATCAAAAAGAAGAAGATCGTCGTAACTACCCAACCAA TACTTACAAAACGCTTGAACTTGAAATTGCTGAGTTCGATGTGAAAGTTA AAGAAGCGGAGCTTGAACTAGTAAAAGAGGAAGCTAAAGAAtCTCGAAAC GAGGGCACAATTAAGCAAGCAAAAGAGAAAGTTGAGAGTAAAAAAGCTG AGGCTACAAGGTTAGAAAACAtCAAGACAGATCGTAAAAAAGCAGAAGA AGAAGCTAAACGAAAAGCAGATGCT (SEQ ID NO 21).

This invention is directed to an isolated polypeptide, wherein the isolated polypeptide consists of the amino acid sequence as set forth in SEQ ID NOS 22 or 23, including fragments, mutants, variants, or analogs, or derivatives, thereof.

SPSLKPEKKVAEAEKKVEEAKKKAEDQKEEDRRNYPTNTYKTLELEIAESDV EVKKAELELVKEEAKEPRNEEKVKQAKAEVESKKAEATRLEKIKTDRKKAEE EAKRKAAEEDKVKEKPA (SEQ ID NO 22; serotype 4; position 323-434); or PSSSLKSGKKVAEAEKKVEEAEKKAKDQKEEDRRNYPTNTYKTLDLEIAESD VKVKEAELELVKEEAKEPRDEEKIKQAKAKVESKKAEATRLENIKTDRKKAE EEAKRKAAEEDKVKEKRA (SEQ ID NO 23, serotype R6x; position 322-434).

"Polypeptide B/R2" means a polypeptide comprising the amino acid sequences from position 270 to position 326 of the N-terminal truncate of choline binding protein A (CbpA) serotype type 4 as set forth in Figure 2. "Polypeptide B/R2 serotype - R6x" means an polypeptide comprising the amino acid sequences from position 264 to

position 326 of the N-terminal truncate of Choline Binding Protein A (CbpA) serotype R6x as set forth in Figure 2. This invention contemplates a polypeptide having the amino acid sequence of regions A, B, C, A+B, B+C, A+C as shown in Figure 1.

Further, this invention provides an isolated polypeptide comprising an amino acid sequence of a N-terminal choline binding protein A truncate, wherein the polypeptide has the amino acid KXXE (SEQ ID NO 6).

This invention is directed to a polypeptide comprising an amino acid sequence of a N-terminal choline binding protein A truncate, wherein the amino acid sequence is set forth in Figure 2. In one embodiment, the polypeptide has an amino acid sequence which is a conserved region as set forth in Figure 2. For example, conserved regions include but are not limited to amino acid sequence 158 to 172; 300 to 321; 331 to 339; 355 to 365; 367 to 374; 379 to 389; 409 to 427; and 430 to 447 Figure 2 sets forth homologies of various serotypes of the nucleic acid and amino acid sequence of the N-terminal region of CbpA which are contemplated by this invention.

This invention provides an isolated polypeptide comprising an amino acid sequence of a N-terminal choline binding protein A truncate, wherein the polypeptide has lectin activity and does not bind to choline. In one embodiment the polypeptide has the amino acid sequence as set forth in any of SEQ ID NO 1, 3-5, 7, or 9-11 including fragments, mutants, variants, analogs, or derivatives, thereof.

As used herein, "a polypeptide having a lectin activity" means a polypeptide, peptide or protein which binds noncovalently to a carbohydrate. As defined herein, "adhesin" means noncovalent binding of a bacteria to a human cell or secretion that is stable enough to withstand washing. As defined herein, "binds to the LNnT" means binds to Lacto-N-neotetraose coated substrates more than albumin-control.

This invention provides an isolated immunogenic polypeptide comprising an amino acid sequence of an N-terminal choline binding protein A truncate. It is contemplated by this

invention that the immunogenic polypeptide has the amino acid sequence as set forth in any of SEQ ID NOS 1, 3-7, or 9-11, including fragments, mutants, variants, analogs, or derivatives, thereof. This invention provides an isolated polypeptide comprising an amino acid sequence of a N-terminal choline binding protein A truncate as set forth in Figure 2. In one embodiment, the polypeptide has an amino acid sequence which is a conserved region as set forth in Figure 2.

This invention is directed to analogs of the polypeptide which comprise the amino acid sequence as set forth above. The analog polypeptide may have an N-terminal methionine or an N-terminal polyhistidine optionally attached to the N or COOH terminus of the polypeptide which comprise the amino acid sequence.

In another embodiment, this invention contemplates peptide fragments of the polypeptide which result from proteolytic digestion products of the polypeptide. In another embodiment, the derivative of the polypeptide has one or more chemical moieties attached thereto. In another embodiment the chemical moiety is a water soluble polymer. In another embodiment the chemical moiety is polyethylene glycol. In another embodiment the chemical moiety is mon-, di-, tri- or tetrapegylated. In another embodiment the chemical moiety is N-terminal monopegylated.

Attachment of polyethylene glycol (PEG) to compounds is particularly useful because PEG has very low toxicity in mammals (Carpenter et al., 1971). For example, a PEG adduct of adenosine deaminase was approved in the United States for use in humans for the treatment of severe combined immunodeficiency syndrome. A second advantage afforded by the conjugation of PEG is that of effectively reducing the immunogenicty and antigenicity of heterologous compounds. For example, a PEG adduct of a human protein might be useful for the treatment of disease in other mammalian species without the risk of triggering a severe immune response. The compound of the present invention may be delivered in a microencapsulation device so as to reduce or prevent an host immune response against the compound or against cells which may produce the

compound. The compound of the present invention may also be delivered microencapsulated in a membrane, such as a liposome.

Numerous activated forms of PEG suitable for direct reaction with proteins have been described. Useful PEG reagents for reaction with protein amino groups include active esters of carboxylic acid or carbonate derivatives, particularly those in which the leaving groups are N-hydroxysuccinimide, p-nitrophenol, imidazole or 1-hydroxy-2-nitrobenzene-4-sulfonate. PEG derivatives containing maleimido or haloacetyl groups are useful reagents for the modification of protein free sulfhydryl groups. Likewise, PEG reagents containing amino hydrazine or hydrazide groups are useful for reaction with aldehydes generated by periodate oxidation of carbohydrate groups in proteins.

In one embodiment, the amino acid residues of the polypeptide described herein are preferred to be in the "L" isomeric form. In another embodiment, the residues in the "D" isomeric form can be substituted for any L-amino acid residue, as long as the desired functional property of lectin activity is retained by the polypeptide. NH_2 refers to the free amino group present at the amino terminus of a polypeptide. COOH refers to the free carboxy group present at the carboxy terminus of a polypeptide. Abbreviations used herein are in keeping with standard polypeptide nomenclature, *J. Biol. Chem.*, **243**:3552-59 (1969).

It should be noted that all amino-acid residue sequences are represented herein by formulae whose left and right orientation is in the conventional direction of amino-terminus to carboxy-terminus. Furthermore, it should be noted that a dash at the beginning or end of an amino acid residue sequence indicates a peptide bond to a further sequence of one or more amino-acid residues.

Synthetic polypeptide, prepared using the well known techniques of solid phase, liquid phase, or peptide condensation techniques, or any combination thereof, can include natural and unnatural amino acids. Amino acids used for peptide synthesis may be standard Boc (N^{α}-amino protected N^{α}-t-butyloxycarbonyl) amino acid resin with the

standard deprotecting, neutralization, coupling and wash protocols of the original solid phase procedure of Merrifield (1963, J. Am. Chem. Soc. 85:2149-2154), or the base-labile N^{α}-amino protected 9-fluorenylmethoxycarbonyl (Fmoc) amino acids first described by Carpino and Han (1972, J. Org. Chem. 37:3403-3409). Thus, polypeptide of the invention may comprise D-amino acids, a combination of D- and L-amino acids, and various "designer" amino acids (*e.g.*, β-methyl amino acids, Cα-methyl amino acids, and Nα-methyl amino acids, etc.) to convey special properties. Synthetic amino acids include ornithine for lysine, fluorophenylalanine for phenylalanine, and norleucine for leucine or isoleucine. Additionally, by assigning specific amino acids at specific coupling steps, α-helices, β turns, β sheets, γ-turns, and cyclic peptides can be generated.

In one aspect of the invention, the peptides may comprise a special amino acid at the C-terminus which incorporates either a CO_2H or $CONH_2$ side chain to simulate a free glycine or a glycine-amide group. Another way to consider this special residue would be as a D or L amino acid analog with a side chain consisting of the linker or bond to the bead. In one embodiment, the pseudo-free C-terminal residue may be of the D or the L optical configuration; in another embodiment, a racemic mixture of D and L-isomers may be used.

In an additional embodiment, pyroglutamate may be included as the N-terminal residue of the peptide. Although pyroglutamate is not amenable to sequence by Edman degradation, by limiting substitution to only 50% of the peptides on a given bead with N-terminal pyroglutamate, there will remain enough non-pyroglutamate peptide on the bead for sequencing. One of ordinary skill would readily recognize that this technique could be used for sequencing of any peptide that incorporates a residue resistant to Edman degradation at the N-terminus. Other methods to characterize individual peptides that demonstrate desired activity are described in detail *infra*. Specific activity of a peptide that comprises a blocked N-terminal group, *e.g.*, pyroglutamate, when the particular N-terminal group is present in 50% of the peptides, would readily be

demonstrated by comparing activity of a completely (100%) blocked peptide with a non-blocked (0%) peptide.

In addition, the present invention envisions preparing peptides that have more well defined structural properties, and the use of peptidomimetics, and peptidomimetic bonds, such as ester bonds, to prepare peptides with novel properties. In another embodiment, a peptide may be generated that incorporates a reduced peptide bond, i.e., R_1-**CH_2-NH-** R_2, where R_1 and R_2 are amino acid residues or sequences. A reduced peptide bond may be introduced as a dipeptide subunit. Such a molecule would be resistant to peptide bond hydrolysis, *e.g.*, protease activity. Such peptides would provide ligands with unique function and activity, such as extended half-lives *in vivo* due to resistance to metabolic breakdown, or protease activity. Furthermore, it is well known that in certain systems constrained peptides show enhanced functional activity (Hruby, 1982, Life Sciences 31:189-199; Hruby et al., 1990, Biochem J. 268:249-262); the present invention provides a method to produce a constrained peptide that incorporates random sequences at all other positions.

A constrained, cyclic or rigidized peptide may be prepared synthetically, provided that in at least two positions in the sequence of the peptide an amino acid or amino acid analog is inserted that provides a chemical functional group capable of cross-linking to constrain, cyclise or rigidize the peptide after treatment to form the cross-link. Cyclization will be favored when a turn-inducing amino acid is incorporated. Examples of amino acids capable of cross-linking a peptide are cysteine to form disulfide, aspartic acid to form a lactone or a lactase, and a chelator such as γ-carboxyl-glutamic acid (Gla) (Bachem) to chelate a transition metal and form a cross-link. Protected γ-carboxyl glutamic acid may be prepared by modifying the synthesis described by Zee-Cheng and Olson (1980, Biophys. Biochem. Res. Commun. 94:1128-1132). A peptide in which the peptide sequence comprises at least two amino acids capable of cross-linking may be treated, *e.g.*, by oxidation of cysteine residues to form a disulfide or addition of a metal ion to form a chelate, so as to cross-link the peptide and form a constrained, cyclic or rigidized peptide.

The present invention provides strategies to systematically prepare cross-links. For example, if four cysteine residues are incorporated in the peptide sequence, different protecting groups may be used (Hiskey, 1981, in The Peptides: Analysis, Synthesis, Biology, Vol. 3, Gross and Meienhofer, eds., Academic Press: New York, pp. 137-167; Ponsanti et al., 1990, Tetrahedron 46:8255-8266). The first pair of cysteine may be deprotected and oxidized, then the second set may be deprotected and oxidized. In this way a defined set of disulfide cross-links may be formed. Alternatively, a pair of cysteine and a pair of collating amino acid analogs may be incorporated so that the cross-links are of a different chemical nature.

The following non-classical amino acids may be incorporated in the peptide in order to introduce particular conformational motifs: 1,2,3,4-tetrahydroisoquinoline-3-carboxylate (Kazmierski et al., 1991, J. Am. Chem. Soc. 113:2275-2283); (2S,3S)-methyl-phenylalanine, (2S,3R)-methyl-phenylalanine, (2R,3S)-methyl-phenylalanine and (2R,3R)-methyl-phenylalanine (Kazmierski and Hruby, 1991, Tetrahedron Lett.); 2-aminotetrahydronaphthalene-2-carboxylic acid (Landis, 1989, Ph.D. Thesis, University of Arizona); hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (Miyake et al., 1989, J. Takeda Res. Labs. 43:53-76); β-carboline (D and L) (Kazmierski, 1988, Ph.D. Thesis, University of Arizona); HIC (histidine isoquinoline carboxylic acid) (Zechel et al., 1991, Int. J. Pep. Protein Res. 43); and HIC (histidine cyclic urea) (Dharanipragada).

The following amino acid analogs and peptidomimetics may be incorporated into a peptide to induce or favor specific secondary structures: LL-Acp (LL-3-amino-2-propenidone-6-carboxylic acid), a β-turn inducing dipeptide analog (Kemp et al., 1985, J. Org. Chem. 50:5834-5838); β-sheet inducing analogs (Kemp et al., 1988, Tetrahedron Lett. 29:5081-5082); β-turn inducing analogs (Kemp et al., 1988, Tetrahedron Lett. 29:5057-5060); ∝-helix inducing analogs (Kemp et al., 1988, Tetrahedron Lett. 29:4935-4938); γ-turn inducing analogs (Kemp et al., 1989, J. Org. Chem. 54:109:115); and analogs provided by the following references: Nagai and Sato, 1985, Tetrahedron Lett. 26:647-650; DiMaio et al., 1989, J. Chem. Soc. Perkin Trans. p. 1687; also a Gly-Ala

turn analog (Kahn et al., 1989, Tetrahedron Lett. 30:2317); amide bond isostere (Jones et al., 1988, Tetrahedron Lett. 29:3853-3856); tretrazol (Zabrocki et al., 1988, J. Am. Chem. Soc. 110:5875-5880); DTC (Samanen et al., 1990, Int. J. Protein Pep. Res. 35:501:509); and analogs taught in Olson et al., 1990, J. Am. Chem. Sci. 112:323-333 and Garvey et al., 1990, J. Org. Chem. 56:436. Conformationally restricted mimetics of beta turns and beta bulges, and peptides containing them, are described in U.S. Patent No. 5,440,013, issued August 8, 1995 to Kahn.

The present invention further provides for modification or derivatization of the polypeptide or peptide of the invention. Modifications of peptides are well known to one of ordinary skill, and include phosphorylation, carboxymethylation, and acylation. Modifications may be effected by chemical or enzymatic means. In another aspect, glycosylated or fatty acylated peptide derivatives may be prepared. Preparation of glycosylated or fatty acylated peptides is well known in the art. Fatty acyl peptide derivatives may also be prepared. For example, and not by way of limitation, a free amino group (N-terminal or lysyl) may be acylated, *e.g.*, myristoylated. In another embodiment an amino acid comprising an aliphatic side chain of the structure -$(CH_2)_nCH_3$ may be incorporated in the peptide. This and other peptide-fatty acid conjugates suitable for use in the present invention are disclosed in U.K. Patent GB-8809162.4, International Patent Application PCT/AU89/00166, and reference 5, *supra*.

Mutations can be made in a nucleic acid encoding the polypeptide such that a particular codon is changed to a codon which codes for a different amino acid. Such a mutation is generally made by making the fewest nucleotide changes possible. A substitution mutation of this sort can be made to change an amino acid in the resulting protein in a non-conservative manner (i.e., by changing the codon from an amino acid belonging to a grouping of amino acids having a particular size or characteristic to an amino acid belonging to another grouping) or in a conservative manner (i.e., by changing the codon from an amino acid belonging to a grouping of amino acids having a particular size or characteristic to an amino acid belonging to the same grouping). Such a conservative change generally leads to less change in the structure and function of the resulting

protein. A non-conservative change is more likely to alter the structure, activity or function of the resulting protein. The present invention should be considered to include sequences containing conservative changes which do not significantly alter the activity or binding characteristics of the resulting protein. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. Amino acids containing aromatic ring structures are phenylalanine, tryptophan, and tyrosine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Such alterations will not be expected to affect apparent molecular weight as determined by polyacrylamide gel electrophoresis, or isoelectric point.

Particularly preferred substitutions are:

- Lys for Arg and vice versa such that a positive charge may be maintained;
- Glu for Asp and vice versa such that a negative charge may be maintained;
- Ser for Thr such that a free -OH can be maintained; and
- Gln for Asn such that a free NH_2 can be maintained.

Synthetic DNA sequences allow convenient construction of genes which will express analogs or "muteins". A general method for site-specific incorporation of unnatural amino acids into proteins is described in Noren, et al. *Science*, **244**:182-188 (April 1989). This method may be used to create analogs with unnatural amino acids.

In accordance with the present invention there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook et al, "Molecular Cloning: A Laboratory Manual" (1989); "Current Protocols in Molecular Biology" Volumes I-III [Ausubel, R. M., ed. (1994)]; "Cell Biology: A Laboratory Handbook" Volumes I-III [J. E. Celis, ed. (1994))]; "Current Protocols in Immunology" Volumes I-

III [Coligan, J. E., ed. (1994)]; "Oligonucleotide Synthesis" (M.J. Gait ed. 1984); "Nucleic Acid Hybridization" [B.D. Hames & S.J. Higgins eds. (1985)]; "Transcription And Translation" [B.D. Hames & S.J. Higgins, eds. (1984)]; "Animal Cell Culture" [R.I. Freshney, ed. (1986)]; "Immobilized Cells And Enzymes" [IRL Press, (1986)]; B. Perbal, "A Practical Guide To Molecular Cloning" (1984).

In an additional embodiment, pyroglutamate may be included as the N-terminal residue of the peptide. Although pyroglutamate is not amenable to sequence by Edman degradation, by limiting substitution to only 50% of the peptides on a given bead with N-terminal pyroglutatamate, there will remain enough non-pyroglutamate peptide on the bead for sequencing. One of ordinary skill in would readily recognize that this technique could be used for sequencing of any peptide that incorporates a residue resistant to Edman degradation at the N-terminus. Other methods to characterize individual peptides that demonstrate desired activity are described in detail *infra*. Specific activity of a peptide that comprises a blocked N-terminal group, *e.g.*, pyroglutamate, when the particular N-terminal group is present in 50% of the peptides, would readily be demonstrated by comparing activity of a completely (100%) blocked peptide with a non-blocked (0%) peptide.

Chemical Moieties For Derivatization. Chemical moieties suitable for derivatization may be selected from among water soluble polymers. The polymer selected should be water soluble so that the component to which it is attached does not precipitate in an aqueous environment, such as a physiological environment. Preferably, for therapeutic use of the end-product preparation, the polymer will be pharmaceutically acceptable. One skilled in the art will be able to select the desired polymer based on such considerations as whether the polymer/component conjugate will be used therapeutically, and if so, the desired dosage, circulation time, resistance to proteolysis, and other considerations. For the present component or components, these may be ascertained using the assays provided herein.

The water soluble polymer may be selected from the group consisting of, for example, polyethylene glycol, copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide co- polymers, polyoxyethylated polyols and polyvinyl alcohol. Polyethylene glycol propionaldenhyde may have advantages in manufacturing due to its stability in water.

The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 2kDa and about 100kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (*e.g.*, the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog).

The number of polymer molecules so attached may vary, and one skilled in the art will be able to ascertain the effect on function. One may mono-derivatize, or may provide for a di-, tri-, tetra- or some combination of derivatization, with the same or different chemical moieties (*e.g.*, polymers, such as different weights of polyethylene glycols). The proportion of polymer molecules to component or components molecules will vary, as will their concentrations in the reaction mixture. In general, the optimum ratio (in terms of efficiency of reaction in that there is no excess unreacted component or components and polymer) will be determined by factors such as the desired degree of derivatization (*e.g.*, mono, di-, tri-, etc.), the molecular weight of the polymer selected, whether the polymer is branched or unbranched, and the reaction conditions.

The polyethylene glycol molecules (or other chemical moieties) should be attached to the component or components with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, *e.g.*, EP 0 401 384 herein incorporated by reference (coupling PEG to G-CSF), *see also* Malik et al., 1992, Exp. Hematol. 20:1028-1035 (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group include lysine residues and the – terminal amino acid residues; those having a free carboxyl group include aspartic acid residues glutamic acid residues and the C-terminal amino acid residue. Sulfhydrl groups may also be used as a reactive group for attaching the polyethylene glycol molecule(s). Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

This invention provides an isolated nucleic acid encoding a polypeptide comprising an amino acid sequence of a N-terminal choline binding protein A truncate. This invention provides an isolated nucleic acid encoding a polypeptide comprising an amino acid sequence of a N-terminal choline binding protein A truncate as set forth in Figure 2. In one embodiment the nucleic acid is set forth in any of SEQ ID NOS 12, 14-17, or 19-21, including fragments, mutants, variants, analogs, or derivatives, thereof. The nucleic acid is DNA, cDNA, genomic DNA, RNA. Further, the isolated nucleic acid may be operatively linked to a promoter of RNA transcription. It is contemplated that the nucleic acid is used to competitively inhibit the lectin activity.

A "vector" is a replicon, such as plasmid, phage or cosmid, to which another DNA segment may be attached so as to bring about the replication of the attached segment.

A "DNA" refers to the polymeric form of deoxyribonucleotides (adenine, guanine, thymine, or cytosine) in its either single stranded form, or a double-stranded helix. This term refers only to the primary and secondary structure of the molecule, and does not

limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia*, in linear DNA molecules (e.g., restriction fragments), viruses, plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the nontranscribed strand of DNA (*i.e.*, the strand having a sequence homologous to the mRNA).

A DNA sequence is "operatively linked" to an expression control sequence when the expression control sequence controls and regulates the transcription and translation of that DNA sequence. The term "operatively linked" includes having an appropriate start signal (*e.g.*, ATG) in front of the DNA sequence to be expressed and maintaining the correct reading frame to permit expression of the DNA sequence under the control of the expression control sequence and production of the desired product encoded by the DNA sequence. If a gene that one desires to insert into a recombinant DNA molecule does not contain an appropriate start signal, such a start signal can be inserted in front of the gene.

Further this invention also provides a vector which comprises the above-described nucleic acid molecule. The promoter may be, or is identical to, a bacterial, yeast, insect or mammalian promoter. Further, the vector may be a plasmid, cosmid, yeast artificial chromosome (YAC), bacteriophage or eukaryotic viral DNA.

Other numerous vector backbones known in the art as useful for expressing protein may be employed. Such vectors include, but are not limited to: adenovirus (AV), adeno-associated virus (AAV), simian virus 40 (SV40), cytomegalovirus (CMV), mouse mammary tumor virus (MMTV), Moloney murine leukemia virus, DNA delivery systems, *i.e.* liposomes, and expression plasmid delivery systems. Further, one class of vectors comprises DNA elements derived from viruses such as bovine papilloma virus, polyoma virus, baculovirus, retroviruses or Semliki Forest virus. Such vectors may be obtained commercially or assembled from the sequences described by methods well-known in the art.

This invention also provides a host vector system for the production of a polypeptide which comprises the vector of a suitable host cell. Suitable host cells include, but are not

limited to, prokaryotic or eukaryotic cells, *e.g.* bacterial cells (including gram positive cells), yeast cells, fungal cells, insect cells, and animals cells. Numerous mammalian cells may be used as hosts, including, but not limited to, the mouse fibroblast cell NIH 3T3, CHO cells, HeLa cells, Ltk$^-$ cells, Cos cells, etc.

A wide variety of host/expression vector combinations may be employed in expressing the DNA sequences of this invention. Useful expression vectors, for example, may consist of segments of chromosomal, non-chromosomal and synthetic DNA sequences. Suitable vectors include derivatives of SV40 and known bacterial plasmids, *e.g.*, *E. coli* plasmids col El, pCR1, pBR322, pMB9 and their derivatives, plasmids such as RP4; phage DNAS, *e.g.*, the numerous derivatives of phage λ, *e.g.*, NM989, and other phage DNA, *e.g.*, M13 and filamentous single stranded phage DNA; yeast plasmids such as the 2μ plasmid or derivatives thereof; vectors useful in eukaryotic cells, such as vectors useful in insect or mammalian cells; vectors derived from combinations of plasmids and phage DNAs, such as plasmids that have been modified to employ phage DNA or other expression control sequences; and the like.

Any of a wide variety of expression control sequences -- sequences that control the expression of a DNA sequence operatively linked to it -- may be used in these vectors to express the DNA sequences of this invention. Such useful expression control sequences include, for example, the early or late promoters of SV40, CMV, vaccinia, polyoma or adenovirus, the *lac* system, the *trp* system, the *TAC* system, the *TRC* system, the *LTR* system, the major operator and promoter regions of phage λ, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase or other glycolytic enzymes, the promoters of acid phosphatase (*e.g.*, Pho5), the promoters of the yeast α-mating factors, and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells or their viruses, and various combinations thereof.

A wide variety of unicellular host cells are also useful in expressing the DNA sequences of this invention. These hosts may include well known eukaryotic and prokaryotic hosts, such as strains of *E. coli*, *Pseudomonas*, *Bacillus*, *Streptomyces*, fungi such as yeasts, and animal cells, such as CHO, Rl.l, B-W and L-M cells, African Green Monkey kidney

cells (e.g., COS 1, COS 7, BSC1, BSC40, and BMT10), insect cells (*e.g.*, Sf9), and human cells and plant cells in tissue culture.

It will be understood that not all vectors, expression control sequences and hosts will function equally well to express the DNA sequences of this invention. Neither will all hosts function equally well with the same expression system. However, one skilled in the art will be able to select the proper vectors, expression control sequences, and hosts without undue experimentation to accomplish the desired expression without departing from the scope of this invention. For example, in selecting a vector, the host must be considered because the vector must function in it. The vector's copy number, the ability to control that copy number, and the expression of any other proteins encoded by the vector, such as antibiotic markers, will also be considered.

In selecting an expression control sequence, a variety of factors will normally be considered. These include, for example, the relative strength of the system, its controllability, and its compatibility with the particular DNA sequence or gene to be expressed, particularly as regards potential secondary structures. Suitable unicellular hosts will be selected by consideration of, *e.g.*, their compatibility with the chosen vector, their secretion characteristics, their ability to fold proteins correctly, and their fermentation requirements, as well as the toxicity to the host of the product encoded by the DNA sequences to be expressed, and the ease of purification of the expression products.

This invention further provides a method of producing a polypeptide which comprises growing the above-described host vector system under suitable conditions permitting the production of the polypeptide and recovering the polypeptide so produced.

This invention further provides an antibody capable of specifically recognizing or binding to the isolated polypeptide. The antibody may be a monoclonal or polyclonal antibody. Further, the antibody may be labeled with a detectable marker that is either a radioactive, colorimetric, fluorescent, or a luminescent marker. The labeled antibody may be a polyclonal or monoclonal antibody. In one embodiment, the labeled antibody is a purified labeled antibody. Methods of labeling antibodies are well known in the art.

The term "antibody" includes, by way of example, both naturally occurring and non-naturally occurring antibodies. Specifically, the term "antibody" includes polyclonal and monoclonal antibodies, and fragments thereof. Furthermore, the term "antibody" includes chimeric antibodies and wholly synthetic antibodies, and fragments thereof.Such antibodies include but are not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments, and an Fab expression library.

Various procedures known in the art may be used for the production of polyclonal antibodies to polypeptide or derivatives or analogs thereof (*see, e.g., Antibodies -- A Laboratory Manual*, Harlow and Lane, eds., Cold Spring Harbor Laboratory Press: Cold Spring Harbor, Ne York, 1988). For the production of antibody, various host animals can be immunized by injection with the truncated CbpA, or a derivative (*e.g.*, fragment or fusion protein) thereof, including but not limited to rabbits, mice, rats, sheep, goats, etc. In one embodiment, the polypeptide can be conjugated to an immunogenic carrier, *e.g.*, bovine serum albumin (BSA) or keyhole limpet hemocyanin (KLH). Various adjuvant may be used to increase the immunological response, depending on the host species.

For preparation of monoclonal antibodies, or fragment, analog, or derivative thereof, any technique that provides for the production of antibody molecules by continuous cell lines in culture may be used (*see, e.g., Antibodies -- A Laboratory Manual*, Harlow and Lane, eds., Cold Spring Harbor Laboratory Press: Cold Spring Harbor, Ne York, 1988). These include but are not limited to the hybridoma technique originally developed by Kohler and Milstein (1975, Nature 256:495-497), as well as the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., 1985, in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96). In an additional embodiment of the invention, monoclonal antibodies can be produced in germ-free animals utilizing recent technology (PCT/US90/02545). According to the invention, human antibodies may be used and can be obtained by using human hybridomas (Cote et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:2026-2030) or by transforming human B cells with EBV virus *in vitro* (Cole et al., 1985, in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, pp. 77-96). In fact, according to the invention, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, J. Bacteriol.

159-870; Neuberger et al., 1984, Nature 312:604-608; Takeda et al., 1985, Nature 314:452-454) by splicing the genes from a mouse antibody molecule specific for a polypeptide together with genes from a human antibody molecule of appropriate biological activity can be used; such antibodies are within the scope of this invention. Such human or humanized chimeric antibodies are preferred for use in therapy of human diseases or disorders (described *infra*), since the human or humanized antibodies are much less likely than xenogenic antibodies to induce an immune response, in particular an allergic response, themselves. An additional embodiment of the invention utilizes the techniques described for the construction of Fab expression libraries (Huse et al., 1989, Science 246:1275-1281) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity for the polypeptide, or its derivatives, or analogs.

Antibody fragments which contain the idiotype of the antibody molecule can be generated by known techniques. For example, such fragments include but are not limited to: the $F(ab')_2$ fragment which can be produced by pepsin digestion of the antibody molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragment, and the Fab fragments which can be generated by treating the antibody molecule with papain and a reducing agent.

In the production of antibodies, screening for the desired antibody can be accomplished by techniques known in the art, *e.g.*, radioimmunoassay, ELISA (enzyme-linked immunosorbant assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitin reactions, immunodiffusion assays, *in situ* immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (*e.g.*, gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

Antibodies can be labeled for detection *in vitro*, *e.g.*, with labels such as enzymes, fluorophores, chromophores, radioisotopes, dyes, colloidal gold, latex particles, and chemiluminescent agents. Alternatively, the antibodies can be labeled for detection *in vivo*, *e.g.*, with radioisotopes (preferably technetium or iodine); magnetic resonance shift reagents (such as gadolinium and manganese); or radio-opaque reagents.

The labels most commonly employed for these studies are radioactive elements, enzymes, chemicals which fluoresce when exposed to ultraviolet light, and others.A number of fluorescent materials are known and can be utilized as labels. These include, for example, fluorescein, rhodamine, auramine, Texas Red, AMCA blue and Lucifer Yellow. A particular detecting material is anti-rabbit antibody prepared in goats and conjugated with fluorescein through an isothiocyanate. The polypeptide can also be labeled with a radioactive element or with an enzyme. The radioactive label can be detected by any of the currently available counting procedures. The preferred isotope may be selected from ^{3}H, ^{14}C, ^{32}P, ^{35}S, ^{36}Cl, ^{51}Cr, ^{57}Co, ^{58}Co, ^{59}Fe, ^{90}Y, ^{125}I, ^{131}I, and ^{186}Re.

Enzyme labels are likewise useful, and can be detected by any of the presently utilized colorimetric, spectrophotometric, fluorospectrophotometric, amperometric or gasometric techniques. The enzyme is conjugated to the selected particle by reaction with bridging molecules such as carbodiimides, diisocyanates, glutaraldehyde and the like. Many enzymes which can be used in these procedures are known and can be utilized. The preferred are peroxidase, ß-glucuronidase, ß-D-glucosidase, ß-D-galactosidase, urease, glucose oxidase plus peroxidase and alkaline phosphatase. U.S. Patent Nos. 3,654,090; 3,850,752; and 4,016,043 are referred to by way of example for their disclosure of alternate labeling material and methods.

In a further embodiment of this invention, commercial test kits suitable for use by a medical specialist may be prepared to determine the presence or absence of predetermined binding activity or predetermined binding activity capability to suspected target cells. In accordance with the testing techniques discussed above, one class of such kits will contain at least the labeled polypeptide or its binding partner, for instance an antibody specific thereto, and directions, of course, depending upon the method selected,

e.g., "competitive," "sandwich," "DASP" and the like. The kits may also contain peripheral reagents such as buffers, stabilizers, etc.

Accordingly, a test kit may be prepared for the demonstration of the presence or capability of cells for predetermined bacterial binding activity, comprising:

(a) a predetermined amount of at least one labeled immunochemically reactive component obtained by the direct or indirect attachment of the present the polypeptide or a specific binding partner thereto, to a detectable label;

(b) other reagents; and

(c) directions for use of said kit.

This invention provides antagonist or blocking agents which include but are not limited to: peptide fragments, mimetics, a nucleic acid molecule, a ribozyme, a polypeptide, a small molecule, a carbohydrate molecule, a monosaccharide, an oligosaccharide or an antibody. Also, agents which competitively block or inhibit pneumococcal bacterium are contemplmated by this invention. This invention provides an agent which comprises an inorganic compound, a nucleic acid molecule, an oligonucleotide, an organic compound, a peptide, a peptidomimetic compound, or a protein which inhibits the polypeptide.

This invention provides a vaccine which comprises the polypeptide having the amino acid sequence as set forth in any of SEQ ID NOS: 1, 3-7, 9-11, 22, and 23 and a pharmaceutically acceptable adjuvant or carrier. The polypeptide may comprise an amino acid sequence of a N-terminal choline binding protein A truncate as set forth in Figure 2. This invention provides a vaccine which comprises the polypeptide having the amino acid sequence which comprises a conserved region as set forth in Figure 2 and a pharmaceutically acceptable adjuvant or carrier. For example, conserved regions include but are not limited to amino acid sequence 158 to 172; 300 to 321; 331 to 339; 355 to 365; 367 to 374; 379 to 389; 409 to 427; and 430 to 447. This invention provides a vaccine comprising the isolated nucleic acid encoding the polypeptide and a pharmaceutically acceptable adjuvant or carrier.

Active immunity against Gram positive bacteria, particularly pneumococcus, can be induced by immunization (vaccination) with an immunogenic amount of the polypeptide,

or peptide derivative or fragment thereof, and an adjuvant, wherein the polypeptide, or antigenic derivative or fragment thereof, is the antigenic component of the vaccine.

The polypeptides of the present invention, or derivatives or fragments thereof, can be prepared in an admixture with an adjuvant to prepare a vaccine. Preferably, the derivative or fragment thereof, used as the antigenic component of the vaccine is an adhesin. More preferably, the polypeptide or peptide derivative or fragment thereof, used as the antigenic component of the vaccine is an antigen common to all or many strains of a species of Gram positive bacteria, or common to closely related species of bacteria. Most preferably, the antigenic component of the vaccine is an adhesin that is a common antigen.

Vectors containing the nucleic acid-based vaccine of the invention can be introduced into the desired host by methods known in the art, *e.g.*, transfection, electroporation, microinjection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, lipofection (lysosome fusion), use of a gene gun, or a DNA vector transporter (see, *e.g.*, Wu et al., 1992, J. Biol. Chem. 267:963-967; Wu and Wu, 1988, J. Biol. Chem. 263:14621-14624; Hartmut et al., Canadian Patent Application No. 2,012,311, filed March 15, 1990).

The vaccine can be administered via any parenteral route, including but not limited to intramuscular, intraperitoneal, intravenous, and the like. Preferably, since the desired result of vaccination is to elucidate an immune response to the antigen, and thereby to the pathogenic organism, administration directly, or by targeting or choice of a viral vector, indirectly, to lymphoid tissues, *e.g.*, lymph nodes or spleen, is desirable. Since immune cells are continually replicating, they are ideal target for retroviral vector-based nucleic acid vaccines, since retroviruses require replicating cells.

Passive immunity can be conferred to an animal subject suspected of suffering an infection with a Gram positive bacterium, preferably streptococcal, and more preferably pneumoccal, by administering antiserum, polyclonal antibodies, or a neutralizing monoclonal antibody against a polypeptide of the invention to the patient. Although passive immunity does not confer long term protection, it can be a valuable tool for the

treatment of a bacterial infection of a subject who has not been vaccinated. Passive immunity is particularly important for the treatment of antibiotic resistant strains of Gram positive bacteria, since no other therapy may be available. Preferably, the antibodies administered for passive immune therapy are autologous antibodies. For example, if the subject is a human, preferably the antibodies are of human origin or have been "humanized," in order to minimize the possibility of an immune response against the antibodies. The active or passive vaccines of the invention, or the administration of an adhesin, can be used to protect an animal subject from infection of a Gram positive bacteria, preferably streptococcus, and more preferably, pneumococcus.

This invention provides a pharmaceutical composition comprising an amount of the polypeptide as described and a pharmaceutically acceptable carrier or diluent.

For example, such pharmaceutical composition for preventing pneumococcal attachment to mucosal surface may include antibody to lectin domain and/or soluble excess lectin domain proteins. Blocking adherence by either mechanism blocks the initial step in infection thereby reducing colonization. This in turn decreases person to person transmission and prevents development of symptomatic disease.

This invention provides a method of inducing an immune response in a subject which has been exposed to or infected with a pneumococcal bacterium comprising administering to the subject an amount of the pharmaceutical composition, thereby inducing an immune response.

This invention provides a method for preventing infection by a pneumococcal bacterium in a subject comprising administering to the subject an amount of the pharmaceutical composition effective to prevent pneumococcal bacterium attachment, thereby preventing infection by a pneumococcal bacterium.

This invention provides a method for preventing infection by a pneumococcal bacterium in a subject comprising administering to the subject an amount of a pharmaceutical composition comprising the antibody and a pharmaceutically acceptable carrier or diluent, thereby preventing infection by a pneumococcal bacterium.

This invention provides a method for treating a subject infected with or exposed to pneumococcal bacterium comprising administering to the subject a therapeutically effective amount of a vaccine of the invention, thereby treating the subject.

This invention provides a method of inhibiting colonization of host cells in a subject which has been exposed to or infected with a pneumococcal bacterium comprising administering to the subject an amount of the pharmaceutical composition comprising the polypeptide consisting of the amino acid sequence as set forth in any of SEQ ID NOS 1, 3-5, 7, or 9-11, thereby inducing an immune response. The therapeutic peptide that blocks colonization is delivered by the respiratory mucosa. The pharmaceutical composition comprising the polypeptide consisting of the amino acid sequence as set forth in Figure 2.

As used herein, "pharmaceutical composition" could mean therapeutically effective amounts of polypeptide products of the invention together with suitable diluents, preservatives, solubilizers, emulsifiers, adjuvant and/or carriers useful in to confer a therapeutic effect or benefit by *e.g.* preventing pneumococcal colonization. A "therapeutically effective amount" as used herein refers to that amount which provides a therapeutic effect for a given condition and administration regimen. Such compositions are liquids or lyophilized or otherwise dried formulations and include diluents of various buffer content (*e.g.*, Tris-HCl., acetate, phosphate), pH and ionic strength, additives such as albumin or gelatin to prevent absorption to surfaces, detergents (e.g., Tween 20, Tween 80, Pluronic F68, bile acid salts). solubilizing agents (e.g., glycerol, polyethylene glycerol), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite), preservatives (e.g., Thimerosal, benzyl alcohol, parabens), bulking substances or tonicity modifiers (e.g., lactose, mannitol), covalent attachment of polymers such as polyethylene glycol to the protein, complexation with metal ions, or incorporation of the material into or onto particulate preparations of polymeric compounds such as polylactic acid, polglycolic acid, hydrogels, etc, or onto liposomes, microemulsions, micelles, unilamellar or multilamellar vesicles, erythrocyte ghosts, or spheroplasts. Such compositions will influence the physical state, solubility, stability, rate of in vivo release, and rate of in vivo clearance of the therapeutic agent. The choice of compositions will depend on the physical and chemical properties of the protein having therapeutic activity. For example,

a product derived from a membrane-bound form of the active may require a formulation containing detergent. Controlled or sustained release compositions include formulation in lipophilic depots (e.g., fatty acids, waxes, oils). Also comprehended by the invention are particulate compositions coated with polymers (e.g., poloxamers or poloxamines) and actives coupled to antibodies directed against tissue-specific receptors, ligands or antigens or coupled to ligands of tissue-specific receptors. Other embodiments of the compositions of the invention incorporate particulate forms protective coatings, protease inhibitors or permeation enhancers for various routes of administration, including parenteral, pulmonary, nasal and oral.

Further, as used herein "pharmaceutically acceptable carrier" are well known to those skilled in the art and include, but are not limited to, 0.01-0.1M and preferably 0.05M phosphate buffer or 0.8% saline. Additionally, such pharmaceutically acceptable carriers may be aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present, such as, for example, antimicrobials, antioxidants, collating agents, inert gases and the like.

The term "adjuvant" refers to a compound or mixture that enhances the immune response to an antigen. An adjuvant can serve as a tissue depot that slowly releases the antigen and also as a lymphoid system activator that non-specifically enhances the immune response (Hood et al., *Immunology, Second Ed.*, 1984, Benjamin/Cummings: Menlo Park, California, p. 384). Often, a primary challenge with an antigen alone, in the absence of an adjuvant, will fail to elicit a humoral or cellular immune response. Adjuvant include, but are not limited to, complete Freund's adjuvant, incomplete Freund's adjuvant, saponin, mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil or hydrocarbon

emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvant such as BCG (*bacille Calmette-Guerin*) and *Corynebacterium parvum*. Preferably, the adjuvant is pharmaceutically acceptable.

Controlled or sustained release compositions include formulation in lipophilic depots (e.g. fatty acids, waxes, oils). Also comprehended by the invention are particulate compositions coated with polymers (e.g. poloxamers or poloxamines) and the compound coupled to antibodies directed against tissue-specific receptors, ligands or antigens or coupled to ligands of tissue-specific receptors. Other embodiments of the compositions of the invention incorporate particulate forms protective coatings, protease inhibitors or permeation enhancers for various routes of administration, including parenteral, pulmonary, nasal and oral.

When administered, compounds are often cleared rapidly from mucosal surfaces or the circulation and may therefore elicit relatively short-lived pharmacological activity. Consequently, frequent administrations of relatively large doses of bioactive compounds may by required to sustain therapeutic efficacy. Compounds modified by the covalent attachment of water-soluble polymers such as polyethylene glycol, copolymers of polyethylene glycol and polypropylene glycol, carboxymethyl cellulose, dextran, polyvinyl alcohol, polyvinylpyrrolidone or polyproline are known to exhibit substantially longer half-lives in blood following intravenous injection than do the corresponding unmodified compounds (Abuchowski et al., 1981; Newmark et al., 1982; and Katre et al., 1987). Such modifications may also increase the compound's solubility in aqueous solution, eliminate aggregation, enhance the physical and chemical stability of the compound, and greatly reduce the immunogenicity and reactivity of the compound. As a result, the desired *in vivo* biological activity may be achieved by the administration of such polymer-compound abducts less frequently or in lower doses than with the unmodified compound.

Dosages. The sufficient amount may include but is not limited to from about 1 μg/kg to about 1000 mg/kg. The amount may be 10 mg/kg. The pharmaceutically acceptable form of the composition includes a pharmaceutically acceptable carrier.

As noted above, the present invention provides therapeutic compositions comprising pharmaceutical compositions comprising vectors, vaccines, polypeptides, nucleic acids and antibodies, anti-antibodies, and agents, to compete with the pneumococcus bacterium for pathogenic activities, such as adherence to host cells.

The preparation of therapeutic compositions which contain an active component is well understood in the art. Typically, such compositions are prepared as an aerosol of the polypeptide delivered to the nasopharynx or as injectables, either as liquid solutions or suspensions, however, solid forms suitable for solution in, or suspension in, liquid prior to injection can also be prepared. The preparation can also be emulsified. The active therapeutic ingredient is often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents which enhance the effectiveness of the active ingredient.

An active component can be formulated into the therapeutic composition as neutralized pharmaceutically acceptable salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the polypeptide or antibody molecule) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed from the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

A composition comprising "A" (where "A" is a single protein, DNA molecule, vector, etc.) is substantially free of "B" (where "B" comprises one or more contaminating proteins, DNA molecules, vectors, etc.) when at least about 75% by weight of the proteins, DNA, vectors (depending on the category of species to which A and B belong) in the composition is "A". Preferably, "A" comprises at least about 90% by weight of the A+B species in the composition, most preferably at least about 99% by weight.

The phrase "therapeutically effective amount" is used herein to mean an amount sufficient to reduce by at least about 15 percent, preferably by at least 50 percent, more preferably by at least 90 percent, and most preferably prevent, a clinically significant deficit in the activity, function and response of the host. Alternatively, a therapeutically effective amount is sufficient to cause an improvement in a clinically significant condition in the host. In the context of the present invention, a deficit in the response of the host is evidenced by continuing or spreading bacterial infection. An improvement in a clinically significant condition in the host includes a decrease in bacterial load, clearance of bacteria from colonized host cells, reduction in fever or inflammation associated with infection, or a reduction in any symptom associated with the bacterial infection.

According to the invention, the component or components of a therapeutic composition of the invention may be introduced parenterally, transmucosally, *e.g.*, orally, nasally, pulmonarailly, or rectally, or transdermally. Preferably, administration is parenteral, *e.g.*, via intravenous injection, and also including, but is not limited to, intra-arterial, intramuscular, intradermal, subcutaneous, intraperitoneal, intraventricular, and intracranial administration. Oral or pulmonary delivery may be preferred to activate mucosal immunity; since pneumococci generally colonize the nasopharyngeal and pulmonary mucosa, mucosal immunity may be a particularly effective preventive treatment. The term "unit dose" when used in reference to a therapeutic composition of the present invention refers to physically discrete units suitable as unitary dosage for humans, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required diluent; *i.e.*, carrier, or vehicle.

In another embodiment, the active compound can be delivered in a vesicle, in particular a liposome (see Langer, Science 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, ibid., pp. 317-327; see generally ibid).

In yet another embodiment, the therapeutic compound can be delivered in a controlled release system. For example, the polypeptide may be administered using intravenous

infusion, an implantable osmotic pump, a transdermal patch, liposomes, or other modes of administration. In one embodiment, a pump may be used (see Langer, supra; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)). In another embodiment, polymeric materials can be used (see *Medical Applications of Controlled Release*, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J. Macromol. Sci. Rev. Macromol. Chem. 23:61 (1983); see also Levy et al., Science 228:190 (1985); During et al., Ann. Neurol. 25:351 (1989); Howard et al., J. Neurosurg. 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984)). Preferably, a controlled release device is introduced into a subject in proximity of the site of inappropriate immune activation or a tumor. Other controlled release systems are discussed in the review by Langer (Science 249:1527-1533 (1990)).

A subject in whom administration of an active component as set forth above is an effective therapeutic regimen for a bacterial infection is preferably a human, but can be any animal. Thus, as can be readily appreciated by one of ordinary skill in the art, the methods and pharmaceutical compositions of the present invention are particularly suited to administration to any animal, particularly a mammal, and including, but by no means limited to, domestic animals, such as feline or canine subjects, farm animals, such as but not limited to bovine, equine, caprine, ovine, and porcine subjects, wild animals (whether in the wild or in a zoological garden), research animals, such as mice, rats, rabbits, goats, sheep, pigs, dogs, cats, etc., *i.e.*, for veterinary medical use.

In the therapeutic methods and compositions of the invention, a therapeutically effective dosage of the active component is provided. A therapeutically effective dosage can be determined by the ordinary skilled medical worker based on patient characteristics (age, weight, sex, condition, complications, other diseases, etc.), as is well known in the art. Furthermore, as further routine studies are conducted, more specific information will emerge regarding appropriate dosage levels for treatment of various conditions in various

patients, and the ordinary skilled worker, considering the therapeutic context, age and general health of the recipient, is able to ascertain proper dosing. Generally, for intravenous injection or infusion, dosage may be lower than for intraperitoneal, intramuscular, or other route of administration. The dosing schedule may vary, depending on the circulation half-life, and the formulation used. The compositions are administered in a manner compatible with the dosage formulation in the therapeutically effective amount. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are peculiar to each individual. However, suitable dosages may range from about 0.1 to 20, preferably about 0.5 to about 10, and more preferably one to several, milligrams of active ingredient per kilogram body weight of individual per day and depend on the route of administration. Suitable regimes for initial administration and booster shots are also variable, but are typified by an initial administration followed by repeated doses at one or more hour intervals by a subsequent injection or other administration. Alternatively, continuous intravenous infusion sufficient to maintain concentrations of ten nanomolar to ten micromolar in the blood are contemplated.

Administration with other compounds. For treatment of a bacterial infection, one may administer the present active component in conjunction with one or more pharmaceutical compositions used for treating bacterial infection, including but not limited to (1) antibiotics; (2) soluble carbohydrate inhibitors of bacterial adhesin; (3) other small molecule inhibitors of bacterial adhesin; (4) inhibitors of bacterial metabolism, transport, or transformation; (5) stimulators of bacterial lysis, or (6) anti-bacterial antibodies or vaccines directed at other bacterial antigens. Other potential active components include anti-inflammatory agents, such as steroids and non-steroidal anti-inflammatory drugs. Administration may be simultaneous (for example, administration of a mixture of the present active component and an antibiotic), or may be *in seriatim*.

Accordingly, in specific embodiment, the therapeutic compositions may further include an effective amount of the active component, and one or more of the following active ingredients: an antibiotic, a steroid, etc. Exemplary formulations are given below:

Formulations

Intravenous Formulation I

Ingredient	mg/ml
cefotaxime	250.0
Polypeptide	10.0
dextrose USP	45.0
sodium bisulfite USP	3.2
edetate disodium USP	0.1
water for injection q.s.a.d.	1.0 ml

Intravenous Formulation II

Ingredient	mg/ml
ampicillin	250.0
Polypeptide	10.0
sodium bisulfite USP	3.2
disodium edetate USP	0.1
water for injection q.s.a.d.	1.0 ml

Intravenous Formulation III

Ingredient	mg/ml
gentamicin (charged as sulfate)	40.0
Polypeptide	10.0
sodium bisulfite USP	3.2
disodium edetate USP	0.1
water for injection q.s.a.d.	1.0 ml

Intravenous Formulation IV

Ingredient	mg/ml
Polypeptide	10.0
dextrose USP	45.0
sodium bisulfite USP	3.2
edetate disodium USP	0.1
water for injection q.s.a.d.	1.0 ml

Intravenous Formulation V

Ingredient	mg/ml
Polypeptide antagonist	5.0
sodium bisulfite USP	3.2
disodium edetate USP	0.1
water for injection q.s.a.d.	1.0 ml

Thus, in a specific instance where it is desired to reduce or inhibit the infection resulting from a bacterium mediated binding of bacteria to a host cell, or an antibody thereto, or a ligand thereof or an antibody to that ligand, the polypeptide is introduced to block the interaction of the bacteria with the host cell.

Also contemplated herein is pulmonary delivery of the present polypeptide having lectin activity which acts as an adhesin inhibitory agent (or derivatives thereof), of the invention. The adhesin inhibitory agent (or derivative) is delivered to the lungs of a mammal, where it can interfere with bacterial, *i.e.*, streptococcal, and preferably pneumococcal binding to host cells. Other reports of preparation of proteins for pulmonary delivery are found in the art [Adjei et al. *Pharmaceutical Research*, **7**:565-569 (1990); Adjei et al., *International Journal of Pharmaceutics*, **63**:135-144 (1990) (leuprolide acetate); Braquet et al., *Journal of Cardiovascular Pharmacology*, **13**(suppl. 5):143-146 (1989) (endothelin-1); Hubbard et al., *Annals of Internal Medicine*, **Vol. III**, pp. 206-212 (1989) (α1-antitrypsin); Smith et al., *J. Clin. Invest.* **84**:1145-1146 (1989) (α-1-proteinase); Oswein et al., "Aerosolization of Proteins", *Proceedings of Symposium on Respiratory Drug Delivery II*, Keystone, Colorado, March, (1990) (recombinant human growth hormone); Debs et al., *J. Immunol.* **140**:3482-3488 (1988) (interferon-γ and tumor necrosis factor alpha); Platz et al., U.S. Patent No. 5,284,656 (granulocyte colony stimulating factor)]. A method and composition for pulmonary delivery of drugs is described in U.S. Patent No. 5,451,569, issued September 19, 1995 to Wong et al.

All such devices require the use of formulations suitable for the dispensing of adhesin inhibitory agent (or derivative). Typically, each formulation is specific to the type of device employed and may involve the use of an appropriate propellant material, in

addition to the usual diluents, adjuvant and/or carriers useful in therapy. Also, the use of liposomes, microcapsules or microspheres, inclusion complexes, or other types of carriers is contemplated. Chemically modified adhesin inhibitory agent may also be prepared in different formulations depending on the type of chemical modification or the type of device employed.

Formulations suitable for use with a nebulizer, either jet or ultrasonic, will typically comprise adhesin inhibitory agent (or derivative) dissolved in water at a concentration of about 0.1 to 25 mg of biologically active adhesin inhibitory agent per ml of solution. The formulation may also include a buffer and a simple sugar (*e.g.*, for adhesin inhibitory agent stabilization and regulation of osmotic pressure). The nebulizer formulation may also contain a surfactant, to reduce or prevent surface induced aggregation of the adhesin inhibitory agent caused by atomization of the solution in forming the aerosol.

Formulations for use with a metered-dose inhaler device will generally comprise a finely divided powder containing the adhesin inhibitory agent (or derivative) suspended in a propellant with the aid of a surfactant. The propellant may be any conventional material employed for this purpose, such as a chlorofluorocarbon, a hydrochlorofluorocarbon, a hydrofluorocarbon, or a hydrocarbon, including trichlorofluoromethane, dichlorodifluoromethane, dichlorotetrafluoroethanol, and 1,1,1,2-tetrafluoroethane, or combinations thereof. Suitable surfactants include sorbitan trioleate and soya lecithin. Oleic acid may also be useful as a surfactant.

The liquid aerosol formulations contain adhesin inhibitory agent and a dispersing agent in a physiologically acceptable diluent. The dry powder aerosol formulations of the present invention consist of a finely divided solid form of adhesin inhibitory agent and a dispersing agent. With either the liquid or dry powder aerosol formulation, the formulation must be aerosolized. That is, it must be broken down into liquid or solid particles in order to ensure that the aerosolized dose actually reaches the mucous membranes of the nasal passages or the lung. The term "aerosol particle" is used herein to describe the liquid or solid particle suitable for nasal or pulmonary administration, *i.e.*, that will reach the mucous membranes. Other considerations, such as construction of the delivery device, additional components in the formulation, and particle characteristics are

important. These aspects of pulmonary administration of a drug are well known in the art, and manipulation of formulations, aerosolization means and construction of a delivery device require at most routine experimentation by one of ordinary skill in the art. In a particular embodiment, the mass median dynamic diameter will be 5 micrometers or less in order to ensure that the drug particles reach the lung alveoli [Wearley, L.L., *Crit. Rev. in Ther. Drug Carrier Systems* **8**:333 (1991)].

Systems of aerosol delivery, such as the pressurized metered dose inhaler and the dry powder inhaler are disclosed in Newman, S.P., *Aerosols and the Lung*, Clarke, S.W. and Davia, D. editors, pp. 197-22 and can be used in connection with the present invention.

In a further embodiment, as discussed in detail *infra*, an aerosol formulation of the present invention can include other therapeutically or pharmacologically active ingredients in addition to adhesin inhibitory agent, such as but not limited to an antibiotic, a steroid, a non-steroidal anti-inflammatory drug, etc.

Liquid Aerosol Formulations. The present invention provides aerosol formulations and dosage forms for use in treating subjects suffering from bacterial, *e.g.*, streptococcal, in particularly pneumococcal, infection. In general such dosage forms contain adhesin inhibitory agent in a pharmaceutically acceptable diluent. Pharmaceutically acceptable diluents include but are not limited to sterile water, saline, buffered saline, dextrose solution, and the like. In a specific embodiment, a diluent that may be used in the present invention or the pharmaceutical formulation of the present invention is phosphate buffered saline, or a buffered saline solution generally between the pH 7.0-8.0 range, or water.

The liquid aerosol formulation of the present invention may include, as optional ingredients, pharmaceutically acceptable carriers, diluents, solubilizing or emulsifying agents, surfactants and excipients. The formulation may include a carrier. The carrier is a macromolecule which is soluble in the circulatory system and which is physiologically acceptable where physiological acceptance means that those of skill in the art would accept injection of said carrier into a patient as part of a therapeutic regime. The carrier preferably is relatively stable in the circulatory system with an acceptable

plasma half life for clearance. Such macromolecules include but are not limited to Soya lecithin, oleic acid and sorbitan trioleate, with sorbitan trioleate preferred.

The formulations of the present embodiment may also include other agents useful for pH maintenance, solution stabilization, or for the regulation of osmotic pressure. Examples of the agents include but are not limited to salts, such as sodium chloride, or potassium chloride, and carbohydrates, such as glucose, galactose or mannose, and the like.

The present invention further contemplates liquid aerosol formulations comprising adhesin inhibitory agent and another therapeutically effective drug, such as an antibiotic, a steroid, a non-steroidal anti-inflammatory drug, etc.

Aerosol Dry Powder Formulations. It is also contemplated that the present aerosol formulation can be prepared as a dry powder formulation comprising a finely divided powder form of adhesin inhibitory agent and a dispersant.

Formulations for dispensing from a powder inhaler device will comprise a finely divided dry powder containing adhesin inhibitory agent (or derivative) and may also include a bulking agent, such as lactose, sorbitol, sucrose, or mannitol in amounts which facilitate dispersal of the powder from the device, *e.g.*, 50 to 90% by weight of the formulation. The adhesin inhibitory agent (or derivative) should most advantageously be prepared in particulate form with an average particle size of less than 10 mm (or microns), most preferably 0.5 to 5 mm, for most effective delivery to the distal lung. In another embodiment, the dry powder formulation can comprise a finely divided dry powder containing adhesin inhibitory agent, a dispersing agent and also a bulking agent. Bulking agents useful in conjunction with the present formulation include such agents as lactose, sorbitol, sucrose, or mannitol, in amounts that facilitate the dispersal of the powder from the device.

The present invention further contemplates dry powder formulations comprising adhesin inhibitory agent and another therapeutically effective drug, such as an antibiotic, a steroid, a non-steroidal anti-inflammatory drug, etc.

Contemplated for use herein are oral solid dosage forms, which are described generally in Remington's Pharmaceutical Sciences, 18th Ed.1990 (Mack Publishing Co. Easton PA 18042) at Chapter 89, which is herein incorporated by reference. Solid dosage forms include tablets, capsules, pills, troches or lozenges, cachets or pellets. Also, liposomal or proteinoid encapsulation may be used to formulate the present compositions (as, for example, proteinoid microspheres reported in U.S. Patent No. 4,925,673). Liposomal encapsulation may be used and the liposomes may be derivatized with various polymers (*e.g.*, U.S. Patent No. 5,013,556). A description of possible solid dosage forms for the therapeutic is given by Marshall, K. In: *Modern Pharmaceutics* Edited by G.S. Banker and C.T. Rhodes Chapter 10, 1979, herein incorporated by reference. In general, the formulation will include the component or components (or chemically modified forms thereof) and inert ingredients which allow for protection against the stomach environment, and release of the biologically active material in the intestine.

Also specifically contemplated are oral dosage forms of the above derivatized component or components. The component or components may be chemically modified so that oral delivery of the derivative is efficacious. Generally, the chemical modification contemplated is the attachment of at least one moiety to the component molecule itself, where said moiety permits (a) inhibition of proteolysis; and (b) uptake into the blood stream from the stomach or intestine. Also desired is the increase in overall stability of the component or components and increase in circulation time in the body. Examples of such moieties include: polyethylene glycol, copolymers of ethylene glycol and propylene glycol, carboxymethyl cellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone and polyproline. Abuchowski and Davis, 1981, "Soluble Polymer-Enzyme Adducts" In: *Enzymes as Drugs*, Hocenberg and Roberts, eds., Wiley-Interscience, New York, NY, pp. 367-383; Newmark, et al., 1982, J. Appl. Biochem. 4:185-189. Other polymers that could be used are poly-1,3-dioxolane and poly-1,3,6-tioxocane. Preferred for pharmaceutical usage, as indicated above, are polyethylene glycol moieties.

For the component (or derivative) the location of release may be the stomach, the small intestine (the duodenum, the jejunem, or the ileum), or the large intestine. One skilled in the art has available formulations which will not dissolve in the stomach, yet will release the material in the duodenum or elsewhere in the intestine. Preferably, the release

will avoid the deleterious effects of the stomach environment, either by protection of the protein (or derivative) or by release of the biologically active material beyond the stomach environment, such as in the intestine.

To ensure full gastric resistance a coating impermeable to at least pH 5.0 is essential. Examples of the more common inert ingredients that are used as enteric coatings are cellulose acetate trimellitate (CAT), hydroxypropylmethylcellulose phthalate (HPMCP), HPMCP 50, HPMCP 55, polyvinyl acetate phthalate (PVAP), Eudragit L30D, Aquateric, cellulose acetate phthalate (CAP), Eudragit L, Eudragit S, and Shellac. These coatings may be used as mixed films.

A coating or mixture of coatings can also be used on tablets, which are not intended for protection against the stomach. This can include sugar coatings, or coatings which make the tablet easier to swallow. Capsules may consist of a hard shell (such as gelatin) for delivery of dry therapeutic i.e. powder; for liquid forms, a soft gelatin shell may be used. The shell material of cachets could be thick starch or other edible paper. For pills, lozenges, molded tablets or tablet triturates, moist massing techniques can be used.

The peptide therapeutic can be included in the formulation as fine multiparticulates in the form of granules or pellets of particle size about 1mm. The formulation of the material for capsule administration could also be as a powder, lightly compressed plugs or even as tablets. The therapeutic could be prepared by compression.

Colorants and flavoring agents may all be included. For example, the protein (or derivative) may be formulated (such as by liposome or microsphere encapsulation) and then further contained within an edible product, such as a refrigerated beverage containing colorants and flavoring agents.

One may dilute or increase the volume of the therapeutic with an inert material. These diluents could include carbohydrates, especially mannitol, a-lactose, anhydrous lactose, cellulose, sucrose, modified dextran and starch. Certain inorganic salts may be also be used as fillers including calcium triphosphate, magnesium carbonate and sodium

chloride. Some commercially available diluents are Fast-Flo, Emdex, STA-Rx 1500, Emcompress and Avicell.

Disintegrants may be included in the formulation of the therapeutic into a solid dosage form. Materials used as disintegrates include but are not limited to starch, including the commercial disintegrant based on starch, Explotab. Sodium starch glycolate, Amberlite, sodium carboxymethylcellulose, ultramylopectin, sodium alginate, gelatin, orange peel, acid carboxymethyl cellulose, natural sponge and bentonite may all be used. Another form of the disintegrants are the insoluble cationic exchange resins. Powdered gums may be used as disintegrants and as binders and these can include powdered gums such as agar, Karaya or tragacanth. Alginic acid and its sodium salt are also useful as disintegrants. Binders may be used to hold the therapeutic agent together to form a hard tablet and include materials from natural products such as acacia, tragacanth, starch and gelatin. Others include methyl cellulose (MC), ethyl cellulose (EC) and carboxymethyl cellulose (CMC). Polyvinyl pyrrolidone (PVP) and hydroxypropylmethyl cellulose (HPMC) could both be used in alcoholic solutions to granulate the therapeutic.

An antifrictional agent may be included in the formulation of the therapeutic to prevent sticking during the formulation process. Lubricants may be used as a layer between the therapeutic and the die wall, and these can include but are not limited to; stearic acid including its magnesium and calcium salts, polytetrafluoroethylene (PTFE), liquid paraffin, vegetable oils and waxes. Soluble lubricants may also be used such as sodium lauryl sulfate, magnesium lauryl sulfate, polyethylene glycol of various molecular weights, Carbowax 4000 and 6000.

Glidants that might improve the flow properties of the drug during formulation and to aid rearrangement during compression might be added. The glidants may include starch, talc, pyrogenic silica and hydrated silicoaluminate.

To aid dissolution of the therapeutic into the aqueous environment a surfactant might be added as a wetting agent. Surfactants may include anionic detergents such as sodium lauryl sulfate, dioctyl sodium sulfosuccinate and dioctyl sodium sulfonate. Cationic detergents might be used and could include benzalkonium chloride or benzethomium

chloride. The list of potential nonionic detergents that could be included in the formulation as surfactants are lauromacrogol 400, polyoxyl 40 stearate, polyoxyethylene hydrogenated castor oil 10, 50 and 60, glycerol monostearate, polysorbate 40, 60, 65 and 80, sucrose fatty acid ester, methyl cellulose and carboxymethyl cellulose. These surfactants could be present in the formulation of the protein or derivative either alone or as a mixture in different ratios.

Additives which potentially enhance uptake of the polypeptide (or derivative) are for instance the fatty acids oleic acid, linoleic acid and linolenic acid.

Pulmonary Delivery. Also contemplated herein is pulmonary delivery of the present polypeptide (or derivatives thereof). The polypeptide (or derivative) is delivered to the lungs of a mammal while inhaling and coats the mucosal surface of the alveoli. Other reports of this include Adjei et al., 1990, Pharmaceutical Research, 7:565-569; Adjei et al., 1990, International Journal of Pharmaceutics, 63:135-144 (leuprolide acetate); Braquet et al., 1989, Journal of Cardiovascular Pharmacology, 13(suppl. 5):143-146 (endothelin-1); Hubbard et al., 1989, Annals of Internal Medicine, Vol. III, pp. 206-212 (a1- antitrypsin); Smith et al., 1989, J. Clin. Invest. 84:1145-1146 (a-1-proteinase); Oswein et al., 1990, "Aerosolization of Proteins", Proceedings of Symposium on Respiratory Drug Delivery II, Keystone, Colorado, March, (recombinant human growth hormone); Debs et al., 1988, J. Immunol. 140:3482-3488 (interferon-g and tumor necrosis factor alpha) and Platz et al., U.S. Patent No. 5,284,656 (granulocyte colony stimulating factor). A method and composition for pulmonary delivery of drugs for systemic effect is described in U.S. Patent No. 5,451,569, issued September 19, 1995 to Wong et al.

Contemplated for use in the practice of this invention are a wide range of mechanical devices designed for pulmonary delivery of therapeutic products, including but not limited to nebulizers, metered dose inhalers, and powder inhalers, all of which are familiar to those skilled in the art.

Formulations suitable for use with a nebulizer, either jet or ultrasonic, will typically comprise polypeptide (or derivative) dissolved in water at a concentration of about 0.1

to 25 mg of biologically active protein per mL of solution. The formulation may also include a buffer and a simple sugar (*e.g.*, for protein stabilization and regulation of osmotic pressure). The nebulizer formulation may also contain a surfactant, to reduce or prevent surface induced aggregation of the protein caused by atomization of the solution in forming the aerosol.

Formulations for use with a metered-dose inhaler device will generally comprise a finely divided powder containing the polypeptide (or derivative) suspended in a propellant with the aid of a surfactant. The propellant may be any conventional material employed for this purpose, such as a chlorofluorocarbon, a hydrochlorofluorocarbon, a hydrofluorocarbon, or a hydrocarbon, including trichlorofluoromethane, dichlorodifluoromethane, dichlorotetrafluoroethanol, and 1,1,1,2-tetrafluoroethane, or combinations thereof. Suitable surfactants include sorbitan trioleate and soya lecithin. Oleic acid may also be useful as a surfactant.

Formulations for dispensing from a powder inhaler device will comprise a finely divided dry powder containing polypeptide (or derivative) and may also include a bulking agent, such as lactose, sorbitol, sucrose, or mannitol in amounts which facilitate dispersal of the powder from the device, *e.g.*, 50 to 90% by weight of the formulation. The protein (or derivative) should most advantageously be prepared in particulate form with an average particle size of less than 10 mm (or microns), most preferably 0.5 to 5 mm, for most effective delivery to the distal lung.

Nasal Delivery. Nasal or nasopharyngeal delivery of the polypeptide (or derivative) is also contemplated. Nasal delivery allows the passage of the polypeptide directly over the upper respiratory tract mucosal after administering the therapeutic product to the nose, without the necessity for deposition of the product in the lung. Formulations for nasal delivery include those with dextran or cyclodextran.

The following examples are presented in order to more fully illustrate the preferred embodiments of the invention. They should in no way be construed, however, as limiting the broad scope of the invention.

EXPERIMENTAL DETAILS SECTION

EXAMPLE 1: **Peptide truncates of choline binding protein A (CbpA)**

A polypeptide comprising a truncated N-terminal fragment of the CbpA (serotype 4) was generated. Full length CbpA was amplified with PCR primers SJ533 and SJ537, the primers were designed based on the derived N-terminal amino acid sequence of the CbpA polypeptide. 5' forward primer SJ533 = 5' GGC GGA TCC ATG GA(A,G) AA(C,T) GA(A,G) GG 3'. This degenerate primer designed from the amino acid sequence XENEG, incorporates both BamHI and NcoI restriction sites and an ATG start codon. 3' reverse primer SJ537 = 5' GCC GTC GAC TTA GTT TAC CCA TTC ACC ATT GGC 3'. This primer incorporates a SalI restriction site for cloning purposes, and the natural stop codon from CbpA, and is based on both type 4 and R6x sequence.

PCR product was generated from genomic DNA as a template with primers SJ533 and SJ537 amplified 30 cycles with an annealing temperature of 50°C using High Fidelity enzyme (Boehringer Mannheim). The resulting PCR products were purified using QIAquick PCR Purification Kit (Qiagen, Inc.) then digested with BamHI and SalI restriction enzymes and cloned into the pQE30 expression vector (Qiagen, Inc.) digested with BamHI, XbaI, and SmaI restriction enzymes.

Polypeptide R2:

The naturally occuring PvuII site at the end of the second repeat region, namely the C region as shown in Figure 1, (nucleic acid 1228 of Type 4 sequence) was exploited to create a truncated version of the cbpA gene, containing only the 5' portion of the gene. To create the truncate clone, the full length clone PMI580 (Type 4) or PMI581(R6x) was digested with PvuII and XbaI, the resulting fragment was ligated into the expression vector, PQE30 and transformed into the appropriate host. The protein was expressed and purified. In this instance the stop codon utilized by the expression vector is downstream of the insert, so the protein expressed is larger than the predicted size of the insert due to additional nucleic acids at the 5' end of the cloning site. The amino acid sequence of polypeptide R2 is set forth in SEQ ID NO 1.

Polypeptide R1:

A similar strategy was used to express only the first repeat region within the N-terminal region of CbpA, namely the A region of polypeptide R1. Here the naturally occuring XmnI site between the two amino repeats (nucleic acid 856 of the Type 4 sequence) was utilized. cbpA full length clone PMI580 was digested with XmnI and AatII. The vector pQE30 was digested with AatII and SmaI. Once again the two sized fragments were ligated, transformed into E. coli and clones screened for inserts. One positive clone was selected and recombinant protein purified from this stain.

All polypeptides were expressed and purified with the Qia Expression System (Qiagen) using an E. coli the pQE30 vector. The amino terminus of the His tagged proteins are detected by host and Western analysis using both anti-histidine antibodies and protein specific antibodies.

Purification of R1 and R2 :

To induce production of and purify recombinant proteins from *E. coli* a single colony was selected from plated bacteria containing the recombinant plasmid and grown overnight at 37° in 6.0 mls LB buffer with 50μg/ml kanamycin and 100μg/ml ampicillin. This 6.0 ml culture was added to 1L LB with antibiotics at above concentrations. The culture was shaken at 37° C until A_{600} = ~0.400. 1M IPTG was added to the 1L culture to a final concentration of 1mM. The culture was then shaken at 37°C for 3-4 hrs. The 1L culture was spun for 15 min at 4000 rpm in a model J-6B centrifuge. The supernatant was discarded and the pellet stored at -20°C.

The 1L pellet was re-suspended in 25 ml 50 mM NaH_2PO_4, 10mM Tris, 6M GuC1, 300mM NaC1, pH 8.0 (Buffer A). This mixture was rotated at room temperature for 30 minutes and sonicated on a (VibraCell Sonicator (Sonics and Materials, Inc., Danbury, CT) using the micro tip, two times, for 30 secs, at 50% Cuty Cycle and with the output setting at 7. The mixture was spun 5 min at 10K in a JA20 rotor and the supernatant removed and discarded. The supernatant was loaded onto a 10 ml Talon (Clonetech, Palo

Alto, CA) resin column attached to a GradiFrac System (Pharmacia Biotech, Upsala, Sweden). The column was equilibrated with 100 ml Buffer A and washed with an additional 200 ml of this buffer. A volume based pH gradient using 100% 50 mM NaH_2PO_4, 8M Urea, 20mM MES, pH6.0 (Buffer B) as the final target buffer was run over a total volume of 100 ml. Protein eluted at ~30% Buffer B. Eluted peaks were collected and pooled.

For refolding, dialysis was carried out with a 2L volume of PBS at room temperature for approximately 3 hrs using dialysis tubing with a molecular weight cutoff of 14,000. The sample was then dialyzed overnight in 2L of PBS at 4°C. Additional buffer exchange was accomplished during the concentration of the protein using Centriprep-30 spin columns by adding PBS to the spun retenate and re-spinning. The protein concentration was determined using the BCA protein assay and the purity visualized using a Coomassie stained 4-20% SDS-PAGE gel (Figure 3).

EXAMPLE 2: **Lectin activity of polypeptides R1 and R2**

LNnt is a carbohydrate analog of the receptors for pneumococci present on eukaryotic cells. It has been shown that a CbpA defective pneumococcal mutant failed to adhere to either eukaryotic cells or immobilized sugar indicating that CbpA is the adhesive ligand. CbpA is a modular protein that can be divided into two regions: the N-Terminal functional domain and the C-terminal choline binding domain (Figure 1). Polypeptides R1 and R2 were analyzed for biological activity to determine if the activities of the entire CbpA were localized in the unique N terminus (modelled by R2) or a fragment thereof (modelled by R1). It was determined whether or not the N-terminal domain alone (R2) contained the lectin binding biological activity in the absence of the choline binding domain (CBD). This was tested using the full length CbpA and polypeptide R2 (truncate missing the CBD region beyond the Pvu II site in the proline rich region).

The assay was to coat tissue culture wells with glycoconjugates known to be recognized by CbpA: LNnT-albumin, 3′ sialyl lactose-albumin, and the negative control albumin. The plates were then blocked with the albumin, washed and either full length CbpA Polypeptide R2, or polypeptide R1 were added for 15 minutes (0.8 μg/ml), then, without

washing, fluoresein labelled R6 pneumococci were added for 30 minutes, washed and adherent bacteria counted visually.

Binding of R6 to carbohydrate without any peptide addition was the positive control and was calibrated at 100% (Table 1). In three separate experiments, CbpA full length or Polypeptide R2 competitively inhibited binding of pneumococci to LNnT coated surfaces. CbpA full length inhibited to 71, 64% and 63% of control; polypeptide R2 inhibited to 65%, 53% and 74% of control. The equivalent activity of CbpA and R2 indicates the choline binding domain is not necessary for LNnT lectin activity of CbpA, and that R2 is a candidate LNnT lectin.

In contrast to binding to LNnT, binding of pneumococci to 3' sialyl lactose was not inhibited by R2 (79 and 101%) compared to the full length CbpA (74 and 66%). This indicates that the sialic acid recognition activity is lost when the CBD is missing. In contrast R1 seems to be active in recognition of sialic acid, a property shared with CbpA but apparently masked in R2. This indicates that folding of polypeptide into functional domains is influenced by the composition and length of the polypeptide. Slight sequence variation is found in other strains (see Figure 2). Given the high degree of homology of sequence between R1 and R2, it is further possible that both R1 and R2 are needed for lectin activity or that they are both lectin with slightly different specificities (± sialic acid).

Table 1

Inhibition of Binding of R6 pneumococci to purified glycoconjugate by soluble forms of CbpA

	LNnT		3′ sialyl lactose	
Cbp form	# pneumococci per monolayer (SD)	% control	# pneumococci per monolayer	% control perwell
No peptide	3282 2421 (489) 2210 (350)	100%	2611 2115 (125)	100%
Full length CbpA	2075 1740 (167) 1415 (50)	63, 71, 64	1933 1405 (240)	74 66
Polypeptide R2	2461 1288 (672) 1440 (530)	74, 53, 65	2639 1670 (420)	101 79
Polypeptide R1	3002 2245 (182) 2500 (310)	91, 92, 112	1052 1445 (526)	40 68

N=3 experiments LNnt each 3 wells

N=2 experiments SiL each 3 wells

Lectin activity correlates with cell binding activity

Human cells bear surface molecules that contain carbohydrates (glycoprotein, and glycolipid) and bacteria bind to these glycoconjugates by the carbohydrate despite very different protein or lipid backbones. Thus, bacteria bearing polypeptide with lectin activity in vitro can adhere to human cell surfaces. This direct correlation between in vitro lectin activity and cell binding action is known for pneumococci. For example, LNnT competitively inhibits binding of pneumococci to TNF activated A549 human lung cells and blocks the progression of pneumonia in vivo. To establish that the lectin activity of truncates of CbpA reflects cell binding activity, CbpA and truncates were tested for inhibition of binding of pneumococci to lung cells (Table 2). Full length

CbpA and polypeptide R2 competitively inhibited adherence of pneumococci to lung cells to 58% and 63% of controls respectively. Polypeptide R1 was not effective, indicating the LNnt binding activity of R2 is needed for and explains pneumococcal binding to lung cells.

Table 2 Binding of R6 pneumococci to TNF activated human lung cells

	A549 Lung	
Cbp form	# pneumococci per monolayer (mean)	% control
No peptide	697,704,674 702,722 (700)	100%
Full length CbpA	376,431 (403)	58%
Polypeptide R2	517,693 314,342,350 (443)	63%
Polypeptide R1	696,642,552 (630)	90%

N=2 experiments of 2 or 3 wells each

LNnT Lectin activity is dependent on R2

The N-terminal region of CbpA contains two repeats of ~110 amino acids each (see Figure 1, regions A and C within polypeptide R2). To study the relative contribution of the two domains to bio-activity R1, containing only domain A was compared to R2 and full length CbpA. When tested in the adherence assay, polypeptide R1 did not inhibit adherence to LNnT at all (91, 92, and 112% of wild type). However, polypeptide R1 demonstrated some inhibition of binding to Sialyl lactose (68 and 40% of control). This demonstrates that the polypeptide R2 is required for LNnT lectin activity and R2 is a candidate LNnT lectin domain. In contrast R1 seems to be active in recognition of sialic acid.

Antibodies to N-terminal Domain of CbpA Block Cell Binding:

Given that the N-terminal domain of CbpA binds cells, interference with the N-terminal domain activity will prevent or reverse bacterial binding to cells or purified glycoconjugates. One such mechanism of interference is antibody.

Table 3 Inhibition of binding of R6 pneumococci to LNnT coated surfaces by anti-CbpA R2 antibodies

	# pneumococci per monolayer (SD)	% control (mean)
Prelmmune Antibody	198 (64); 88 (4)	100%
Antibody to Truncate R2	56 (11); 9 (2)	28%; 10%

5 µl of rabbit antibody undiluted + 5 µl 2 x 10^7 R6x Preincubate, 6 at RT x 30 min, then add to LNnT coated wells for adherence assay. Two independent experiments are shown.

Antisera raised to the recombinant N-terminal domain of CbpA (R2) was tested for the ability to block adherence of pneumococci to LNnT. Rabbit polyclonal anti CbpA antisera (5µl) plus 5µl of 2 x 10^7 of labeled bacteria were incubated at room temperature for 30 min. This mixture was overlaid onto immobilized LNnT for 30 min., and then washed 3 times with PBS to remove unbound bacteria. Bacteria bound to the plates were enumerated microscopically and results are presented as the mean values plus the standard deviation from six wells. Results shown in Table 3 demonstrate that antisera raised against the R2 polypeptide blocked the binding of pneumococci to LNnT. Figure 5 demonstrates a titration curve of prelmmune versus anti-CbpA R2 antibody for inhibition of binding of pneumococci R6x to the model receptor LNnT. Greater than 70% of pneumococcal adherence was blocked by anti-R2 at dilutions of 1:100 and 1:200. Further dilution to 1:400 eliminated activity indicating the specificity of the effect.

The CbpA used to prepare the antisera shown in Table 3 and Figure 5 was raised against CbpA from serotype 4. The R6x strain pneumococci used in the inhibition of adherence

assay was derived from serotype 2. The ability of the antibody to block adherence of a heterologous serotype of bacteria indicates cross protective activity across serotypes. Such activity is highly desired for an effective vaccine immunogen.

Activity of antibodies to native conformation of N terminus of CbpA:

CbpA can be purified over a choline affinity column from its natural host, the pneumococcus, as described by Rosenow et al. Alternatively, a polyhistidine tag can be engineered onto the end of the gene such that the transcribed protein is extended by several histidine residues. These residues facilitate purification over a nickel affinity matrix Purification of full length polypeptides as opposed to shorter truncates favors retention of the native tertiary structure. CbpA purified especially from pneumococcus but also from E. coli or other host bacteria by these biochemical means retains its native tertiary structure. Used as an immunogen, natively folded CbpA engenders antibodies that potentially differ from those elicited by immunization with a truncate which may fold differently. Similarly, CbpA used as a therapeutic may have tertiary structure differing from the truncate which would improve its ability to block adherence. Given these considerations, it may be advantageous to produce CbpA as full length protein allowing it to fold to its native tertiary structure and then cleave the C terminal (CBD) away biochemically. For example, treatment with hydroxylamine will cleave CbpA at amino acid position 475 of serotype R6x and of serotype 4 of choline binding protein A, separating the N and C termini. The N terminal fragment is then suitable as a therapeutic or an immunogen.

Alternatively, native CbpA can be used as an immunogen and antisera to the active structure. The bioactive anti-N terminal antibodies in this mixture can be enriched by removing antibodies to the BD by absorption. Such an antibody was prepared by incubating 200, ul serum with 1 x 10^8 CbpA defective - bacteria for 1 hour at R1. The other choline binding proteins on this mutant absorb out anti-CBD antibodies which are then removed from the antiserum by centrifuging and removing the bacteria.

To demonstrate the bioactivity of absorbed anti CbpA antibodies, the ability of the absorbed antiserum to block pneumococcal adherence to the model receptor LNnT was

determined. R6x pneumococci were incubated with 1:600 dilution of antiserum and then added to wells coated with LNnT albumin.

Table 4 Absorbed anti CbpA antiserum blocks adherence

Antisera (1:600)	Number of pneumococci per well ± SD (% of control)
No antibody	563 ± 11 (100%)
PreImmune antiserum	479 ± 11 (85%)
Anti CbpA antiserum	294 ± 72 (52%)
Anti CbpA antiserum absorbed to remove CBD antibodies	175 ± 38 (31%)

These results indicate that antibodies to the N terminal domain of Cbp/A in its native conformation strongly block adherence. This activity is greater than that to the truncate of Figure 5 which was inactive at 1:600 dilution. Further demonstration of this activity of absorbed anti CbpA antiserum is shown by the titration study of Figure 5. Baseline adherence of pneumococci Type 4 to LNnT coated wells is shown by the triangles. Pre-incubation of pneumoccoci with unabsorbed (squares) or absorbed (diamonds) antiserum at the various dilutions indicated yielded decreased adherence. The fact that both antisera showed similar decreases in adherence demonstrates that the majority of the blocking activity of antibody to CbpA resides in the N-terminus (*i.e.*, removal of antibodies to the choline binding domain by absorption does not decrease bioactivity.

EXAMPLE 3: Passive Protection With Anti-R2 Antiserum

Generation of Rabbit Immune Sera:

Rabbit immune sera against polypeptide R2 (CbpA truncate) and CbpA were generated at Covance (Denver, PA). Following collection of pre-immune serum, a New Zealand white rabbit was immunized with 250 μg R2 containing both amino terminal repeats (preparation 483:58 above), in Complete Freund's Adjuvant. The rabbit was given a

boost of 125 μg R2 in Incomplete Freund's Adjuvant on day 21 and bled on day 31. A second rabbit was similarly immunized with purified CbpA.

Passive Protection in Mice:

C3H/HeJ mice (5/group) were passively immunized intraperitoneally by with 100 μl of a 1:2 dilution of rabbit anti R2 or preimmune sera in sterile PBS (pre-immune and day 31 immune sera). One hour after administration of serum, mice were challenged with 1600 CFU *Streptococcus pneumoniae* serotype 6B (strain SP317). Mice were monitored for 14 days for survival. Eighty percent of the mice immunized with rabbit immune serum raised against polypeptide R2 survived challenge (Figure 4). All mice immunized with pre-immune rabbit serum were dead by day 7.

This data demonstrates that antibodies specific for CbpA are protective against systemic pneumococcal infection. The data further indicate that the choline-binding region is not necessary for protection, as antibody specific for the truncated protein polypeptide R2, lacking the conserved choline binding repeats, was sufficient for protection. In addition, serum directed to CbpA of serotype 4 was protective against challenge with serotype 6B.

EXAMPLE 4: Active Protection With Anti-R1 Antiserum

C3H/HeJ mice (10/group) were immunized intraperitoneally with CbpA truncate protein R1 (15 μg in 50 μl PBS, plus 50 μl Complete Freund's Adjuvant). A group of 10 sham immunized mice received PBS and adjuvant. A second immunization was administered four weeks later, 15 μg protein i.p. with Incomplete Freund's Adjuvant (sham received PBS plus IFA). Blood was drawn (retro-orbital bleed) at weeks 3, 6, and 9 for analysis of immune response. The ELISA end point anti-CbpA truncate titer of pooled sera from the 10 CbpA immunized mice at 9 weeks was 4,096,000. No antibody was detected in sera from sham immunized mice. Mice were challenged at week 10 with 560 CFU *Streptococcus pneumoniae* serotype 6B (strain SPSJ2p, provided by P. Flynn, St. Jude Children's Research Hospital, Memphis, TN). Mice were monitored for 14 days for survival. Eighty percent of the mice immunized with

CbpA truncate protein R1 survived challenge. All sham immunized mice were dead by day 8 (Figure 7).

This data demonstrates that immunization with a recombinant fragment of CbpA elicits production of specific antibodies capable of protecting against systemic pneumococcal infection and death. The data further indicates that the choline-binding region is not necessary for protection, as the immunogen is the truncated protein R1. Additionally, the results suggest that a single amino terminal repeat may be sufficient to elicit a protective response. Cross protection is also demonstrated as the recombinant pneumococcal protein was generated based on serotype 4 DNA sequence and protection was observed following challenge with a serotype 6B isolate.

EXAMPLE 5 Prophylaxis against nasopharyngeal colonization in the infant rat

In vitro the N terminal domain of CbpA competitively inhibited pneumococcal attachment. To demonstrate the therapeutic utility of peptides with this activity, infant rats were administered truncate peptides, then challenged with pneumococci and colonization of the nasopharynx was evaluated.

Rats were treated intranasally with 10 µl of PBS containing 0.8 µg of polypeptide R2 or R1 or no protein. 15 min later Type 3 pneumococci (Strain SIII) (10 µl containing 1 x 10^5 cfer) were introduced intranasally. To determine the ability of the polypeptide to competitively inhibit pneumococcal adherence and colonization, nasal washing was performed at 72 hours and the number of pneumococci recovered was quantitated in each of 4 animals per group. Rats receiving SIII alone displayed 2200, 6500, 6900 and 8700 (mean 6075) colonies per 10µl. Animals treated with truncate R2 showed the greatest decrease (3600, 3500, 2500, 2100) to mean 2925 bacteria 10µl (48% of control). Animals treated with truncate R1 also showed decreased colonization (5000, 4800, 3500, 1600) to mean 3725 (61% of control).

This experiment demonstrates that administration of the peptide of the instant invention to animals in a therapeutic study design to animals can protect against subsequent pneumococcal challenge.

Discussion:

As demonstrated by the experiments, polypeptide R2 when: 1) administered as a vaccine antigen elicits protective antibodies and is a preferred composition for a vaccine formulation; and 2) delivered as a peptide to the respiratory tract and/or nasopharynx receptor, competitively prevents pneumococcal attachment and is a preferred composition for a prophylactic and therapeutic agent against colonization or invasive disease. Also, truncates of CbpA function as lectins without the CBD. Two carbohydrates are recognized: LNnT by a peptide containing both N-terminal repeats (A and C) in Figure 1 and sialic acid by a peptide containing only the single most N-terminal repeat (A). The truncate containing the N-terminal repeat polypeptide R1 and R2 demonstrates lectin activity in cell culture assays as well.

Important features of polypeptide R2 activity include: 1) complete correlation of bioactivity of polypeptide R2 and full length CbpA for recognition of purified glycoconjugate receptor analogs, lung cells and animal models. Correlation is also demonstrated for antibodies to them; and 2) cross protection between type 4 derived agents and bacteria in *in vitro* assays using other serotype (e.g. 6B and 2) which is important for useful vaccine, prophylactic and therapeutic modalities.

While the invention has been described and illustrated herein by references to various specific material, procedures and examples, it is understood that the invention is not restricted to the particular material combinations of material, and procedures selected for that purpose. Numerous variations of such details can be implied as will be appreciated by those skilled in the art. Likewise, any references cited herein are, to the extent relevant to the disclosure of the invention, to be considered as incorporated herein by reference.

WHAT IS CLAIMED IS:

1. An isolated polypeptide comprising an amino acid sequence of a N-terminal choline binding protein A truncate.

2. The isolated polypeptide of claim 1, wherein the amino acid sequence is set forth in SEQ ID NO 1, including fragments, mutants, variants, analogs, or derivatives, thereof.

3. The isolated polypeptide of claim 1, wherein the amino acid sequence is set forth in SEQ ID NO 3, including fragments, mutants, variants, analogs, or derivatives, thereof.

4. The isolated polypeptide of claim 1, wherein the amino acid sequence is set forth in SEQ ID NO 6.

5. The isolated polypeptide of claim 1, wherein the amino acid sequence is set forth in SEQ ID NO 7, including fragments, mutants, variants, analogs, or derivatives, thereof.

6. The isolated polypeptide of claim 1, wherein the amino acid sequence is set forth in SEQ ID NO 9, including fragments, mutants, variants, analogs, or derivatives, thereof.

7. An isolated polypeptide comprising an amino acid sequence of a N-terminal choline binding protein A truncate having the amino acid as set forth in SEQ ID NO 24, wherein the polypeptide exhibits its tertiary structure.

8. The isolated polypeptide of claim 7, wherein the tertiary structure corresponds to that present in the native protein.

9. The isolated polypeptide of claim 7, wherein the polypeptide is made by cleaving a full length choline binding protein A with hydroxylamine, wherein the hydroxylamine cleaves the choline binding protein A at amino acid 475 thereby creating the N-terminal choline binding protein A truncate.

10. An isolated analog of the polypeptide of claim 1.

11. An isolated polypeptide according to claim 10, wherein the analog comprises the amino acid sequence having an N-terminal methionine or an N-terminal polyhistidine.

12. The isolated polypeptide according to claim 1, wherein said fragments are proteolytic digestion products of the polypeptide.

13. An isolated polypeptide comprising an amino acid sequence of a N-terminal choline binding protein A truncate, wherein the polypeptide has lectin activity and does not bind to choline.

14. An isolated immunogenic polypeptide comprising an amino acid sequence of a N-terminal choline binding protein A truncate.

15. The immunogenic polypeptide of claim 14, wherein the amino acid sequence is set forth in SEQ ID NO 1, including fragments, mutants, variants, analogs, or derivatives, thereof.

16. The immunogenic polypeptide of claim 14, wherein the amino acid sequence is set forth in SEQ ID NO 3, including fragments, mutants, variants, analogs, or derivatives, thereof.

17. The immunogenic polypeptide of claim 14, wherein the amino acid sequence is set forth in SEQ ID NO 7, including fragments, mutants, variants, analogs, or derivatives, thereof.

18. The immunogenic polypeptide of claim 14, wherein the amino acid sequence is set forth in SEQ ID NO 9, including fragments, mutants, variants, analogs, or derivatives, thereof.

19. An isolated nucleic acid encoding a polypeptide comprising an amino acid sequence of a N-terminal choline binding protein A truncate.

20. The isolated nucleic acid of claim 19, wherein the nucleic acid is set forth in SEQ ID NO 12, including fragments, mutants, variants, analogs, or derivatives, thereof.

21. The isolated nucleic acid of claim 19, wherein the nucleic acid is set forth in SEQ ID NO 14, including fragments, mutants, variants, analogs, or derivatives, thereof.

22. The isolated nucleic acid of claim 19, wherein the nucleic acid is set forth in SEQ ID NO 17, including fragments, mutants, variants, analogs, or derivatives, thereof.

23. The isolated nucleic acid of claim 19, wherein the nucleic acid is set forth in SEQ ID NO 19, including fragments, mutants, variants, analogs, or derivatives, thereof.

24. The isolated nucleic acid of claim 19, wherein the nucleic acid is DNA.

25. The isolated nucleic acid of claim 19, wherein the nucleic acid is cDNA.

26. The isolated nucleic acid of claim 19, wherein the nucleic acid is genomic DNA.

27. The isolated nucleic acid of claim 19, wherein the nucleic acid is RNA.

28. An isolated nucleic acid of claim 19 operatively linked to a promoter of RNA transcription.

29. A vector which comprises the nucleic acid molecule of claim 19.

30. The vector of claim 29, wherein the promoter comprises a bacterial, yeast, insect or mammalian promoter.

31. The vector of claim 30, wherein the vector is a plasmid, cosmid, yeast artificial chromosome (YAC), bacteriophage or eukaryotic viral DNA.

32. A host vector system for the production of a polypeptide which comprises the vector of claim 30 in a suitable host cell.

33. The host vector system of claim 32, wherein the suitable host cell comprises a prokaryotic or eukaryotic cell.

34. A cell line comprising the nucleic acid of claim 19.

35. A method of obtaining a polypeptide in purified form which comprises:
(a) introducing the vector of claim 19 into a suitable host cell;
(b) culturing the resulting host cell so as to produce the polypeptide;
(c) recovering the polypeptide produced in step (b); and
(d) purifying the polypeptide so recovered in step (c).

36. An antibody capable of specifically binding to the polypeptide of claim 1 or 7.

37. The antibody of claim 36, wherein the antibody is a monoclonal antibody.

38. The antibody of claim 36, wherein the antibody is a polyclonal antibody.

39. The antibody of claim 36, wherein the antibody is a chimeric (bispecific) antibody.

40. A pharmaceutical composition comprising an amount of the polypeptide of claim 1 and a pharmaceutically acceptable carrier or diluent.

41. A method of inducing an immune response in a subject which has been exposed to or infected with a pneumococcal bacterium comprising administering to the subject an amount of the pharmaceutical composition of claim 40, thereby inducing an immune response.

42. A method for preventing infection by a pneumococcal bacterium in a subject comprising administering to the subject an amount of the pharmaceutical composition of claim 40 effective to prevent pneumococcal bacterium attachment, thereby preventing infection by a pneumococcal bacterium.

43. The method of claim 42, wherein the pharmaceutical composition is delivered to the respiratory tract or nasopharynx.

44. A method for preventing infection by a pneumococcal bacterium in a subject comprising administering to the subject an amount of a pharmaceutical composition comprising the antibody of claim 36 and a pharmaceutically acceptable carrier or diluent, thereby preventing infection by a pneumococcal bacterium.

45. A vaccine which comprises the polypeptide of Claim 1 and a pharmaceutically acceptable adjuvant or carrier.

46. The vaccine of Claim 45, wherein the polypeptide has the amino acid sequence as set forth in any of SEQ ID NOS: 1, 3-7, 9-11, 22, and 23.

47. The vaccine of Claim 45 wherein the polypeptide comprises an amino acid sequence of a N-terminal choline binding protein A truncate as set forth in Figure 2.

48. A vaccine which comprises the polypeptide having the amino acid sequence which comprises a conserved region as set forth in Figure 2 and a pharmaceutically acceptable adjuvant or carrier.

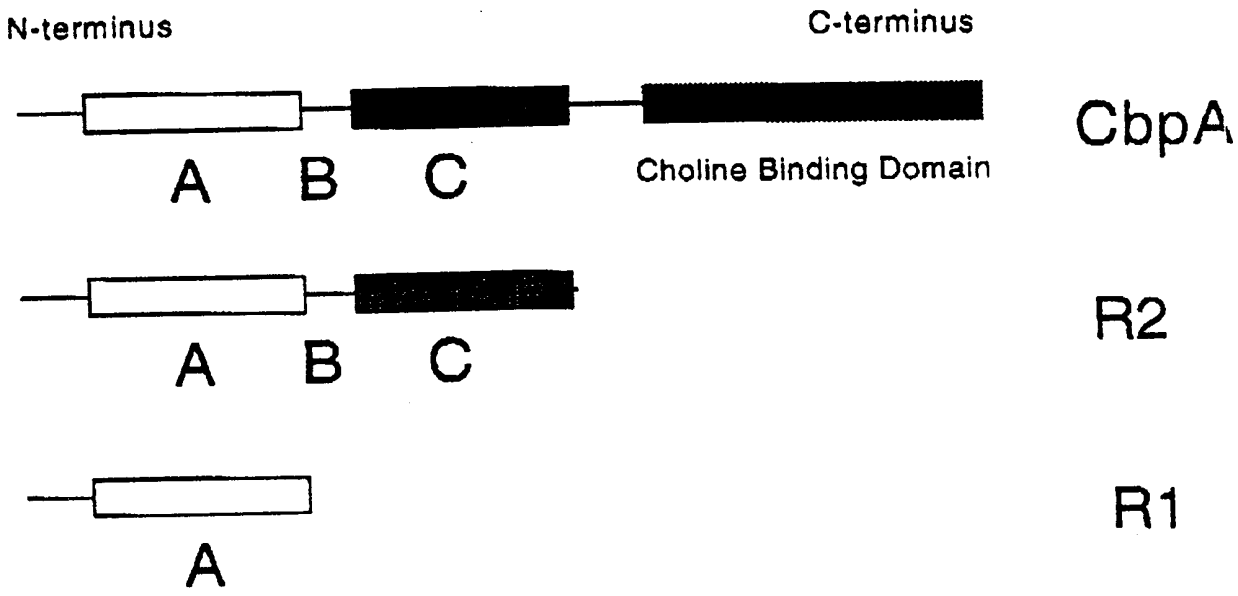
49. The vaccine of Claim 48 wherein the conserved region is selected from amino acid sequence 158 to 172; 300 to 321; 331 to 339; 355 to 365; 367 to 374; 379 to 389; 409 to 427; and 430 to 447.

50. A vaccine comprising an isolated nucleic acid encoding the polypeptide of Claim 1 and a pharmaceutically acceptable adjuvant or carrier.

51. A vaccine comprising an isolated nucleic acid of Claim 19 and a pharmaceutically acceptable adjuvant or carrier.

52. A vaccine comprising the vector of Claim 29 and a pharmaceutically acceptable adjuvant or carrier.

53. A method for treating a subject infected with or exposed to pneumococcal bacterium comprising administering to the subject a therapeutically effective amount of the vaccine of any of Claims 45-51 or 52, thereby treating the subject.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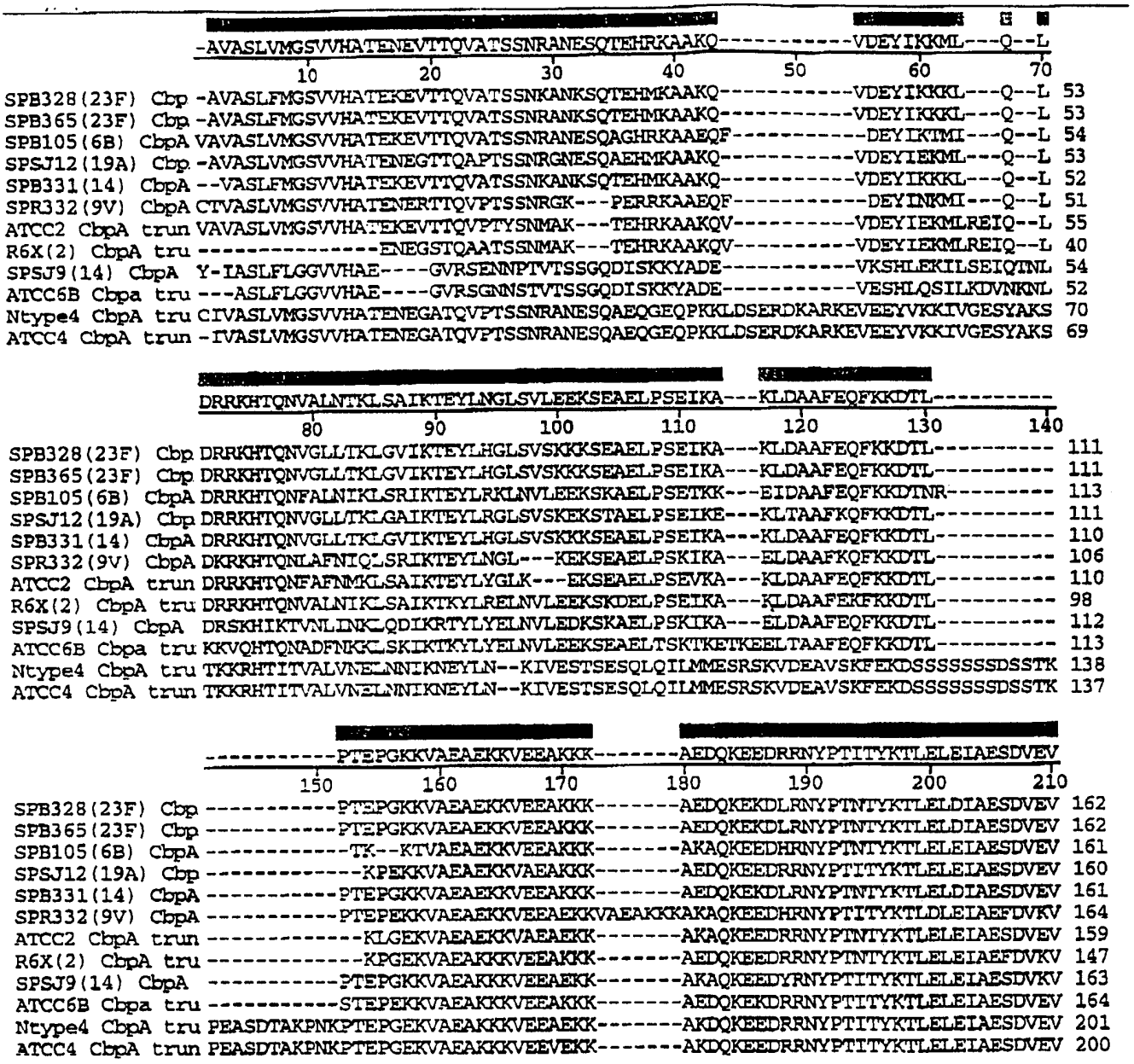
C-terminus
A
B
C
Choline Binding Domain
CbpA
A
B
C
R2
A
R1

Figure 1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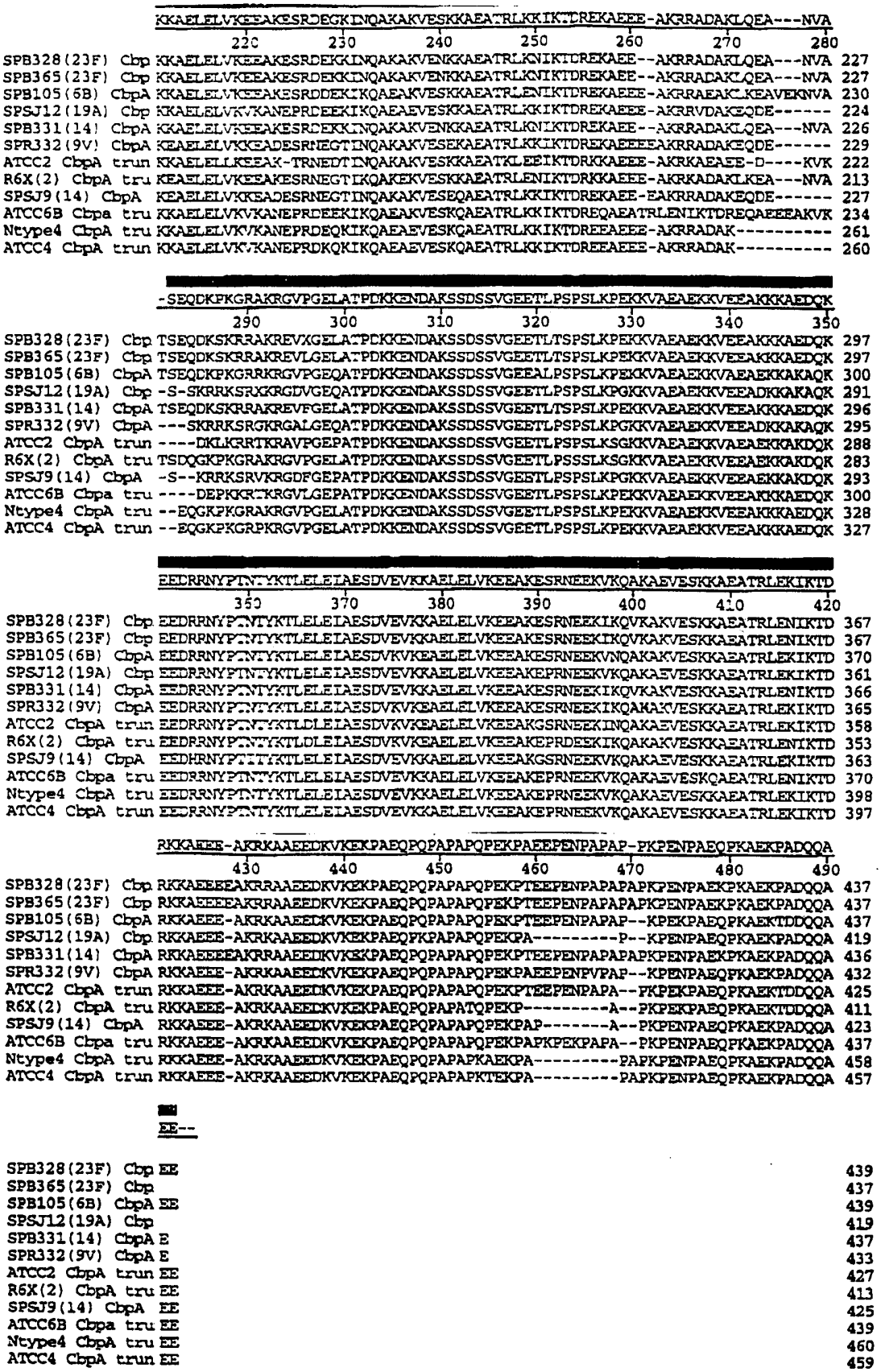
SPB365(23F) Cbp
SPB105(6B) CbpA
SPSJ12(19A) Cbp
SPB331(14) CbpA
SPR332(9V) CbpA
ATCC2 CbpA trun
R6X(2) CbpA tru
SPSJ9(14) CbpA
ATCC6B Cbpa tru
Ntype4 CbpA tru
ATCC4 CbpA trun

Figure 2A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SPB365(23F) Cbp KKAELELVKEEAKESRDEKKINQAKAKVENKKAEATRLKNIKTDREKAEE--AKRRADAKLQEA---NVA 227
SPB105(6B) CbpA KKAELELVKEEAKESRDDEKIKQAEAKVESKKAEATRLENIKTDREKAEEE-AKRRAEAKLKEAVEKNVA 230
SPSJ12(19A) Cbp KKAELELVKVKANEPRDEEKIKQAEAEVESKKAEATRLKKIKTDREKAEEE-AKRRVDAKEQDE------ 224
SPB331(14) CbpA KKAELELVKEEAKESRDEKKINQAKAKVENKKAEATRLKNIKTDREKAEE--AKRRADAKLQEA---NVA 226
SPR332(9V) CbpA KEAELELVKKEADESRNEGTINQAKAKVESEKAEATRLKKIKTDREKAEEEEAKRRADAKEQDE------ 229
ATCC2 CbpA trun KKAELELLKEEAK-TRNEDTINQAKAKVESKKAEATKLEEIKTDRKKAEEE-AKRKAEAEE-D----KVK 222
R6X(2) CbpA tru KEAELELVKEEAKESRNEGTIKQAKEKVESKKAEATRLENIKTDRKKAEEE-AKRKADAKLKEA---NVA 213
SPSJ9(14) CbpA KEAELELVKKEADESRNEGTINQAKAKVESEQAEATRLKKIKTDREKAEE-EAKRRADAKEQDE------ 227
ATCC6B Cbpa tru KKAELELVKVKANEPRDEEKIKQAEAKVESKQAEATRLKKIKTDREQAEATRLENIKTDREQAEEEAKVK 234
Ntype4 CbpA tru KKAELELVKVKANEPRDEQKIKQAEAEVESKQAEATRLKKIKTDREEAEEE-AKRRADAK---------- 261
ATCC4 CbpA trun KKAELELVKVKANEPRDKQKIKQAEAEVESKQAEATRLKKIKTDREEAEEE-AKRRADAK---------- 260
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SPB331(14) CbpA TSEQDKSKRRAKREVFGELATPDKKENDAKSSDSSVGEETLTSPSLKPEKKVAEAEKKVEEAKKKAEDQK 296
SPR332(9V) CbpA ---SKRRKSRGKRGALGEQATPDKKENDAKSSDSSVGEETLPSPSLKPGKKVAEAEKKVEEADKKAKAQK 295
ATCC2 CbpA trun ----DKLKRRTKRAVPGEPATPDKKENDAKSSDSSVGEETLPSPSLKSGKKVAEAEKKVAEAEKKAKDQK 288
R6X(2) CbpA tru TSDQGKPKGRAKRGVPGELATPDKKENDAKSSDSSVGEETLPSSSLKSGKKVAEAEKKVEEAEKKAKDQK 283
SPSJ9(14) CbpA -S--KRRKSRVKRGDFGEPATPDKKENDAKSSDSSVGEETLPSPSLKPGKKVAEAEKKVEEAEKKAKDQK 293
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Ntype4 CbpA tru --EQGKPKGRAKRGVPGELATPDKKENDAKSSDSSVGEETLPSPSLKPEKKVAEAEKKVEEAKKKAEDQK 328
ATCC4 CbpA trun --EQGKPKGRPKRGVPGELATPDKKENDAKSSDSSVGEETLPSPSLKPEKKVAEAEKKVEEAKKKAEDQK 327
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SPB105(6B) CbpA EEDRRNYPTNTYKTLELEIAESDVKVKEAELELVKEEAKESRNEEKVNQAKAKVESKKAEATRLEKIKTD 370
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SPR332(9V) CbpA EEDRRNYPTNTYKTLELEIAESDVKVKEAELELVKEEAKESRNEEKIKQAKAKVESKKAEATRLEKIKTD 365
ATCC2 CbpA trun EEDRRNYPTNTYKTLDLEIAESDVKVKEAELELVKEEAKGSRNEEKINQAKAEVESKKAEATRLEKIKTD 358
R6X(2) CbpA tru EEDRRNYPTNTYKTLDLEIAESDVKVKEAELELVKEEAKEPRDEEKIKQAKAKVESKKAEATRLEKIKTD 353
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Ntype4 CbpA tru EEDRRNYPTNTYKTLELEIAESDVEVKKAELELVKEEAKEPRNEEKVKQAKAEVESKKAEATRLEKIKTD 398
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SPB365(23F) Cbp RKKAEEEEAKRRAAEEDKVKEKPAEQPQPAPAPQPEKPTEEPENPAPAPAPKPENPAEKPKAEKPADQQA 437
SPB105(6B) CbpA RKKAEEE-AKRKAAEEDKVKEKPAEQPQPAPAPQPEKPTEEPENPAPAP--KPEKPAEQPKAEKTDDQQA 437
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SPB331(14) CbpA RKKAEEEEAKRRAAEEDKVKEKPAEQPQPAPAPQPEKPTEEPENPAPAPAPKPENPAEKPKAEKPADQQA 436
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ATCC2 CbpA trun RKKAEEE-AKRKAAEEDKVKEKPAEQPQPAPAPQPEKPTEEPENPAPA--PKPEKPAEQPKAEKTDDQQA 425
R6X(2) CbpA tru RKKAEEE-AKRKAAEEDKVKEKPAEQPQPAPATQPEKP---------A--PKPEKPAEQPKAEKTDDQQA 411
SPSJ9(14) CbpA RKKAEEE-AKRKAAEEDKVKEKPAEQPQPAPAPQPEKPAP-------A--PKPENPAEQPKAEKPADQQA 423
ATCC6B Cbpa tru RKKAEEE-AKRKAAEEDKVKEKPAEQPQPAPAPQPEKPAPKPEKPAPA--PKPENPAEQPKAEKPADQQA 437
Ntype4 CbpA tru RKKAEEE-AKRKAAEEDKVKEKPAEQPQPAPAPKAEKPA---------PAPKPENPAEQPKAEKPADQQA 458
ATCC4 CbpA trun RKKAEEE-AKRKAAEEDKVKEKPAEQPQPAPAPKTEKPA---------PAPKPENPAEQPKAEKPADQQA 457
EE--
SPB328(23F) Cbp EE 439
SPB365(23F) Cbp 437
SPB105(6B) CbpA EE 439
SPSJ12(19A) Cbp 419
SPB331(14) CbpA E 437
SPR332(9V) CbpA E 433
ATCC2 CbpA trun EE 427
R6X(2) CbpA tru EE 413
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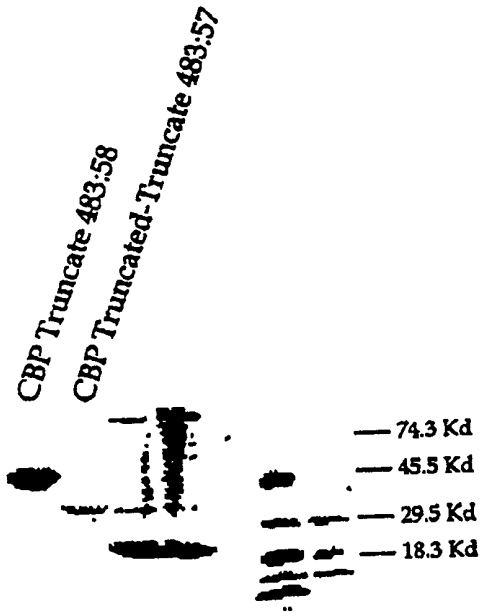
ATCC4 CbpA trun EE 459

Figure 2B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CBP Truncated-Truncate 483:57
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45.5 Kd
29.5 Kd
18.3 Kd

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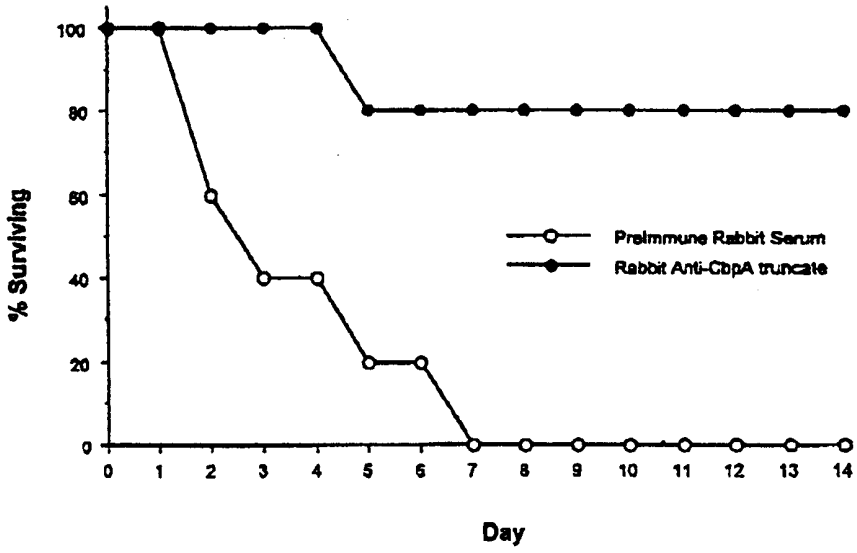
100
80
60
40
20
0
% Surviving
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Day
Preimmune Rabbit Serum
Rabbit Anti-CbpA truncate

Figure 4

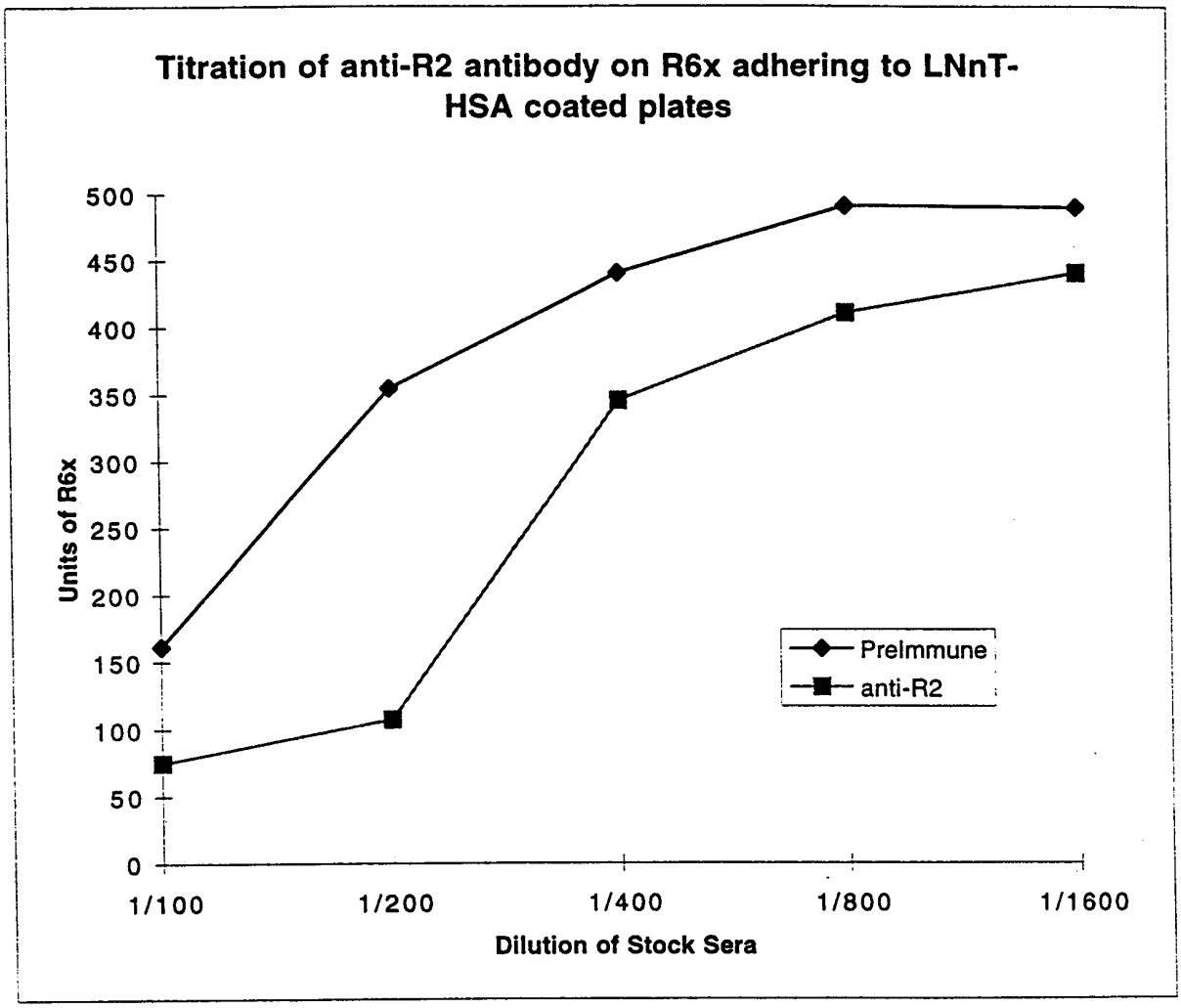
Titration of anti-R2 antibody on R6x adhering to LNnT-HSA coated plates
Units of R6x
500
450
400
350
300
250
200
150
100
50
0
1/100
1/200
1/400
1/800
1/1600
Dilution of Stock Sera
PreImmune
anti-R2

Figure 5

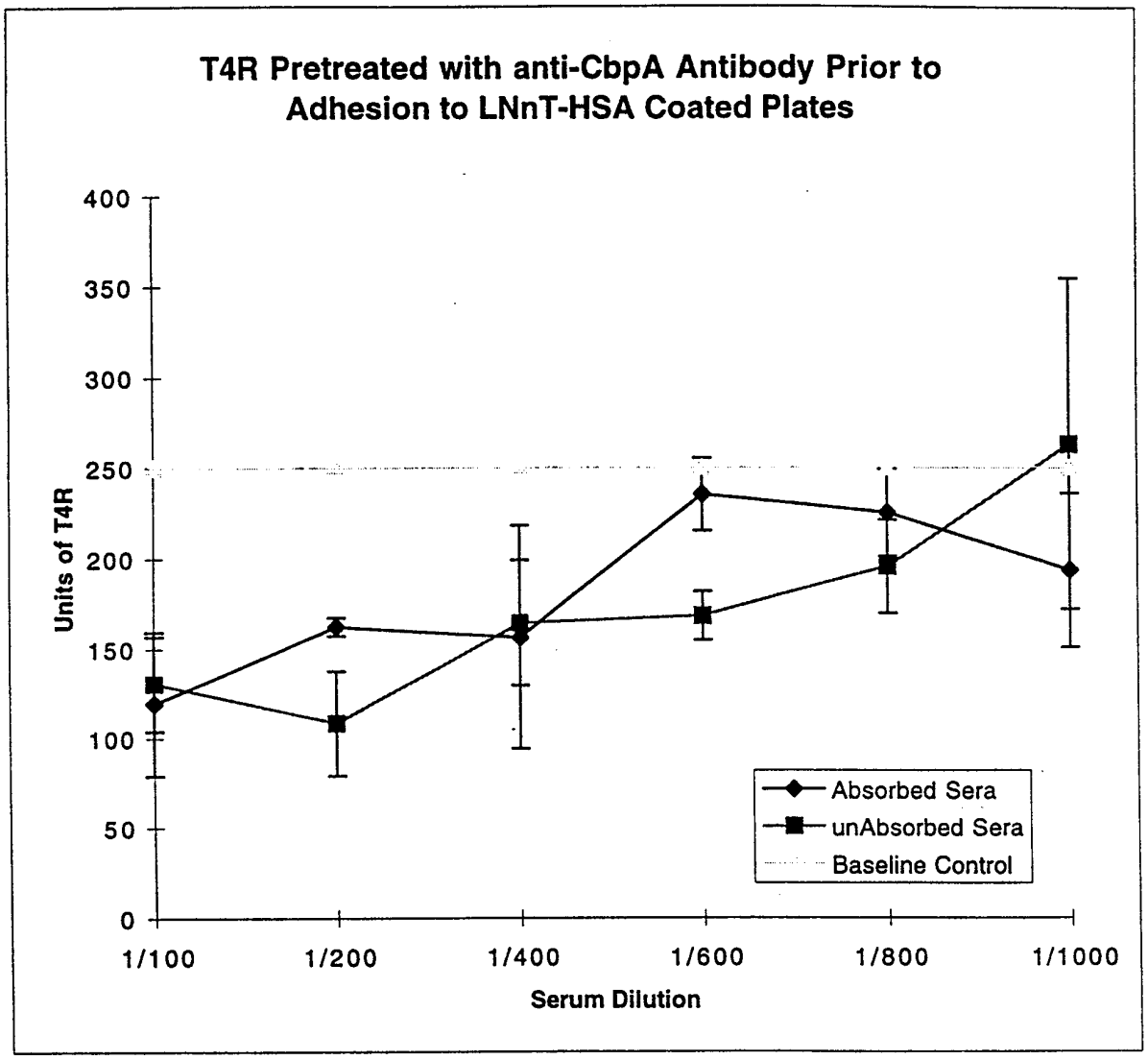
T4R Pretreated with anti-CbpA Antibody Prior to Adhesion to LNnT-HSA Coated Plates
Units of T4R
400
350
300
250
200
150
100
50
0
1/100
1/200
1/400
1/600
1/800
1/1000
Serum Dilution
Absorbed Sera
unAbsorbed Sera
Baseline Control

Figure 6

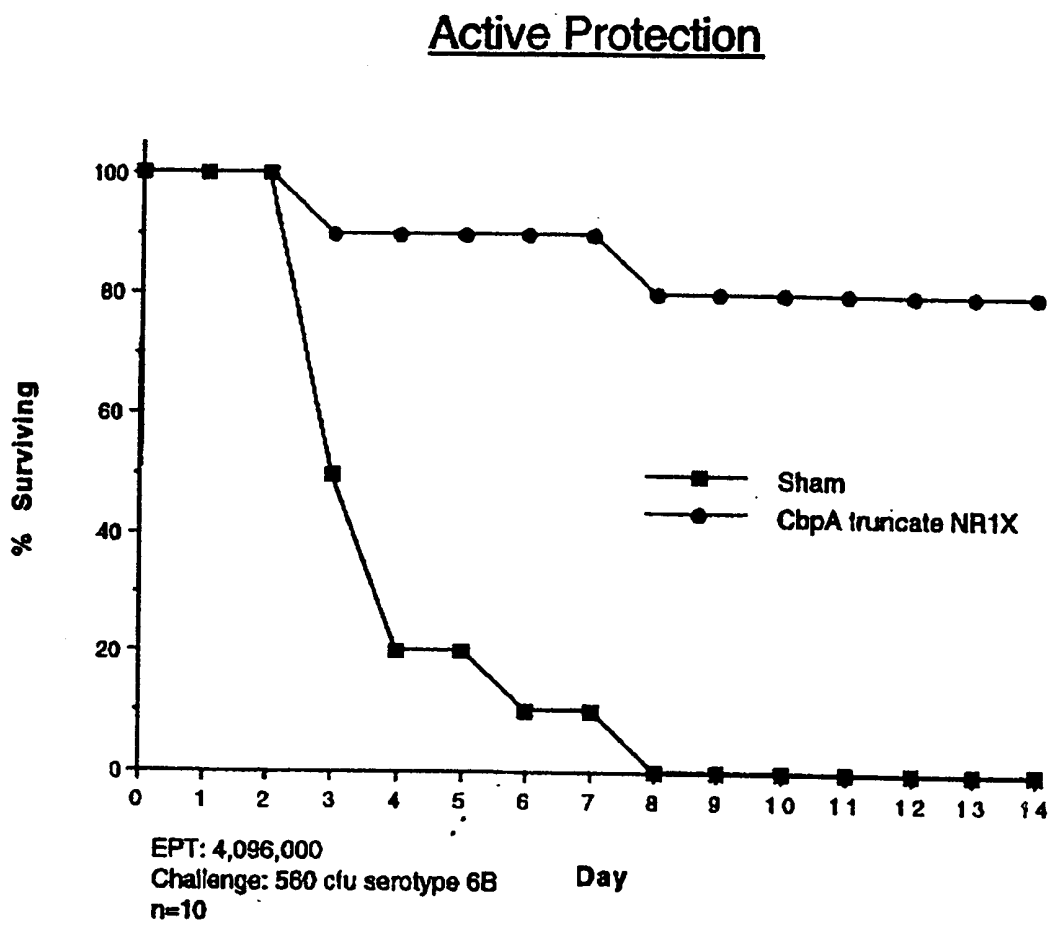
Active Protection
% Surviving
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Day
Sham
CbpA truncate NR1X
EPT: 4,096,000
Challenge: 560 cfu serotype 6B
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Figure 7

SEQUENCE LISTING

<110> Tuomanen, Elaine I.
Wizemann, Theresa
Masure, H. R.
Johnson, Leslie S.
Koenig, Scott

<120> POLYPEPTIDE COMPRISING THE AMINO ACID OF AN N-TERMINAL CHOLINE BINDING PROTEIN A TRUNCATE, VACCINE DERIVED THEREFROM AND USES THEREOF

<130> 1340-1-017 msc

<140> 09/056,019

<141> 1998-04-07

<160> 39

<170> PatentIn Ver. 2.0

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35 40 45

Gly Glu Ser Tyr Ala Lys Ser Thr Lys Lys Arg His Thr Ile Thr Val
50 55 60

Ala Leu Val Asn Glu Leu Asn Asn Ile Lys Asn Glu Tyr Leu Asn Lys
65 70 75 80

Ile Val Glu Ser Thr Ser Glu Ser Gln Leu Gln Ile Leu Met Met Glu
85 90 95

Ser Arg Ser Lys Val Asp Glu Ala Val Ser Lys Phe Glu Lys Asp Ser
100 105 110

Ser Ser Ser Ser Ser Ser Asp Ser Ser Thr Lys Pro Glu Ala Ser Asp
115 120 125

Thr Ala Lys Pro Asn Lys Pro Thr Glu Pro Gly Glu Lys Val Ala Glu
130 135 140

Ala Lys Lys Lys Val Glu Glu Ala Glu Lys Lys Ala Lys Asp Gln Lys
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165 170 175

Leu Glu Ile Ala Glu Ser Asp Val Glu Val Lys Lys Ala Glu Leu Glu
180 185 190

Leu Val Lys Val Lys Ala Asn Glu Pro Arg Asp Glu Gln Lys Ile Lys
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Gln Ala Glu Ala Glu Val Glu Ser Lys Gln Ala Glu Ala Thr Arg Leu
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Lys Lys Ile Lys Thr Asp Arg Glu Glu Ala Glu Glu Glu Ala Lys Arg
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Arg Ala Asp Ala Lys Glu Gln Gly Lys Pro Lys Gly Arg Ala Lys Arg
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Gly Val Pro Gly Glu Leu Ala Thr Pro Asp Lys Lys Glu Asn Asp Ala
260 265 270

Lys Ser Ser Asp Ser Ser Val Gly Glu Glu Thr Leu Pro Ser Pro Ser
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Leu Lys Pro Glu Lys Lys Val Ala Glu Ala Glu Lys Lys Val Glu Glu
290 295 300

Ala Lys Lys Lys Ala Glu Asp Gln Lys Glu Glu Asp Arg Arg Asn Tyr
305 310 315 320

Pro Thr Asn Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Ser Asp
325 330 335

Val Glu Val Lys Lys Ala Glu Leu Glu Leu Val Lys Glu Glu Ala Lys
340 345 350

Glu Pro Arg Asn Glu Glu Lys Val Lys Gln Ala Lys Ala Glu Val Glu
355 360 365

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Val Lys Glu Lys Pro Ala
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35 40 45

Gly Glu Ser Tyr Ala Lys Ser Thr Lys Lys Arg His Thr Ile Thr Val
50 55 60

Ala Leu Val Asn Glu Leu Asn Asn Ile Lys Asn Glu Tyr Leu Asn Lys
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Ile Val Glu Ser Thr Ser Glu Ser Gln Leu Gln Ile Leu Met Met Glu
85 90 95

Ser Arg Ser Lys Val Asp Glu Ala Val Ser Lys Phe Glu Lys Asp Ser
100 105 110

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Gln Ala Glu Ala Glu Val Glu Ser Lys Gln Ala Glu Ala Thr Arg Leu
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275 280 285

Leu Lys Pro Glu Lys Lys Val Ala Glu Ala Glu Lys Lys Val Glu Glu
290 295 300

Ala Lys Lys Lys Ala Glu Asp Gln Lys Glu Glu Asp Arg Arg Asn Tyr
305 310 315 320

Pro Thr Asn Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Ser Asp
325 330 335

Val Glu Val Lys Lys Ala Glu Leu Glu Leu Val Lys Glu Glu Ala Lys
340 345 350

Glu Pro Arg Asn Glu Glu Lys Val Lys Gln Ala Lys Ala Glu Val Glu
355 360 365

Ser Lys Lys Ala Glu Ala Thr Arg Leu Glu Lys Ile Lys Thr Asp Arg
370 375 380

Lys Lys Ala Glu Glu Glu Ala Lys Arg Lys Ala Ala Glu Glu Asp Lys
385 390 395 400

Val Lys Glu Lys Pro Ala Glu Gln Pro Gln Pro Ala Pro Ala Pro Lys
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Ala Glu Lys Pro Ala Pro Ala Pro Lys Pro Glu Asn Pro Ala Glu Gln
420 425 430

Pro Lys Ala Glu Lys Pro Ala Asp Gln Gln Ala Glu Glu Asp Tyr Ala
435 440 445

Arg Arg Ser Glu Glu Glu Tyr Asn Arg Leu Thr Gln Gln Gln Pro Pro
450 455 460

Lys Thr Glu Lys Pro Ala Gln Pro Ser Thr Pro Lys Thr Gly Trp Lys
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Gln Glu Asn Gly Met Trp Tyr Phe Tyr Asn Thr Asp Gly Ser Met Ala

485 490 495

Thr Gly Trp Leu Gln Asn Asn Gly Ser Trp Tyr Tyr Leu Asn Ser Asn
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Leu Asn Ala Asn Gly Ser Met Ala Thr Gly Trp Leu Gln Asn Asn Gly
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Ser Trp Tyr Tyr Leu Asn Ala Asn Gly Ser Met Ala Thr Gly Trp Leu
545 550 555 560

Gln Tyr Asn Gly Ser Trp Tyr Tyr Leu Asn Ala Asn Gly Ser Met Ala
565 570 575

Thr Gly Trp Leu Gln Tyr Asn Gly Ser Trp Tyr Tyr Leu Asn Ala Asn
580 585 590

Gly Asp Met Ala Thr Gly Trp Val Lys Asp Gly Asp Thr Trp Tyr Tyr
595 600 605

Leu Glu Ala Ser Gly Ala Met Lys Ala Ser Gln Trp Phe Lys Val Ser
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<213> Streptococcus pneumoniae

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35 40 45

Gly Glu Ser Tyr Ala Lys Ser Thr Lys Lys Arg His Thr Ile Thr Val
50 55 60

Ala Leu Val Asn Glu Leu Asn Asn Ile Lys Asn Glu Tyr Leu Asn Lys
65 70 75 80

Ile Val Glu Ser Thr Ser Glu Ser Gln Leu Gln Ile Leu Met Met Glu
85 90 95

Ser Arg Ser Lys Val Asp Glu Ala Val Ser Lys Phe Glu Lys Asp Ser

100 105 110

Ser Ser Ser Ser Ser Ser Asp Ser Ser Thr Lys Pro Glu Ala Ser Asp
115 120 125

Thr Ala Lys Pro Asn Lys Pro Thr Glu Pro Gly Glu Lys Val Ala Glu
130 135 140

Ala Lys Lys Lys Val Glu Glu Ala Glu Lys Lys Ala Lys Asp Gln Lys
145 150 155 160

Glu Glu Asp Arg Arg Asn Tyr Pro Thr Ile Thr Tyr Lys Thr Leu Glu
165 170 175

Leu Glu Ile Ala Glu Ser Asp Val Glu Val Lys Lys Ala Glu Leu Glu
180 185 190

Leu Val Lys Val Lys Ala Asn Glu Pro Arg Asp Glu Gln Lys Ile Lys
195 200 205

Gln Ala Glu Ala Glu Val Glu Ser Lys Gln Ala Glu Ala Thr Arg Leu
210 215 220

Lys Lys Ile Lys Thr Asp Arg Glu Glu Ala Glu Glu Glu Ala Lys Arg
225 230 235 240

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

Glu Val Lys Lys Ala Glu Leu Glu Leu Val Lys Glu Glu Ala Lys Glu
50 55 60

Pro Arg Asn Glu Glu Lys Val Lys Gln Ala Lys Ala Glu Val Glu Ser
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<213> Streptococcus pneumoniae

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35 40 45

Val Glu Val Lys Lys Ala Glu Leu Glu Leu Val Lys Val Lys Ala Asn
50 55 60

Glu Pro Arg Asp Glu Gln Lys Ile Lys Gln Ala Glu Ala Glu Val Glu
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Ser Lys Gln Ala Glu Ala Thr Arg Leu Lys Lys Ile Lys Thr Asp Arg
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20 25 30

Lys Met Leu Arg Glu Ile Gln Leu Asp Arg Arg Lys His Thr Gln Asn
35 40 45

Val Ala Leu Asn Ile Lys Leu Ser Ala Ile Lys Thr Lys Tyr Leu Arg
50 55 60

Glu Leu Asn Val Leu Glu Glu Lys Ser Lys Asp Glu Leu Pro Ser Glu
65 70 75 80

Ile Lys Ala Lys Leu Asp Ala Ala Phe Glu Lys Phe Lys Lys Asp Thr
85 90 95

Leu Lys Pro Gly Glu Lys Val Ala Glu Ala Lys Lys Lys Val Glu Glu
100 105 110

Ala Lys Lys Lys Ala Glu Asp Gln Lys Glu Glu Asp Arg Arg Asn Tyr
115 120 125

Pro Thr Asn Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Phe Asp
130 135 140

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145 150 155 160

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35 40 45

Val Ala Leu Asn Ile Lys Leu Ser Ala Ile Lys Thr Lys Tyr Leu Arg
50 55 60

Glu Leu Asn Val Leu Glu Glu Lys Ser Lys Asp Glu Leu Pro Ser Glu
65 70 75 80

Ile Lys Ala Lys Leu Asp Ala Ala Phe Glu Lys Phe Lys Lys Asp Thr

85 90 95

Leu Lys Pro Gly Glu Lys Val Ala Glu Ala Lys Lys Lys Val Glu Glu
100 105 110

Ala Lys Lys Lys Ala Glu Asp Gln Lys Glu Glu Asp Arg Arg Asn Tyr
115 120 125

Pro Thr Asn Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Phe Asp
130 135 140

Val Lys Val Lys Glu Ala Glu Leu Glu Leu Val Lys Glu Glu Ala Lys
145 150 155 160

Glu Ser Arg Asn Glu Gly Thr Ile Lys Gln Ala Lys Glu Lys Val Glu
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Ser Lys Lys Ala Glu Ala Thr Arg Leu Glu Asn Ile Lys Thr Asp Arg
180 185 190

Lys Lys Ala Glu Glu Glu Ala Lys Arg Lys Ala Asp Ala Lys Leu Lys
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Glu Ala Asn Val Ala Thr Ser Asp Gln Gly Lys Pro Lys Gly Arg Ala
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245 250 255

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260 265 270

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275 280 285

Asn Tyr Pro Thr Asn Thr Tyr Lys Thr Leu Asp Leu Glu Ile Ala Glu
290 295 300

Ser Asp Val Lys Val Lys Glu Ala Glu Leu Glu Leu Val Lys Glu Glu
305 310 315 320

Ala Lys Glu Pro Arg Asp Glu Glu Lys Ile Lys Gln Ala Lys Ala Lys
325 330 335

Val Glu Ser Lys Lys Ala Glu Ala Thr Arg Leu Glu Asn Ile Lys Thr
340 345 350

Asp Arg Lys Lys Ala Glu Glu Glu Ala Lys Arg Lys Ala Ala Glu Glu
355 360 365

Asp Lys Val Lys Glu Lys Pro Ala Glu Gln Pro Gln Pro Ala Pro Ala
370 375 380

Thr Gln Pro Glu Lys Pro Ala Pro Lys Pro Glu Lys Pro Ala Glu Gln
385 390 395 400

Pro Lys Ala Glu Lys Thr Asp Asp Gln Gln Ala Glu Glu Asp Tyr Ala
405 410 415

Arg Arg Ser Glu Glu Glu Tyr Asn Arg Leu Thr Gln Gln Gln Pro Pro
420 425 430

Lys Thr Glu Lys Pro Ala Gln Pro Ser Thr Pro Lys Thr Gly Trp Lys
435 440 445

Gln Glu Asn Gly Met Trp Tyr Phe Tyr Asn Thr Asp Gly Ser Met Ala
450 455 460

Thr Gly Trp Leu Gln Asn Asn Gly Ser Trp Tyr Tyr Leu Asn Ala Asn
465 470 475 480

Gly Ala Met Ala Thr Gly Trp Leu Gln Asn Asn Gly Ser Trp Tyr Tyr
485 490 495

Leu Asn Ala Asn Gly Ser Met Ala Thr Gly Trp Leu Gln Asn Asn Gly
500 505 510

Ser Trp Tyr Tyr Leu Asn Ala Asn Gly Ala Met Ala Thr Gly Trp Leu
515 520 525

Gln Tyr Asn Gly Ser Trp Tyr Tyr Leu Asn Ser Asn Gly Ala Met Ala
530 535 540

Thr Gly Trp Leu Gln Tyr Asn Gly Ser Trp Tyr Tyr Leu Asn Ala Asn

545 550 555 560

Gly Asp Met Ala Thr Gly Trp Leu Gln Asn Asn Gly Ser Trp Tyr Tyr
565 570 575

Leu Asn Ala Asn Gly Asp Met Ala Thr Gly Trp Leu Gln Tyr Asn Gly
580 585 590

Ser Trp Tyr Tyr Leu Asn Ala Asn Gly Asp Met Ala Thr Gly Trp Val
595 600 605

Lys Asp Gly Asp Thr Trp Tyr Tyr Leu Glu Ala Ser Gly Ala Met Lys
610 615 620

Ala Ser Gln Trp Phe Lys Val Ser Asp Lys Trp Tyr Tyr Val Asn Gly
625 630 635 640

Ser Gly Ala Leu Ala Val Asn Thr Thr Val Asp Gly Tyr Gly Val Asn
645 650 655

Ala Asn Gly Glu Trp Val Asn
660

<210> 9

<211> 254

<212> PRT

<213> Streptococcus pneumoniae

<400> 9

Glu Asn Glu Gly Ser Thr Gln Ala Ala Thr Ser Ser Asn Met Ala Lys
1 5 10 15

Thr Glu His Arg Lys Ala Ala Lys Gln Val Val Asp Glu Tyr Ile Glu
20 25 30

Lys Met Leu Arg Glu Ile Gln Leu Asp Arg Arg Lys His Thr Gln Asn
35 40 45

Val Ala Leu Asn Ile Lys Leu Ser Ala Ile Lys Thr Lys Tyr Leu Arg
50 55 60

Glu Leu Asn Val Leu Glu Glu Lys Ser Lys Asp Glu Leu Pro Ser Glu
65 70 75 80

Ile Lys Ala Lys Leu Asp Ala Ala Phe Glu Lys Phe Lys Lys Asp Thr
85 90 95

Leu Lys Pro Gly Glu Lys Val Ala Glu Ala Lys Lys Lys Val Glu Glu
100 105 110

Ala Lys Lys Lys Ala Glu Asp Gln Lys Glu Glu Asp Arg Arg Asn Tyr
115 120 125

Pro Thr Asn Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Phe Asp
130 135 140

Val Lys Val Lys Glu Ala Glu Leu Glu Leu Val Lys Glu Glu Ala Lys

145 150 155 160

Glu Ser Arg Asn Glu Gly Thr Ile Lys Gln Ala Lys Glu Lys Val Glu
165 170 175

Ser Lys Lys Ala Glu Ala Thr Arg Leu Glu Asn Ile Lys Thr Asp Arg
180 185 190

Lys Lys Ala Glu Glu Glu Ala Lys Arg Lys Ala Asp Ala Lys Leu Lys
195 200 205

Glu Ala Asn Val Ala Thr Ser Asp Gln Gly Lys Pro Lys Gly Arg Ala
210 215 220

Lys Arg Gly Val Pro Gly Glu Leu Ala Thr Pro Asp Lys Lys Glu Asn
225 230 235 240

Asp Ala Lys Ser Ser Asp Ser Ser Val Gly Glu Glu Thr Leu
245 250

<210> 10

<211> 106

<212> PRT

<213> Streptococcus pneumoniae

<400> 10

Lys Ser Gly Lys Lys Val Ala Glu Ala Glu Lys Lys Val Glu Glu Ala
1 5 10 15

Glu Lys Lys Ala Lys Asp Gln Lys Glu Glu Asp Arg Arg Asn Tyr Pro
20 25 30

Thr Asn Thr Tyr Lys Thr Leu Asp Leu Glu Ile Ala Glu Ser Asp Val
35 40 45

Lys Val Lys Glu Ala Glu Leu Glu Leu Val Lys Glu Glu Ala Lys Glu
50 55 60

Pro Arg Asp Glu Glu Lys Ile Lys Gln Ala Lys Ala Lys Val Glu Ser
65 70 75 80

Lys Lys Ala Glu Ala Thr Arg Leu Glu Asn Ile Lys Thr Asp Arg Lys
85 90 95

Lys Ala Glu Glu Glu Ala Lys Arg Lys Ala
100 105

<210> 11

<211> 107

<212> PRT

<213> Streptococcus pneumoniae

<400> 11

Pro Gly Glu Lys Val Ala Glu Ala Lys Lys Lys Val Glu Glu Ala Lys
1 5 10 15

Lys Lys Ala Glu Asp Gln Lys Glu Glu Asp Arg Arg Asn Tyr Pro Thr
20 25 30

Asn Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Phe Asp Val Lys
35 40 45

Val Lys Glu Ala Glu Leu Glu Leu Val Lys Glu Glu Ala Lys Glu Ser
50 55 60

Arg Asn Glu Gly Thr Ile Lys Gln Ala Lys Glu Lys Val Glu Ser Lys
65 70 75 80

Lys Ala Glu Ala Thr Arg Leu Glu Asn Ile Lys Thr Asp Arg Lys Lys
85 90 95

Ala Glu Glu Glu Ala Lys Arg Lys Ala Asp Ala
100 105

<210> 12

<211> 1219

<212> DNA

<213> Streptococcus pneumoniae

<400> 12

gagaacgagg gagctaccca agtacccact tcttctaata gggcaaatga aagtcaggca 60

gaacaaggag aacaacctaa aaaactcgat tcagaacgag ataaggcaag gaaagaggtc 120

gaggaatatg taaaaaaaat agtgggtgag agctatgcaa aatcaactaa aaagcgacat 180

acaattactg tagctctagt taacgagttg aacaacatta agaacgagta tttgaataaa 240

atagttgaat caacctcaga aagccaacta cagatactga tgatggagag tcgatcaaaa 300

gtagatgaag ctgtgtctaa gtttgaaaag gactcatctt cttcgtcaag ttcagactct 360

tccactaaac cggaagcttc agatacagcg aagccaaaca agccgacaga accaggagaa 420

aaggtagcag aagctaagaa gaaggttgaa gaagctgaga aaaaagccaa ggatcaaaaa 480

gaagaagatc gtcgtaacta cccaaccatt acttacaaaa cgcttgaact tgaaattgct 540

gagtccgatg tggaagttaa aaaagcggag cttgaactag taaaagtgaa agctaacgaa 600

cctcgagacg agcaaaaaat taagcaagca gaagcggaag ttgagagtaa acaagctgag 660

gctacaaggt taaaaaaaat caagacagat cgtgaagaag cagaagaaga agctaaacga 720

agagcagatg ctaaagagca aggtaaacca aaggggcggg caaaacgagg agttcctgga 780

gagctagcaa cacctgataa aaaagaaaat gatgcgaagt cttcagattc tagcgtaggt 840

gaagaaactc ttccaagccc atccctgaaa ccagaaaaaa aggtagcaga agctgagaag 900

aaggttgaag aagctaagaa aaaagccgag gatcaaaaag aagaagatcg ccgtaactac 960

ccaaccaata cttacaaaac gcttgaactt gaaattgctg agtccgatgt ggaagttaaa 1020

aaagcggagc ttgaactagt aaaagaggaa gctaaggaac ctcgaaacga ggaaaaagtt 1080

aagcaagcaa aagcggaagt tgagagtaaa aaagctgagg ctacaaggtt agaaaaaatc 1140

aagacagatc gtaaaaaagc agaagaagaa gctaaacgaa aagcagcaga agaagataaa 1200

gttaaagaaa aaccagctg 1219

<210> 13

<211> 1969

<212> DNA

<213> Streptococcus pneumoniae

<400> 13

gagaacgagg gagctaccca agtacccact tcttctaata gggcaaatga aagtcaggca 60

gaacaaggag aacaacctaa aaaactcgat tcagaacgag ataaggcaag gaaagaggtc 120

gaggaatatg taaaaaaaat agtgggtgag agctatgcaa aatcaactaa aaagcgacat 180

acaattactg tagctctagt taacgagttg aacaacatta agaacgagta tttgaataaa 240

atagttgaat caacctcaga aagccaacta cagatactga tgatggagag tcgatcaaaa 300

gtagatgaag ctgtgtctaa gtttgaaaag gactcatctt cttcgtcaag ttcagactct 360

tccactaaac cggaagcttc agatacagcg aagccaaaca agccgacaga accaggagaa 420

aaggtagcag aagctaagaa gaaggttgaa gaagctgaga aaaaagccaa ggatcaaaaa 480

gaagaagatc gtcgtaacta cccaaccatt acttacaaaa cgcttgaact tgaaattgct 540

gagtccgatg tggaagttaa aaaagcggag cttgaactag taaaagtgaa agctaacgaa 600

cctcgagacg agcaaaaaat taagcaagca gaagcggaag ttgagagtaa acaagctgag 660

gctacaaggt taaaaaaaat caagacagat cgtgaagaag cagaagaaga agctaaacga 720

agagcagatg ctaaagagca aggtaaacca aaggggcggg caaaacgagg agttcctgga 780

gagctagcaa cacctgataa aaaagaaaat gatgcgaagt cttcagattc tagcgtaggt 840

gaagaaactc ttccaagccc atccctgaaa ccagaaaaaa aggtagcaga agctgagaag 900

aaggttgaag aagctaagaa aaaagccgag gatcaaaaag aagaagatcg ccgtaactac 960

ccaaccaata cttacaaaac gcttgaactt gaaattgctg agtccgatgt ggaagttaaa 1020

aaagcggagg cttgaactag taaaagagga agctaaggaa cctcgaaacg aggaaaaagt 1080

taagcaagca aaagcggaag ttgagagtaa aaaagctgag gctacaaggt tagaaaaaat 1140

caagacagat cgtaaaaaag cagaagaaga agctaaacga aaagcagcag aagaagataa 1200

agttaaagaa aaaccagctg aacaaccaca accagcgccg gctccaaaag cagaaaaacc 1260

agctccagct ccaaaaccag agaatccagc tgaacaacca aaagcagaaa aaccagctga 1320

tcaacaagct gaagaagact atgctcgtag atcagaagaa gaatataatc gcttgactca 1380

acagcaaccg ccaaaaactg aaaaaccagc acaaccatct actccaaaaa caggctggaa 1440

acaagaaaac ggtatgtggt acttctacaa tactgatggt tcaatggcga caggatggct 1500

ccaaaacaat ggctcatggt actacctcaa cagcaatggc gctatggcga caggatggct 1560

ccaaaacaat ggttcatggt actatctaaa cgctaatggt tcaatggcaa caggatggct 1620

ccaaaacaat ggttcatggt actacctaaa cgctaatggt tcaatggcga caggatggct 1680

ccaatacaat ggctcatggt actacctaaa cgctaatggt tcaatggcga caggatggct 1740

ccaatacaat ggctcatggt actacctaaa cgctaatggt gatatggcga caggttgggt 1800

gaaagatgga gatacctggt actatcttga agcatcaggt gctatgaaag caagccaatg 1860

gttcaaagta tcagataaat ggtactatgt caatggctca ggtgcccttg cagtcaacac 1920

aactgtagat ggctatggag tcaatgccaa tggtgaatgg gtaaactaa 1969

<210> 14

<211> 853

<212> DNA

<213> Streptococcus pneumoniae

<400> 14

gagaacgagg gagctaccca agtacccact tcttctaata gggcaaatga aagtcaggca 60

gaacaaggag aacaacctaa aaaactcgat tcagaacgag ataaggcaag gaaagaggtc 120

gaggaatatg taaaaaaaat agtgggtgag agctatgcaa aatcaactaa aaagcgacat 180

acaattactg tagctctagt taacgagttg aacaacatta agaacgagta tttgaataaa 240

atagttgaat caacctcaga aagccaacta cagatactga tgatggagag tcgatcaaaa 300

gtagatgaag ctgtgtctaa gtttgaaaag gactcatctt cttcgtcaag ttcagactct 360

tccactaaac cggaagcttc agatacagcg aagccaaaca agccgacaga accaggagaa 420

aaggtagcag aagctaagaa gaaggttgaa gaagctgaga aaaaagccaa ggatcaaaaa 480

gaagaagatc gtcgtaacta cccaaccatt acttacaaaa cgcttgaact tgaaattgct 540

gagtccgatg tggaagttaa aaaagcggag cttgaactag taaaagtgaa agctaacgaa 600

cctcgagacg agcaaaaaat taagcaagca gaagcggaag ttgagagtaa acaagctgag 660

gctacaaggt taaaaaaaat caagacagat cgtgaagaag cagaagaaga agctaaacga 720

agagcagatg ctaaagagca aggtaaacca aaggggcggg caaaacgagg agttcctgga 780

gagctagcaa cacctgataa aaaagaaaat gatgcgaagt cttcagattc tagcgtaggt 840

gaagaaactc ttc 853

<210> 15

<211> 318

<212> DNA

<213> Streptococcus pneumoniae

<400> 15

aaaccagaaa aaaaggtagc agaagctgag aagaaggttg aagaagctaa gaaaaaagcc 60

gaggatcaaa aagaagaaga tcgccgtaac tacccaacca atacttacaa aacgcttgaa 120
cttgaaattg ctgagtccga tgtggaagtt aaaaaagcgg agcttgaact agtaaaagag 180
gaagctaagg aacctcgaaa cgaggaaaaa gttaagcaag caaaagcgga agttgagagt 240
aaaaaagctg aggctacaag gttagaaaaa atcaagacag atcgtaaaaa agcagaagaa 300
gaagctaaac gaaaagca 318

<210> 16
<211> 327
<212> DNA
<213> Streptococcus pneumoniae

<400> 16
acagaaccag gagaaaaggt agcagaagct aagaagaagg ttgaagaagc tgagaaaaaa 60
gccaaggatc aaaaagaaga agatcgtcgt aactacccaa ccattactta caaaacgctt 120
gaacttgaaa ttgctgagtc cgatgtggaa gttaaaaaag cggagcttga actagtaaaa 180
gtgaaagcta acgaacctcg agacgagcaa aaaattaagc aagcagaagc ggaagttgag 240
agtaaacaag ctgaggctac aaggttaaaa aaaatcaaga cagatcgtga agaagcagaa 300
gaagaagcta aacgaagagc agatgct 327

<210> 17
<211> 1129
<212> DNA
<213> Streptococcus pneumoniae

<400> 17
gaaaacgaag gaagtaccca agcagccact tcttctaata tggcaaagac agaacatagg 60
aaagctgcta aacaagtcgt cgatgaatat atagaaaaaa tgttgaggga gattcaacta 120
gatagaagaa aacataccca aaatgtcgcc ttaaacataa agttgagcgc aattaaaacg 180
aagtatttgc gtgaattaaa tgttttagaa gagaagtcga aagatgagtt gccgtcagaa 240

ataaaagcaa agttagacgc agcttttgag aagtttaaaa aagatacatt gaaaccagga 300

gaaaaggtag cagaagctaa gaagaaggtt gaagaagcta agaaaaaagc cgaggatcaa 360

aaagaagaag atcgtcgtaa ctacccaacc aatacttaca aaacgcttga acttgaaatt 420

gctgagttcg atgtgaaagt taaagaagcg gagcttgaac tagtaaaaga ggaagctaaa 480

gaatctcgaa acgagggcac aattaagcaa gcaaaagaga aagttgagag taaaaaagct 540

gaggctacaa ggttagaaaa catcaagaca gatcgtaaaa aagcagaaga agaagctaaa 600

cgaaaagcag atgctaagtt gaaggaagct aatgtagcga cttcagatca aggtaaacca 660

aaggggcggg caaaacgagg agttcctgga gagctagcaa cacctgataa aaaagaaaat 720

gatgcgaagt cttcagattc tagcgtaggt gaagaaactc ttccaagctc atccctgaaa 780

tcaggaaaaa aggtagcaga agctgagaag aaggttgaag aagctgagaa aaaagccaag 840

gatcaaaaag aagaagatcg ccgtaactac ccaaccaata cttacaaaac gcttgacctt 900

gaaattgctg agtccgatgt gaaagttaaa gaagcggagc ttgaactagt aaaagaggaa 960

gctaaggaac ctcgagacga ggaaaaaatt aagcaagcaa aagcgaaagt tgagagtaaa 1020

aaagctgagg ctacaaggtt agaaaacatc aagacagatc gtaaaaaagc agaagaagaa 1080

gctaaacgaa aagcagcaga agaagataaa gttaaagaaa aaccagctg 1129

<210> 18

<211> 1992

<212> DNA

<213> Streptococcus pneumoniae

<400> 18

gaaaacgaag gaagtaccca agcagccact tcttctaata tggcaaagac agaacatagg 60

aaagctgcta aacaagtcgt cgatgaatat atagaaaaaa tgttgaggga gattcaacta 120

gatagaagaa aacataccca aaatgtcgcc ttaaacataa agttgagcgc aattaaaacg 180

aagtatttgc gtgaattaaa tgttttagaa gagaagtcga aagatgagtt gccgtcagaa 240

ataaaagcaa agttagacgc agcttttgag aagtttaaaa aagatacatt gaaaccagga 300

gaaaaggtag cagaagctaa gaagaaggtt gaagaagcta agaaaaaagc cgaggatcaa 360

aaagaagaag atcgtcgtaa ctacccaacc aatacttaca aaacgcttga acttgaaatt 420

gctgagttcg atgtgaaagt taaagaagcg gagcttgaac tagtaaaaga ggaagctaaa 480

gaatctcgaa acgagggcac aattaagcaa gcaaaagaga aagttgagag taaaaaagct 540

gaggctacaa ggttagaaaa catcaagaca gatcgtaaaa aagcagaaga agaagctaaa 600

cgaaaagcag atgctaagtt gaaggaagct aatgtagcga cttcagatca aggtaaacca 660

aaggggcggg caaaacgagg agttcctgga gagctagcaa cacctgataa aaaagaaaat 720

gatgcgaagt cttcagattc tagcgtaggt gaagaaactc ttccaagctc atccctgaaa 780

tcaggaaaaa aggtagcaga agctgagaag aaggttgaag aagctgagaa aaaagccaag 840

gatcaaaaag aagaagatcg ccgtaactac ccaaccaata cttacaaaac gcttgacctt 900

gaaattgctg agtccgatgt gaaagttaaa gaagcggagc ttgaactagt aaaagaggaa 960

gctaaggaac ctcgagacga ggaaaaaatt aagcaagcaa aagcgaaagt tgagagtaaa 1020

aaagctgagg ctacaaggtt agaaaacatc aagacagatc gtaaaaaagc agaagaagaa 1080

gctaaacgaa aagcagcaga agaagataaa gttaaagaaa aaccagctga acaaccacaa 1140

ccagcgccgg ctactcaacc agaaaaacca gctccaaaac cagagaagcc agctgaacaa 1200

ccaaaagcag aaaaaacaga tgatcaacaa gctgaagaag actatgctcg tagatcagaa 1260

gaagaatata atcgcttgac tcaacagcaa ccgccaaaaa ctgaaaaacc agcacaacca 1320

tctactccaa aaacaggctg gaaacaagaa aacggtatgt ggtacttcta caatactgat 1380

ggttcaatgg caacaggatg gctccaaaac aacggttcat ggtactatct aaacgctaat 1440

ggtgctatgg cgacaggatg gctccaaaac aatggttcat ggtactatct aaacgctaat 1500

ggttcaatgg caacaggatg gctccaaaac aatggttcat ggtactacct aaacgctaat 1560

ggtgctatgg cgacaggatg gctccaatac aatggttcat ggtactacct aaacagcaat 1620

ggcgctatgg cgacaggatg gctccaatac aatggctcat ggtactacct caacgctaat 1680

ggtgatatgg cgacaggatg gctccaaaac aacggttcat ggtactacct caacgctaat 1740

ggtgatatgg cgacaggatg gctccaatac aacggttcat ggtattacct caacgctaat 1800

ggtgatatgg cgacaggttg ggtgaaagat ggagatacct ggtactatct tgaagcatca 1860

ggtgctatga aagcaagcca atggttcaaa gtatcagata aatggtacta tgtcaatggc 1920

tcaggtgccc ttgcagtcaa cacaactgta gatggctatg gagtcaatgc caatggtgaa 1980

tgggtaaact aa 1992

<210> 19

<211> 763

<212> DNA

<213> Streptococcus pneumoniae

<400> 19

gaaaacgaag gaagtaccca agcagccact tcttctaata tggcaaagac agaacatagg 60

aaagctgcta aacaagtcgt cgatgaatat atagaaaaaa tgttgaggga gattcaacta 120

gatagaagaa aacataccca aaatgtcgcc ttaaacataa agttgagcgc aattaaaacg 180

aagtatttgc gtgaattaaa tgttttagaa gagaagtcga aagatgagtt gccgtcagaa 240

ataaaagcaa agttagacgc agcttttgag aagtttaaaa aagatacatt gaaaccagga 300

gaaaaggtag cagaagctaa gaagaaggtt gaagaagcta agaaaaaagc cgaggatcaa 360

aaagaagaag atcgtcgtaa ctacccaacc aatacttaca aaacgcttga acttgaaatt 420

gctgagttcg atgtgaaagt taaagaagcg gagcttgaac tagtaaaaga ggaagctaaa 480

gaatctcgaa acgagggcac aattaagcaa gcaaaagaga aagttgagag taaaaaagct 540

gaggctacaa ggttagaaaa catcaagaca gatcgtaaaa aagcagaaga agaagctaaa 600

cgaaaagcag atgctaagtt gaaggaagct aatgtagcga cttcagatca aggtaaacca 660

aaggggcggg caaaacgagg agttcctgga gagctagcaa cacctgataa aaaagaaaat 720

gatgcgaagt cttcagattc tagcgtaggt gaagaaactc ttc 763

<210> 20

<211> 318

<212> DNA

<213> Streptococcus pneumoniae

<400> 20

aaatcaggaa aaaaggtagc agaagctgag aagaaggttg aagaagctga gaaaaaagcc 60

aaggatcaaa aagaagaaga tcgccgtaac tacccaacca atacttacaa aacgcttgac 120

cttgaaattg ctgagtccga tgtgaaagtt aaagaagcgg agcttgaact agtaaaagag 180

gaagctaagg aacctcgaga cgaggaaaaa attaagcaag caaaagcgaa agttgagagt 240

aaaaaagctg aggctacaag gttagaaaac atcaagacag atcgtaaaaa agcagaagaa 300

gaagctaaac gaaaagca 318

<210> 21

<211> 321

<212> DNA

<213> Streptococcus pneumoniae

<400> 21

ccaggagaaa aggtagcaga agctaagaag aaggttgaag aagctaagaa aaaagccgag 60

gatcaaaaag aagaagatcg tcgtaactac ccaaccaata cttacaaaac gcttgaactt 120

gaaattgctg agttcgatgt gaaagttaaa gaagcggagc ttgaactagt aaaagaggaa 180

gctaaagaat ctcgaaacga gggcacaatt aagcaagcaa aagagaaagt tgagagtaaa 240

aaagctgagg ctacaaggtt agaaaacatc aagacagatc gtaaaaaagc agaagaagaa 300

gctaaacgaa aagcagatgc t 321

<210> 22

<211> 121

<212> PRT

<213> Streptococcus pneumoniae

<400> 22

Ser Pro Ser Leu Lys Pro Glu Lys Lys Val Ala Glu Ala Glu Lys Lys

1 5 10 15

Val Glu Glu Ala Lys Lys Lys Ala Glu Asp Gln Lys Glu Glu Asp Arg

20 25 30

Arg Asn Tyr Pro Thr Asn Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala

35 40 45

Glu Ser Asp Val Glu Val Lys Lys Ala Glu Leu Glu Leu Val Lys Glu

50 55 60

Glu Ala Lys Glu Pro Arg Asn Glu Glu Lys Val Lys Gln Ala Lys Ala

65 70 75 80

Glu Val Glu Ser Lys Lys Ala Glu Ala Thr Arg Leu Glu Lys Ile Lys

85 90 95

Thr Asp Arg Lys Lys Ala Glu Glu Glu Ala Lys Arg Lys Ala Ala Glu

100 105 110

Glu Asp Lys Val Lys Glu Lys Pro Ala

115 120

<210> 23

<211> 122

<212> PRT

<213> Streptococcus pneumoniae

<400> 23

Pro Ser Ser Ser Leu Lys Ser Gly Lys Lys Val Ala Glu Ala Glu Lys

1 5 10 15

Lys Val Glu Glu Ala Glu Lys Lys Ala Lys Asp Gln Lys Glu Glu Asp

20 25 30

Arg Arg Asn Tyr Pro Thr Asn Thr Tyr Lys Thr Leu Asp Leu Glu Ile
35 40 45

Ala Glu Ser Asp Val Lys Val Lys Glu Ala Glu Leu Glu Leu Val Lys
50 55 60

Glu Glu Ala Lys Glu Pro Arg Asp Glu Glu Lys Ile Lys Gln Ala Lys
65 70 75 80

Ala Lys Val Glu Ser Lys Lys Ala Glu Ala Thr Arg Leu Glu Asn Ile
85 90 95

Lys Thr Asp Arg Lys Lys Ala Glu Glu Glu Ala Lys Arg Lys Ala Ala
100 105 110

Glu Glu Asp Lys Val Lys Glu Lys Arg Ala
115 120

<210> 24

<211> 428

<212> PRT

<213> Streptococcus pneumoniae

<400> 24

Glu Asn Glu Gly Ala Thr Gln Val Pro Thr Ser Ser Asn Arg Ala Asn
1 5 10 15

Glu Ser Gln Ala Glu Gln Gly Glu Gln Pro Lys Lys Leu Asp Ser Glu
20 25 30

Arg Asp Lys Ala Arg Lys Glu Val Glu Glu Tyr Val Lys Lys Ile Val
35 40 45

Gly Glu Ser Tyr Ala Lys Ser Thr Lys Lys Arg His Thr Ile Thr Val
50 55 60

Ala Leu Val Asn Glu Leu Asn Asn Ile Lys Asn Glu Tyr Leu Asn Lys
65 70 75 80

Ile Val Glu Ser Thr Ser Glu Ser Gln Leu Gln Ile Leu Met Met Glu
85 90 95

Ser Arg Ser Lys Val Asp Glu Ala Val Ser Lys Phe Glu Lys Asp Ser
100 105 110

Ser Ser Ser Ser Ser Ser Asp Ser Ser Thr Lys Pro Glu Ala Ser Asp
115 120 125

Thr Ala Lys Pro Asn Lys Pro Thr Glu Pro Gly Glu Lys Val Ala Glu
130 135 140

Ala Lys Lys Lys Val Glu Glu Ala Glu Lys Lys Ala Lys Asp Gln Lys
145 150 155 160

Glu Glu Asp Arg Arg Asn Tyr Pro Thr Ile Thr Tyr Lys Thr Leu Glu
165 170 175

Leu Glu Ile Ala Glu Ser Asp Val Glu Val Lys Lys Ala Glu Leu Glu
180 185 190

Leu Val Lys Val Lys Ala Asn Glu Pro Arg Asp Glu Gln Lys Ile Lys
195 200 205

Gln Ala Glu Ala Glu Val Glu Ser Lys Gln Ala Glu Ala Thr Arg Leu
210 215 220

Lys Lys Ile Lys Thr Asp Arg Glu Glu Ala Glu Glu Glu Ala Lys Arg
225 230 235 240

Arg Ala Asp Ala Lys Glu Gln Gly Lys Pro Lys Gly Arg Ala Lys Arg
245 250 255

Gly Val Pro Gly Glu Leu Ala Thr Pro Asp Lys Lys Glu Asn Asp Ala
260 265 270

Lys Ser Ser Asp Ser Ser Val Gly Glu Glu Thr Leu Pro Ser Pro Ser
275 280 285

Leu Lys Pro Glu Lys Lys Val Ala Glu Ala Glu Lys Lys Val Glu Glu
290 295 300

Ala Lys Lys Lys Ala Glu Asp Gln Lys Glu Glu Asp Arg Arg Asn Tyr
305 310 315 320

Pro Thr Asn Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Ser Asp

325 330 335

Val Glu Val Lys Lys Ala Glu Leu Glu Leu Val Lys Glu Glu Ala Lys
340 345 350

Glu Pro Arg Asn Glu Glu Lys Val Lys Gln Ala Lys Ala Glu Val Glu
355 360 365

Ser Lys Lys Ala Glu Ala Thr Arg Leu Glu Lys Ile Lys Thr Asp Arg
370 375 380

Lys Lys Ala Glu Glu Glu Ala Lys Arg Lys Ala Ala Glu Glu Asp Lys
385 390 395 400

Val Lys Glu Lys Pro Ala Glu Gln Pro Gln Pro Ala Pro Ala Pro Lys
405 410 415

Ala Glu Lys Pro Ala Pro Ala Pro Lys Pro Glu Asn
420 425

<210> 25

<211> 23

<212> DNA

<213> Streptococcus pneumoniae

<400> 25

ggcggatcca tggaraayga rgg 23

<210> 26

<211> 33

<212> DNA

<213> Streptococcus pneumoniae

<400> 26

gccgtcgact tagtttaccc attcaccatt ggc 33

<210> 27

<211> 5

<212> PRT

<213> Streptococcus pneumoniae

<400> 27

Xaa Glu Asn Glu Gly

1 5

<210> 28

<211> 439

<212> PRT

<213> Streptococcus pneumoniae

<400> 28

Ala Val Ala Ser Leu Phe Met Gly Ser Val Val His Ala Thr Glu Lys

1 5 10 15

Glu Val Thr Thr Gln Val Ala Thr Ser Ser Asn Lys Ala Asn Lys Ser

20 25 30

Gln Thr Glu His Met Lys Ala Ala Lys Gln Val Asp Glu Tyr Ile Lys
35 40 45

Lys Lys Leu Gln Leu Asp Arg Arg Lys His Thr Gln Asn Val Gly Leu
50 55 60

Leu Thr Lys Leu Gly Val Ile Lys Thr Glu Tyr Leu His Gly Leu Ser
65 70 75 80

Val Ser Lys Lys Lys Ser Glu Ala Glu Leu Pro Ser Glu Ile Lys Ala
85 90 95

Lys Leu Asp Ala Ala Phe Glu Gln Phe Lys Lys Asp Thr Leu Pro Thr
100 105 110

Glu Pro Gly Lys Lys Val Ala Glu Ala Glu Lys Lys Val Glu Glu Ala
115 120 125

Lys Lys Lys Ala Glu Asp Gln Lys Glu Lys Asp Leu Arg Asn Tyr Pro
130 135 140

Thr Asn Thr Tyr Lys Thr Leu Glu Leu Asp Ile Ala Glu Ser Asp Val
145 150 155 160

Glu Val Lys Lys Ala Glu Leu Glu Leu Val Lys Glu Glu Ala Lys Glu
165 170 175

Ser Arg Asp Glu Lys Lys Ile Asn Gln Ala Lys Ala Lys Val Glu Asn

180 185 190

Lys Lys Ala Glu Ala Thr Arg Leu Lys Asn Ile Lys Thr Asp Arg Glu
195 200 205

Lys Ala Glu Glu Ala Lys Arg Arg Ala Asp Ala Lys Leu Gln Glu Ala
210 215 220

Asn Val Ala Thr Ser Glu Gln Asp Lys Ser Lys Arg Arg Ala Lys Arg
225 230 235 240

Glu Val Xaa Gly Glu Leu Ala Thr Pro Asp Lys Lys Glu Asn Asp Ala
245 250 255

Lys Ser Ser Asp Ser Ser Val Gly Glu Glu Thr Leu Thr Ser Pro Ser
260 265 270

Leu Lys Pro Glu Lys Lys Val Ala Glu Ala Glu Lys Lys Val Glu Glu
275 280 285

Ala Lys Lys Lys Ala Glu Asp Gln Lys Glu Glu Asp Arg Arg Asn Tyr
290 295 300

Pro Thr Asn Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Ser Asp
305 310 315 320

Val Glu Val Lys Lys Ala Glu Leu Glu Leu Val Lys Glu Glu Ala Lys
325 330 335

Glu Ser Arg Asn Glu Glu Lys Ile Lys Gln Val Lys Ala Lys Val Glu
340 345 350

Ser Lys Lys Ala Glu Ala Thr Arg Leu Glu Asn Ile Lys Thr Asp Arg
355 360 365

Lys Lys Ala Glu Glu Glu Glu Ala Lys Arg Arg Ala Ala Glu Glu Asp
370 375 380

Lys Val Lys Glu Lys Pro Ala Glu Gln Pro Gln Pro Ala Pro Ala Pro
385 390 395 400

Gln Pro Glu Lys Pro Thr Glu Glu Pro Glu Asn Pro Ala Pro Ala Pro
405 410 415

Ala Pro Lys Pro Glu Asn Pro Ala Glu Lys Pro Lys Ala Glu Lys Pro
420 425 430

Ala Asp Gln Gln Ala Glu Glu
435

<210> 29

<211> 437

<212> PRT

<213> Streptococcus pneumoniae

<400> 29

Ala Val Ala Ser Leu Phe Met Gly Ser Val Val His Ala Thr Glu Lys

1 5 10 15

Glu Val Thr Thr Gln Val Ala Thr Ser Ser Asn Arg Ala Asn Lys Ser
20 25 30

Gln Thr Glu His Met Lys Ala Ala Lys Gln Val Asp Glu Tyr Ile Lys
35 40 45

Lys Lys Leu Gln Leu Asp Arg Arg Lys His Thr Gln Asn Val Gly Leu
50 55 60

Leu Thr Lys Leu Gly Val Ile Lys Thr Glu Tyr Leu His Gly Leu Ser
65 70 75 80

Val Ser Lys Lys Lys Ser Glu Ala Glu Leu Pro Ser Glu Ile Lys Ala
85 90 95

Lys Leu Asp Ala Ala Phe Glu Gln Phe Lys Lys Asp Thr Leu Pro Thr
100 105 110

Glu Pro Gly Lys Lys Val Ala Glu Ala Glu Lys Lys Val Glu Glu Ala
115 120 125

Lys Lys Lys Ala Glu Asp Gln Lys Glu Lys Asp Leu Arg Asn Tyr Pro
130 135 140

Thr Asn Thr Tyr Lys Thr Leu Glu Leu Asp Ile Ala Glu Ser Asp Val
145 150 155 160

Glu Val Lys Lys Ala Glu Leu Glu Leu Val Lys Glu Glu Ala Lys Glu
165 170 175

Ser Arg Asp Glu Lys Lys Ile Asn Gln Ala Lys Ala Lys Val Glu Asn
180 185 190

Lys Lys Ala Glu Ala Thr Arg Leu Lys Asn Ile Lys Thr Asp Arg Glu
195 200 205

Lys Ala Glu Glu Ala Lys Arg Arg Ala Asp Ala Lys Leu Gln Glu Ala
210 215 220

Asn Val Ala Thr Ser Glu Gln Asp Lys Ser Lys Arg Arg Ala Lys Arg
225 230 235 240

Glu Val Leu Gly Glu Leu Ala Thr Pro Asp Lys Lys Glu Asn Asp Ala
245 250 255

Lys Ser Ser Asp Ser Ser Val Gly Glu Glu Thr Leu Thr Ser Pro Ser
260 265 270

Leu Lys Pro Glu Lys Lys Val Ala Glu Ala Glu Lys Lys Val Glu Glu
275 280 285

Ala Lys Lys Lys Ala Glu Asp Gln Lys Glu Glu Asp Arg Arg Asn Tyr
290 295 300

Pro Thr Asn Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Ser Asp
305 310 315 320

Val Glu Val Lys Lys Ala Glu Leu Glu Leu Val Lys Glu Glu Ala Lys
325 330 335

Glu Ser Arg Asn Glu Glu Lys Ile Lys Gln Val Lys Ala Lys Val Glu
340 345 350

Ser Lys Lys Ala Glu Ala Thr Arg Leu Glu Asn Ile Lys Thr Asp Arg
355 360 365

Lys Lys Ala Glu Glu Glu Glu Ala Lys Arg Arg Ala Ala Glu Glu Asp
370 375 380

Lys Val Lys Glu Lys Pro Ala Glu Gln Pro Gln Pro Ala Pro Ala Pro
385 390 395 400

Gln Pro Glu Lys Pro Thr Glu Glu Pro Glu Asn Pro Ala Pro Ala Pro
405 410 415

Ala Pro Lys Pro Glu Asn Pro Ala Glu Lys Pro Lys Ala Glu Lys Pro
420 425 430

Ala Asp Gln Gln Ala
435

<210> 30

<211> 439

<212> PRT

<213> Streptococcus pneumoniae

<400> 30

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Val Ala Val Ala Ser Leu Val Met Gly Ser Val Val His Ala Thr Glu
 1               5                  10                  15

Lys Glu Val Thr Thr Gln Val Ala Thr Ser Ser Asn Arg Ala Asn Glu
            20                  25                  30

Ser Gln Ala Gly His Arg Lys Ala Ala Glu Gln Phe Asp Glu Tyr Ile
        35                  40                  45

Lys Thr Met Ile Gln Leu Asp Arg Arg Lys His Thr Gln Asn Phe Ala
    50                  55                  60

Leu Asn Ile Lys Leu Ser Arg Ile Lys Thr Glu Tyr Leu Arg Lys Leu
65                  70                  75                  80

Asn Val Leu Glu Glu Lys Ser Lys Ala Glu Leu Pro Ser Glu Thr Lys
                85                  90                  95

Lys Glu Ile Asp Ala Ala Phe Glu Gln Phe Lys Lys Asp Thr Asn Arg
            100                 105                 110

Thr Lys Lys Thr Val Ala Glu Ala Glu Lys Lys Val Glu Glu Ala Lys
        115                 120                 125

Lys Lys Ala Lys Ala Gln Lys Glu Glu Asp His Arg Asn Tyr Pro Thr
    130                 135                 140
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Asn Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Ser Asp Val Glu
145 150 155 160

Val Lys Lys Ala Glu Leu Glu Leu Val Lys Glu Glu Ala Lys Glu Ser
165 170 175

Arg Asp Asp Glu Lys Ile Lys Gln Ala Glu Ala Lys Val Glu Ser Lys
180 185 190

Lys Ala Glu Ala Thr Arg Leu Glu Asn Ile Lys Thr Asp Arg Glu Lys
195 200 205

Ala Glu Glu Glu Ala Lys Arg Arg Ala Glu Ala Lys Leu Lys Glu Ala
210 215 220

Val Glu Lys Asn Val Ala Thr Ser Glu Gln Asp Lys Pro Lys Gly Arg
225 230 235 240

Arg Lys Arg Gly Val Pro Gly Glu Gln Ala Thr Pro Asp Lys Lys Glu
245 250 255

Asn Asp Ala Lys Ser Ser Asp Ser Ser Val Gly Glu Glu Ala Leu Pro
260 265 270

Ser Pro Ser Leu Lys Pro Glu Lys Lys Val Ala Glu Ala Glu Lys Lys
275 280 285

Val Ala Glu Ala Glu Lys Lys Ala Lys Ala Gln Lys Glu Glu Asp Arg

290 295 300

Arg Asn Tyr Pro Thr Asn Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala
305 310 315 320

Glu Ser Asp Val Lys Val Lys Glu Ala Glu Leu Glu Leu Val Lys Glu
325 330 335

Glu Ala Lys Glu Ser Arg Asn Glu Glu Lys Val Asn Gln Ala Lys Ala
340 345 350

Lys Val Glu Ser Lys Lys Ala Glu Ala Thr Arg Leu Glu Lys Ile Lys
355 360 365

Thr Asp Arg Lys Lys Ala Glu Glu Glu Ala Lys Arg Lys Ala Ala Glu
370 375 380

Glu Asp Lys Val Lys Glu Lys Pro Ala Glu Gln Pro Gln Pro Ala Pro
385 390 395 400

Ala Pro Gln Pro Glu Lys Pro Thr Glu Glu Pro Glu Asn Pro Ala Pro
405 410 415

Ala Pro Lys Pro Glu Lys Pro Ala Glu Gln Pro Lys Ala Glu Lys Thr
420 425 430

Asp Asp Gln Gln Ala Glu Glu
435

<210> 31

<211> 419

<212> PRT

<213> Streptococcus pneumoniae

<400> 31

Ala Val Ala Ser Leu Val Met Gly Ser Val Val His Ala Thr Glu Asn
1 5 10 15

Glu Gly Thr Thr Gln Ala Pro Thr Ser Ser Asn Arg Gly Asn Glu Ser
20 25 30

Gln Ala Glu His Met Lys Ala Ala Lys Gln Val Asp Glu Tyr Ile Glu
35 40 45

Lys Met Leu Gln Leu Asp Arg Arg Lys His Thr Gln Asn Val Gly Leu
50 55 60

Leu Thr Lys Leu Gly Ala Ile Lys Thr Glu Tyr Leu Arg Gly Leu Ser
65 70 75 80

Val Ser Lys Glu Lys Ser Thr Ala Glu Leu Pro Ser Glu Ile Lys Glu
85 90 95

Lys Leu Thr Ala Ala Phe Lys Gln Phe Lys Lys Asp Thr Leu Lys Pro
100 105 110

Glu Lys Lys Val Ala Glu Ala Glu Lys Lys Val Ala Glu Ala Lys Lys

115 120 125

Lys Ala Glu Asp Gln Lys Glu Glu Asp Arg Arg Asn Tyr Pro Thr Ile
130 135 140

Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Ser Asp Val Glu Val
145 150 155 160

Lys Lys Ala Glu Leu Glu Leu Val Lys Val Lys Ala Asn Glu Pro Arg
165 170 175

Asp Glu Glu Lys Ile Lys Gln Ala Glu Ala Glu Val Glu Ser Lys Lys
180 185 190

Ala Glu Ala Thr Arg Leu Lys Lys Ile Lys Thr Asp Arg Glu Lys Ala
195 200 205

Glu Glu Glu Ala Lys Arg Arg Val Asp Ala Lys Glu Gln Asp Glu Ser
210 215 220

Ser Lys Arg Arg Lys Ser Arg Val Lys Arg Gly Asp Val Gly Glu Gln
225 230 235 240

Ala Thr Pro Asp Lys Lys Glu Asn Asp Ala Lys Ser Ser Asp Ser Ser
245 250 255

Val Gly Glu Glu Thr Leu Pro Ser Pro Ser Leu Lys Pro Gly Lys Lys
260 265 270

Val Ala Glu Ala Glu Lys Lys Val Glu Glu Ala Asp Lys Lys Ala Lys
275 280 285

Ala Gln Lys Glu Glu Asp Arg Arg Asn Tyr Pro Thr Asn Thr Tyr Lys
290 295 300

Thr Leu Glu Leu Glu Ile Ala Glu Ser Asp Val Glu Val Lys Lys Ala
305 310 315 320

Glu Leu Glu Leu Val Lys Glu Glu Ala Lys Glu Pro Arg Asn Glu Glu
325 330 335

Lys Val Lys Gln Ala Lys Ala Glu Val Glu Ser Lys Lys Ala Glu Ala
340 345 350

Thr Arg Leu Glu Lys Ile Lys Thr Asp Arg Lys Lys Ala Glu Glu Glu
355 360 365

Ala Lys Arg Lys Ala Ala Glu Glu Asp Lys Val Lys Glu Lys Pro Ala
370 375 380

Glu Gln Pro Lys Pro Ala Pro Ala Pro Gln Pro Glu Lys Pro Ala Pro
385 390 395 400

Lys Pro Glu Asn Pro Ala Glu Gln Pro Lys Ala Glu Lys Pro Ala Asp
405 410 415

Gln Gln Ala

<210> 32

<211> 437

<212> PRT

<213> Streptococcus pneumoniae

<400> 32

Val Ala Ser Leu Phe Met Gly Ser Val Val His Ala Thr Glu Lys Glu
1 5 10 15

Val Thr Thr Gln Val Ala Thr Ser Ser Asn Lys Ala Asn Lys Ser Gln
20 25 30

Thr Glu His Met Lys Ala Ala Lys Gln Val Asp Glu Tyr Ile Lys Lys
35 40 45

Lys Leu Gln Leu Asp Arg Arg Lys His Thr Gln Asn Val Gly Leu Leu
50 55 60

Thr Lys Leu Gly Val Ile Lys Thr Glu Tyr Leu His Gly Leu Ser Val
65 70 75 80

Ser Lys Lys Lys Ser Glu Ala Glu Leu Pro Ser Glu Ile Lys Ala Lys
85 90 95

Leu Asp Ala Ala Phe Glu Gln Phe Lys Lys Asp Thr Leu Pro Thr Glu
100 105 110

Pro Gly Lys Lys Val Ala Glu Ala Glu Lys Lys Val Glu Glu Ala Lys
115 120 125

Lys Lys Ala Glu Asp Gln Lys Glu Lys Asp Leu Arg Asn Tyr Pro Thr
130 135 140

Asn Thr Tyr Lys Thr Leu Glu Leu Asp Ile Ala Glu Ser Asp Val Glu
145 150 155 160

Val Lys Lys Ala Glu Leu Glu Leu Val Lys Glu Glu Ala Lys Glu Ser
165 170 175

Arg Asp Glu Lys Lys Ile Asn Gln Ala Lys Ala Lys Val Glu Asn Lys
180 185 190

Lys Ala Glu Ala Thr Arg Leu Lys Asn Ile Lys Thr Asp Arg Glu Lys
195 200 205

Ala Glu Glu Ala Lys Arg Arg Ala Asp Ala Lys Leu Gln Glu Ala Asn
210 215 220

Val Ala Thr Ser Glu Gln Asp Lys Ser Lys Arg Arg Ala Lys Arg Glu
225 230 235 240

Val Phe Gly Glu Leu Ala Thr Pro Asp Lys Lys Glu Asn Asp Ala Lys
245 250 255

Ser Ser Asp Ser Ser Val Gly Glu Glu Thr Leu Thr Ser Pro Ser Leu
260 265 270

Lys Pro Glu Lys Lys Val Ala Glu Ala Glu Lys Lys Val Glu Glu Ala
275 280 285

Lys Lys Lys Ala Glu Asp Gln Lys Glu Glu Asp Arg Arg Asn Tyr Pro
290 295 300

Thr Asn Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Ser Asp Val
305 310 315 320

Glu Val Lys Lys Ala Glu Leu Glu Leu Val Lys Glu Glu Ala Lys Glu
325 330 335

Ser Arg Asn Glu Glu Lys Ile Lys Gln Val Lys Ala Lys Val Glu Ser
340 345 350

Lys Lys Ala Glu Ala Thr Arg Leu Glu Asn Ile Lys Thr Asp Arg Lys
355 360 365

Lys Ala Glu Glu Glu Glu Ala Lys Arg Arg Ala Ala Glu Glu Asp Lys
370 375 380

Val Lys Glu Lys Pro Ala Glu Gln Pro Gln Pro Ala Pro Ala Pro Gln
385 390 395 400

Pro Glu Lys Pro Thr Glu Glu Pro Glu Asn Pro Ala Pro Ala Pro Ala
405 410 415

Pro Lys Pro Glu Asn Pro Ala Glu Lys Pro Lys Ala Glu Lys Pro Ala

420 425 430

Asp Gln Gln Ala Glu
435

<210> 33
<211> 433
<212> PRT
<213> Streptococcus pneumoniae

<400> 33

Cys Thr Val Ala Ser Leu Val Met Gly Ser Val Val His Ala Thr Glu
1 5 10 15

Asn Glu Arg Thr Thr Gln Val Pro Thr Ser Ser Asn Arg Gly Lys Pro
20 25 30

Glu Arg Arg Lys Ala Ala Glu Gln Phe Asp Glu Tyr Ile Asn Lys Met
35 40 45

Ile Gln Leu Asp Lys Arg Lys His Thr Gln Asn Leu Ala Phe Asn Ile
50 55 60

Gln Leu Ser Arg Ile Lys Thr Glu Tyr Leu Asn Gly Leu Lys Glu Lys
65 70 75 80

Ser Glu Ala Glu Leu Pro Ser Lys Ile Lys Ala Glu Leu Asp Ala Ala
85 90 95

Phe Lys Gln Phe Lys Lys Asp Thr Leu Pro Thr Glu Pro Glu Lys Lys
100 105 110

Val Ala Glu Ala Glu Lys Lys Val Glu Glu Ala Glu Lys Lys Val Ala
115 120 125

Glu Ala Lys Lys Lys Ala Lys Ala Gln Lys Glu Glu Asp His Arg Asn
130 135 140

Tyr Pro Thr Ile Thr Tyr Lys Thr Leu Asp Leu Glu Ile Ala Glu Phe
145 150 155 160

Asp Val Lys Val Lys Glu Ala Glu Leu Glu Leu Val Lys Lys Glu Ala
165 170 175

Asp Glu Ser Arg Asn Glu Gly Thr Ile Asn Gln Ala Lys Ala Lys Val
180 185 190

Glu Ser Glu Lys Ala Glu Ala Thr Arg Leu Lys Lys Ile Lys Thr Asp
195 200 205

Arg Glu Lys Ala Glu Glu Glu Glu Ala Lys Arg Arg Ala Asp Ala Lys
210 215 220

Glu Gln Asp Glu Ser Lys Arg Arg Lys Ser Arg Gly Lys Arg Gly Ala
225 230 235 240

Leu Gly Glu Gln Ala Thr Pro Asp Lys Lys Glu Asn Asp Ala Lys Ser

245 250 255

Ser Asp Ser Ser Val Gly Glu Glu Thr Leu Pro Ser Pro Ser Leu Lys
260 265 270

Pro Gly Lys Lys Val Ala Glu Ala Glu Lys Lys Val Glu Glu Ala Asp
275 280 285

Lys Lys Ala Lys Ala Gln Lys Glu Glu Asp Arg Arg Asn Tyr Pro Thr
290 295 300

Asn Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Ser Asp Val Lys
305 310 315 320

Val Lys Glu Ala Glu Leu Glu Leu Val Lys Glu Glu Ala Lys Glu Ser
325 330 335

Arg Asn Glu Glu Lys Ile Lys Gln Ala Lys Ala Lys Val Glu Ser Lys
340 345 350

Lys Ala Glu Ala Thr Arg Leu Glu Lys Ile Lys Thr Asp Arg Lys Lys
355 360 365

Ala Glu Glu Glu Ala Lys Arg Lys Ala Ala Glu Glu Asp Lys Val Lys
370 375 380

Glu Lys Pro Ala Glu Gln Pro Gln Pro Ala Pro Ala Pro Gln Pro Glu
385 390 395 400

Lys Pro Ala Glu Glu Pro Glu Asn Pro Val Pro Ala Pro Lys Pro Glu
405 410 415

Asn Pro Ala Glu Gln Pro Lys Ala Glu Lys Pro Ala Asp Gln Gln Ala
420 425 430

Glu

<210> 34

<211> 427

<212> PRT

<213> Streptococcus pneumoniae

<400> 34

Val Ala Val Ala Ser Leu Val Met Gly Ser Val Val His Ala Thr Glu
1 5 10 15

Lys Glu Val Thr Thr Gln Val Pro Thr Tyr Ser Asn Met Ala Lys Thr
20 25 30

Glu His Arg Lys Ala Ala Lys Gln Val Val Asp Glu Tyr Ile Glu Lys
35 40 45

Met Leu Arg Glu Ile Gln Leu Asp Arg Arg Lys His Thr Gln Asn Phe
50 55 60

Ala Phe Asn Met Lys Leu Ser Ala Ile Lys Thr Glu Tyr Leu Tyr Gly

65 70 75 80

Leu Lys Glu Lys Ser Glu Ala Glu Leu Pro Ser Glu Val Lys Ala Lys
85 90 95

Leu Asp Ala Ala Phe Glu Gln Phe Lys Lys Asp Thr Leu Lys Leu Gly
100 105 110

Glu Lys Val Ala Glu Ala Glu Lys Lys Val Ala Glu Ala Glu Lys Lys
115 120 125

Ala Lys Ala Gln Lys Glu Glu Asp Arg Arg Asn Tyr Pro Thr Asn Thr
130 135 140

Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Ser Asp Val Glu Val Lys
145 150 155 160

Lys Ala Glu Leu Glu Leu Leu Lys Glu Glu Ala Lys Thr Arg Asn Glu
165 170 175

Asp Thr Ile Asn Gln Ala Lys Ala Lys Val Glu Ser Lys Lys Ala Glu
180 185 190

Ala Thr Lys Leu Glu Glu Ile Lys Thr Asp Arg Lys Lys Ala Glu Glu
195 200 205

Glu Ala Lys Arg Lys Ala Glu Ala Glu Glu Asp Lys Val Lys Asp Lys
210 215 220

Leu Lys Arg Arg Thr Lys Arg Ala Val Pro Gly Glu Pro Ala Thr Pro
225 230 235 240

Asp Lys Lys Glu Asn Asp Ala Lys Ser Ser Asp Ser Ser Val Gly Glu
245 250 255

Glu Thr Leu Pro Ser Pro Ser Leu Lys Ser Gly Lys Lys Val Ala Glu
260 265 270

Ala Glu Lys Lys Val Ala Glu Ala Glu Lys Lys Ala Lys Asp Gln Lys
275 280 285

Glu Glu Asp Arg Arg Asn Tyr Pro Thr Asn Thr Tyr Lys Thr Leu Asp
290 295 300

Leu Glu Ile Ala Glu Ser Asp Val Lys Val Lys Glu Ala Glu Leu Glu
305 310 315 320

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

Gln Ala Lys Ala Glu Val Glu Ser Lys Lys Ala Glu Ala Thr Arg Leu
340 345 350

Glu Lys Ile Lys Thr Asp Arg Lys Lys Ala Glu Glu Glu Ala Lys Arg
355 360 365

Lys Ala Ala Glu Glu Asp Lys Val Lys Glu Lys Pro Ala Glu Gln Pro
370 375 380

Gln Pro Ala Pro Ala Pro Gln Pro Glu Lys Pro Thr Glu Glu Pro Glu
385 390 395 400

Asn Pro Ala Pro Ala Pro Lys Pro Glu Lys Pro Ala Glu Gln Pro Lys
405 410 415

Ala Glu Lys Thr Asp Asp Gln Gln Ala Glu Glu
420 425

<210> 35
<211> 413
<212> PRT
<213> Streptococcus pneumoniae

<400> 35
Glu Asn Glu Gly Ser Thr Gln Ala Ala Thr Ser Ser Asn Met Ala Lys
1 5 10 15

Thr Glu His Arg Lys Ala Ala Lys Gln Val Val Asp Glu Tyr Ile Glu
20 25 30

Lys Met Leu Arg Glu Ile Gln Leu Asp Arg Arg Lys His Thr Gln Asn
35 40 45

Val Ala Leu Asn Ile Lys Leu Ser Ala Ile Lys Thr Lys Tyr Leu Arg
50 55 60

Glu Leu Asn Val Leu Glu Glu Lys Ser Lys Asp Glu Leu Pro Ser Glu
65 70 75 80

Ile Lys Ala Lys Leu Asp Ala Ala Phe Glu Lys Phe Lys Lys Asp Thr
85 90 95

Leu Lys Pro Gly Glu Lys Val Ala Glu Ala Lys Lys Lys Val Glu Glu
100 105 110

Ala Lys Lys Lys Ala Glu Asp Gln Lys Glu Glu Asp Arg Arg Asn Tyr
115 120 125

Pro Thr Asn Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Phe Asp
130 135 140

Val Lys Val Lys Glu Ala Glu Leu Glu Leu Val Lys Glu Glu Ala Lys
145 150 155 160

Glu Ser Arg Asn Glu Gly Thr Ile Lys Gln Ala Lys Glu Lys Val Glu
165 170 175

Ser Lys Lys Ala Glu Ala Thr Arg Leu Glu Asn Ile Lys Thr Asp Arg
180 185 190

Lys Lys Ala Glu Glu Glu Ala Lys Arg Lys Ala Asp Ala Lys Leu Lys
195 200 205

Glu Ala Asn Val Ala Thr Ser Asp Gln Gly Lys Pro Lys Gly Arg Ala
210 215 220

Lys Arg Gly Val Pro Gly Glu Leu Ala Thr Pro Asp Lys Lys Glu Asn
225 230 235 240

Asp Ala Lys Ser Ser Asp Ser Ser Val Gly Glu Glu Thr Leu Pro Ser
245 250 255

Ser Ser Leu Lys Ser Gly Lys Lys Val Ala Glu Ala Glu Lys Lys Val
260 265 270

Glu Glu Ala Glu Lys Lys Ala Lys Asp Gln Lys Glu Glu Asp Arg Arg
275 280 285

Asn Tyr Pro Thr Asn Thr Tyr Lys Thr Leu Asp Leu Glu Ile Ala Glu
290 295 300

Ser Asp Val Lys Val Lys Glu Ala Glu Leu Glu Leu Val Lys Glu Glu
305 310 315 320

Ala Lys Glu Pro Arg Asp Glu Glu Lys Ile Lys Gln Ala Lys Ala Lys
325 330 335

Val Glu Ser Lys Lys Ala Glu Ala Thr Arg Leu Glu Asn Ile Lys Thr
340 345 350

Asp Arg Lys Lys Ala Glu Glu Glu Ala Lys Arg Lys Ala Ala Glu Glu
355 360 365

Asp Lys Val Lys Glu Lys Pro Ala Glu Gln Pro Gln Pro Ala Pro Ala

370 375 380

Thr Gln Pro Glu Lys Pro Ala Pro Lys Pro Glu Lys Pro Ala Glu Gln
385 390 395 400

Pro Lys Ala Glu Lys Thr Asp Asp Gln Gln Ala Glu Glu
405 410

<210> 36

<211> 425

<212> PRT

<213> Streptococcus pneumoniae

<400> 36

Tyr Ile Ala Ser Leu Phe Leu Gly Gly Val Val His Ala Glu Gly Val
1 5 10 15

Arg Ser Glu Asn Asn Pro Thr Val Thr Ser Ser Gly Gln Asp Ile Ser
20 25 30

Lys Lys Tyr Ala Asp Glu Val Lys Ser His Leu Glu Lys Ile Leu Ser
35 40 45

Glu Ile Gln Thr Asn Leu Asp Arg Ser Lys His Ile Lys Thr Val Asn
50 55 60

Leu Ile Asn Lys Leu Gln Asp Ile Lys Arg Thr Tyr Leu Tyr Glu Leu
65 70 75 80

Asn Val Leu Glu Asp Lys Ser Lys Ala Glu Leu Pro Ser Lys Ile Lys
85 90 95

Ala Glu Leu Asp Ala Ala Phe Glu Gln Phe Lys Lys Asp Thr Leu Pro
100 105 110

Thr Glu Pro Gly Lys Lys Val Ala Glu Ala Lys Lys Lys Val Glu Glu
115 120 125

Ala Glu Lys Lys Ala Lys Ala Gln Lys Glu Glu Asp Tyr Arg Asn Tyr
130 135 140

Pro Thr Ile Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Ser Asp
145 150 155 160

Val Lys Val Lys Glu Ala Glu Leu Glu Leu Val Lys Lys Glu Ala Asp
165 170 175

Glu Ser Arg Asn Glu Gly Thr Ile Asn Gln Ala Lys Ala Lys Val Glu
180 185 190

Ser Glu Gln Ala Glu Ala Thr Arg Leu Lys Lys Ile Lys Thr Asp Arg
195 200 205

Glu Lys Ala Glu Glu Glu Ala Lys Arg Arg Ala Asp Ala Lys Glu Gln
210 215 220

Asp Glu Ser Lys Arg Arg Lys Ser Arg Val Lys Arg Gly Asp Phe Gly

225 230 235 240

Glu Pro Ala Thr Pro Asp Lys Lys Glu Asn Asp Ala Lys Ser Ser Asp
245 250 255

Ser Ser Val Gly Glu Glu Thr Leu Pro Ser Pro Ser Leu Lys Pro Gly
260 265 270

Lys Lys Val Ala Glu Ala Glu Lys Lys Val Glu Glu Ala Glu Lys Lys
275 280 285

Ala Lys Asp Gln Lys Glu Glu Asp His Arg Asn Tyr Pro Thr Ile Thr
290 295 300

Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Ser Asp Val Glu Val Lys
305 310 315 320

Lys Ala Glu Leu Glu Leu Val Lys Glu Glu Ala Lys Gly Ser Arg Asn
325 330 335

Glu Glu Lys Val Lys Gln Ala Lys Ala Glu Val Glu Ser Lys Lys Ala
340 345 350

Glu Ala Thr Arg Leu Glu Lys Ile Lys Thr Asp Arg Lys Lys Ala Glu
355 360 365

Glu Glu Ala Lys Arg Lys Ala Ala Glu Glu Asp Lys Val Lys Glu Lys
370 375 380

Pro Ala Glu Gln Pro Gln Pro Ala Pro Ala Pro Gln Pro Glu Lys Pro
385 390 395 400

Ala Pro Ala Pro Lys Pro Glu Asn Pro Ala Glu Gln Pro Lys Ala Glu
405 410 415

Lys Pro Ala Asp Gln Gln Ala Glu Glu
420 425

<210> 37

<211> 439

<212> PRT

<213> Streptococcus pneumoniae

<400> 37

Ala Ser Leu Phe Leu Gly Gly Val Val His Ala Glu Gly Val Arg Ser
1 5 10 15

Gly Asn Asn Ser Thr Val Thr Ser Ser Gly Gln Asp Ile Ser Lys Lys
20 25 30

Tyr Ala Asp Glu Val Glu Ser His Leu Gln Ser Ile Leu Lys Asp Val
35 40 45

Asn Lys Asn Leu Lys Lys Val Gln His Thr Gln Asn Ala Asp Phe Asn
50 55 60

Lys Lys Leu Ser Lys Ile Lys Thr Lys Tyr Leu Tyr Glu Leu Asn Val

65 70 75 80

Leu Glu Glu Lys Ser Glu Ala Glu Leu Thr Ser Lys Thr Lys Glu Thr
85 90 95

Lys Glu Glu Leu Thr Ala Ala Phe Glu Gln Phe Lys Lys Asp Thr Leu
100 105 110

Ser Thr Glu Pro Glu Lys Lys Val Ala Glu Ala Lys Lys Lys Val Glu
115 120 125

Glu Ala Lys Lys Lys Ala Glu Asp Gln Lys Glu Lys Asp Arg Arg Asn
130 135 140

Tyr Pro Thr Ile Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Ser
145 150 155 160

Asp Val Glu Val Lys Lys Ala Glu Leu Glu Leu Val Lys Val Lys Ala
165 170 175

Asn Glu Pro Arg Asp Glu Glu Lys Ile Lys Gln Ala Glu Ala Lys Val
180 185 190

Glu Ser Lys Gln Ala Glu Ala Thr Arg Leu Lys Lys Ile Lys Thr Asp
195 200 205

Arg Glu Gln Ala Glu Ala Thr Arg Leu Glu Asn Ile Lys Thr Asp Arg
210 215 220

Glu Gln Ala Glu Glu Glu Ala Lys Val Lys Asp Glu Pro Lys Lys Arg
225 230 235 240

Thr Lys Arg Gly Val Leu Gly Glu Pro Ala Thr Pro Asp Lys Lys Glu
245 250 255

Asn Asp Ala Lys Ser Ser Asp Ser Ser Val Gly Glu Glu Thr Leu Pro
260 265 270

Ser Pro Ser Leu Lys Pro Glu Lys Lys Val Ala Glu Ala Glu Lys Lys
275 280 285

Val Glu Glu Ala Lys Lys Lys Ala Glu Asp Gln Lys Glu Glu Asp Arg
290 295 300

Arg Asn Tyr Pro Thr Asn Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala
305 310 315 320

Glu Ser Asp Val Glu Val Lys Lys Ala Glu Leu Glu Leu Val Lys Glu
325 330 335

Glu Ala Lys Glu Pro Arg Asn Glu Glu Lys Val Lys Gln Ala Lys Ala
340 345 350

Glu Val Glu Ser Lys Gln Ala Glu Ala Thr Arg Leu Glu Asn Ile Lys
355 360 365

Thr Asp Arg Lys Lys Ala Glu Glu Glu Ala Lys Arg Lys Ala Ala Glu
370 375 380

Glu Asp Lys Val Lys Glu Lys Pro Ala Glu Gln Pro Gln Pro Ala Pro
385 390 395 400

Ala Pro Gln Pro Glu Lys Pro Ala Pro Lys Pro Glu Lys Pro Ala Pro
405 410 415

Ala Pro Lys Pro Glu Asn Pro Ala Glu Gln Pro Lys Ala Glu Lys Pro
420 425 430

Ala Asp Gln Gln Ala Glu Glu
435

<210> 38
<211> 460
<212> PRT
<213> Streptococcus pneumoniae

<400> 38
Cys Ile Val Ala Ser Leu Val Met Gly Ser Val Val His Ala Thr Glu
1 5 10 15

Asn Glu Gly Ala Thr Gln Val Pro Thr Ser Ser Asn Arg Ala Asn Glu
20 25 30

Ser Gln Ala Glu Gln Gly Glu Gln Pro Lys Lys Leu Asp Ser Glu Arg
35 40 45

Asp Lys Ala Arg Lys Glu Val Glu Glu Tyr Val Lys Lys Ile Val Gly
50 55 60

Glu Ser Tyr Ala Lys Ser Thr Lys Lys Arg His Thr Ile Thr Val Ala
65 70 75 80

Leu Val Asn Glu Leu Asn Asn Ile Lys Asn Glu Tyr Leu Asn Lys Ile
85 90 95

Val Glu Ser Thr Ser Glu Ser Gln Leu Gln Ile Leu Met Met Glu Ser
100 105 110

Arg Ser Lys Val Asp Glu Ala Val Ser Lys Phe Glu Lys Asp Ser Ser
115 120 125

Ser Ser Ser Ser Ser Asp Ser Ser Thr Lys Pro Glu Ala Ser Asp Thr
130 135 140

Ala Lys Pro Asn Lys Pro Thr Glu Pro Gly Glu Lys Val Ala Glu Ala
145 150 155 160

Lys Lys Lys Val Glu Glu Ala Glu Lys Lys Ala Lys Asp Gln Lys Glu
165 170 175

Glu Asp Arg Arg Asn Tyr Pro Thr Ile Thr Tyr Lys Thr Leu Glu Leu
180 185 190

Glu Ile Ala Glu Ser Asp Val Glu Val Lys Lys Ala Glu Leu Glu Leu
195 200 205

Val Lys Val Lys Ala Asn Glu Pro Arg Asp Glu Gln Lys Ile Lys Gln
210 215 220

Ala Glu Ala Glu Val Glu Ser Lys Gln Ala Glu Ala Thr Arg Leu Lys
225 230 235 240

Lys Ile Lys Thr Asp Arg Glu Glu Ala Glu Glu Glu Ala Lys Arg Arg
245 250 255

Ala Asp Ala Lys Glu Gln Gly Lys Pro Lys Gly Arg Ala Lys Arg Gly
260 265 270

Val Pro Gly Glu Leu Ala Thr Pro Asp Lys Lys Glu Asn Asp Ala Lys
275 280 285

Ser Ser Asp Ser Ser Val Gly Glu Glu Thr Leu Pro Ser Pro Ser Leu
290 295 300

Lys Pro Glu Lys Lys Val Ala Glu Ala Glu Lys Lys Val Glu Glu Ala
305 310 315 320

Lys Lys Lys Ala Glu Asp Gln Lys Glu Glu Asp Arg Arg Asn Tyr Pro
325 330 335

Thr Asn Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Ser Asp Val
340 345 350

Glu Val Lys Lys Ala Glu Leu Glu Leu Val Lys Glu Glu Ala Lys Glu

355 360 365

Pro Arg Asn Glu Glu Lys Val Lys Gln Ala Lys Ala Glu Val Glu Ser
370 375 380

Lys Lys Ala Glu Ala Thr Arg Leu Glu Lys Ile Lys Thr Asp Arg Lys
385 390 395 400

Lys Ala Glu Glu Glu Ala Lys Arg Lys Ala Ala Glu Glu Asp Lys Val
405 410 415

Lys Glu Lys Pro Ala Glu Gln Pro Gln Pro Ala Pro Ala Pro Lys Ala
420 425 430

Glu Lys Pro Ala Pro Ala Pro Lys Pro Glu Asn Pro Ala Glu Gln Pro
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Lys Ala Glu Lys Pro Ala Asp Gln Gln Ala Glu Glu
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<210> 39

<211> 459

<212> PRT

<213> Streptococcus pneumoniae

<400> 39

Ile Val Ala Ser Leu Val Met Gly Ser Val Val His Ala Thr Glu Asn
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Glu Gly Ala Thr Gln Val Pro Thr Ser Ser Asn Arg Ala Asn Glu Ser
20 25 30

Gln Ala Glu Gln Gly Glu Gln Pro Lys Lys Leu Asp Ser Glu Arg Asp
35 40 45

Lys Ala Arg Lys Glu Val Glu Glu Tyr Val Lys Lys Ile Val Gly Glu
50 55 60

Ser Tyr Ala Lys Ser Thr Lys Lys Arg His Thr Ile Thr Val Ala Leu
65 70 75 80

Val Asn Glu Leu Asn Asn Ile Lys Asn Glu Tyr Leu Asn Lys Ile Val
85 90 95

Glu Ser Thr Ser Glu Ser Gln Leu Gln Ile Leu Met Met Glu Ser Arg
100 105 110

Ser Lys Val Asp Glu Ala Val Ser Lys Phe Glu Lys Asp Ser Ser Ser
115 120 125

Ser Ser Ser Ser Asp Ser Ser Thr Lys Pro Glu Ala Ser Asp Thr Ala
130 135 140

Lys Pro Asn Lys Pro Thr Glu Pro Gly Glu Lys Val Ala Glu Ala Lys
145 150 155 160

Lys Lys Val Glu Glu Val Glu Lys Lys Ala Lys Asp Gln Lys Glu Glu

165 170 175

Asp Arg Arg Asn Tyr Pro Thr Ile Thr Tyr Lys Thr Leu Glu Leu Glu
180 185 190

Ile Ala Glu Ser Asp Val Glu Val Lys Lys Ala Glu Leu Glu Leu Val
195 200 205

Lys Val Lys Ala Asn Glu Pro Arg Asp Lys Gln Lys Ile Lys Gln Ala
210 215 220

Glu Ala Glu Val Glu Ser Lys Gln Ala Glu Ala Thr Arg Leu Lys Lys
225 230 235 240

Ile Lys Thr Asp Arg Glu Glu Ala Glu Glu Glu Ala Lys Arg Arg Ala
245 250 255

Asp Ala Lys Glu Gln Gly Lys Pro Lys Gly Arg Pro Lys Arg Gly Val
260 265 270

Pro Gly Glu Leu Ala Thr Pro Asp Lys Lys Glu Asn Asp Ala Lys Ser
275 280 285

Ser Asp Ser Ser Val Gly Glu Glu Thr Leu Pro Ser Pro Ser Leu Lys
290 295 300

Pro Glu Lys Lys Val Ala Glu Ala Glu Lys Lys Val Glu Glu Ala Lys
305 310 315 320

Lys Lys Ala Glu Asp Gln Lys Glu Glu Asp Arg Arg Asn Tyr Pro Thr
325 330 335

Asn Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Ser Asp Val Glu
340 345 350

Val Lys Lys Ala Glu Leu Glu Leu Val Lys Glu Glu Ala Lys Glu Pro
355 360 365

Arg Asn Glu Glu Lys Val Lys Gln Ala Lys Ala Glu Val Glu Ser Lys
370 375 380

Lys Ala Glu Ala Thr Arg Leu Glu Lys Ile Lys Thr Asp Arg Lys Lys
385 390 395 400

Ala Glu Glu Glu Ala Lys Arg Lys Ala Ala Glu Glu Asp Lys Val Lys
405 410 415

Glu Lys Pro Ala Glu Gln Pro Gln Pro Ala Pro Ala Pro Lys Thr Glu
420 425 430

Lys Pro Ala Pro Ala Pro Lys Pro Glu Asn Pro Ala Glu Gln Pro Lys
435 440 445

Ala Glu Lys Pro Ala Asp Gln Gln Ala Glu Glu
450 455