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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

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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			
<p>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.</p>			

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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

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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

20 *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 25 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 30

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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.

5

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

10 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.

15 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

20 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).

25 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

30 inhibition with a peptide mimicking this domain, may be critical to blocking disease.

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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 5 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.

The discussion of the background to the invention herein is included to explain the context of the invention. This is not to be taken as an admission that any of the material referred to was published, known or part of the common general knowledge in Australia as at the priority date of any of the claims.

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

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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 5 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 10 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.

15 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 20 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.

25

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 30 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

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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 5; 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

10 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.

15 **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.

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

25 **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

30 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

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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.

5 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).

10

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 15 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.

20 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 25 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.

30

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 5 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 10 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:

15 ENEGATQVPTSSNRANESQAEQGEQPKLDSERDKARKEVEYVKKIVGESY
AKSTKKRHTITVALVNELNNIKNEYLNKIVESTSESQLQILMMESRSKVDEAV
SKFEKDSSSSSSSDSSTKPEASDTAKPNKPTEPGEKVAEAKKKVEEAEKKAKD
QKEEDRRNYPTITYKTLELEIAESDVEVKKAELELVVKVANEPRDEQKIKQAE
AEVESKQAEATRLKKIKTDREEAEEEAKRRADAKEQGKPKGRAKRGVPGEL
20 ATPDKKENDAKSSDSSVGEETLPSPSLKPEKKVAEAEKKVEEAKKAEDQKE
EDRRNYPTNTYKTLELEIAESDVEVKKAELELVKKEEAKPRNEEKVKQAKAE
VESKKAEATRLEKIKTDRKKAEEEAKRKAEEEDKVKEKPAEQPQPAPAPKAE
KPAKAPKPE (SEQ ID NO 24).

25 "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 :
ENEGATQVPTSSNRANESQAEQGEQPKLDSERDKARKEVEYVKKIVGESY
AKSTKKRHTITVALVNELNNIKNEYLNKIVESTSESQLQILMMESRSKVDEAV
30 SKFEKDSSSSSSSDSSTKPEASDTAKPNKPTEPGEKVAEAKKKVEEAEKKAKD
QKEEDRRNYPTITYKTLELEIAESDVEVKKAELELVVKVANEPRDEQKIKQAE

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AEVESKQAEATRLKKIKTDREEAEEEAKRRADAKEQGKPKGRAKRGVPGEL
ATPDKKENDAKSSDSSVGEETLPSPSLKPEKKVAEAEKKVEEAKKKAFDQKE
EDRRNYPTNTYKTLLEIAESDVEVKKAELELVKEEAKPRNEEKVKQAKAE
VESKKAEATRLEIKITDRKKAEEEAKRKAAEEDVKVEKPA (SEQ ID NO 1).

5

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

GAGAACGAGGGAGCTACCCAAGTACCCACTTCTCTAATAGGGCAATGAAAGTCAGGCAGAACAGGAGAACAACTTAAAAACTCGATTCAAGAACGA

10 GATAAGGCAAGGAAAGAGGTCGAGGAATATGTAaaaaaaaATAGTGGGT
AGAGCTATGCAAAATCAACTAAAAGCGACATACAATTACTGTAGCTCA
GTTAACGAGTTGAAACAACATTAAGAACGAGTATTGAAATAAAATAGTGA
ATCAACCTCAGAAAGCCAACACTACAGATACTGATGATGGAGAGTCGATCAA
AAGTAGATGAAGCTGTCTAAGTTGAAAAGGACTCATCTTCTCGTCAA
15 GTTCAGACTCTTCCACTAAACCGGAAGCTCAGATAACAGCGAAGCCAAAC
AAGCCGACAGAACCCAGGAGAAAAGGTAGCAGAAGCTAAGAAGAAGGTG
AAGAAGCTGAGAAAAAAAGCCAAGGATCAAAAGAAGAAGATCGTCGAA
CTACCCAACCATTACTTACAAAACGCTTGAACCTGAAATTGCTGAGTCG
ATGTGGAAGTTAAAAAAGCGGAGCTGAACTAGTAAAGTGAAGCTAA
20 CGAACCTCGAGACGAGCAAAAAATTAAAGCAAGCAGAAGCGGAAGTGTGAG
AGTAAACAAAGCTGAGGCTACAAGGTTAAAAAAATCAAGACAGATCGTG
AAGAAGCAGAAGAAGAAGCTAACGAAGAGCAGATGCTAAAGAGCAAG
GTAACCAAAGGGCGGGCAAAACGAGGAGTTCTGGAGAGCTAGCAAC
ACCTGATAAAAAGAAAATGATGCGAAGTCTCAGATTCTAGCGTAGGTG
25 AAGAAAACCTTCCAAGCCCACCCCTGAAACCAGAAAAAAAGGTAGCAGA
AGCTGAGAAGAAGGTTGAAAGAAGCTAAGAAAAAAAGCCGAGGATCAAAA
GAAGAAGATGCCGTAACTACCCAAACCAACTTACAAAACGCTTGAAC
TGAAATTGCTGAGTCCGATGTGAAAGTTAAAAAAGCGGAGCTGAACTAG
TAAAAGAGGAAGCTAAGGAACCTCGAAACGAGGAAAAAGTTAAGCAAGC
30 AAAAGCGGAAGTTGAGATAAAAAAGCTGAGGCTACAAAGGTTAGAAAAA

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ATCAAGACAGATCGTAAAAAGCAGAAGAAGAAGCTAACGAAAAGCAG
CAGAAGAAGATAAAGTTAAGAAAAACCGAGCTG (SEQ ID NO 12).

Amino acid sequence of CbpA of serotype 4:

5 ENEGATQVPTSSNRANESQAEQGEQPKKLDSERDKARKEVEEYVKKIVGESY
AKSTKKRHTITVALVNELNNIKNEYLNKIVESTSESQQLQILMMESRSKVDEAV
SKFEKDSSSSSSDSSTKPEASDTAKPNKPTEPGEKVAEAKKKVEEAEKKAKD
QKEEDRRNYPTITYKTLELEIAESDVEVKKAELELVVKVANEPRDEQKIKQAE
AEVESKQAEATRLKKIKTDREEAEEEAKRRADAKEQGKPKGRAKRGVPHEL
10 ATPDKKENDAKSSDSSVGEETLPSPSLKPEKKVAAEAKKVEEAKKKAEDQKE
EDRRNYPTNTYKTLELEIAESDVEVKKAELELVKEEAKPRNEEKVKQAKAE
VESKKAETRLEKIKTDRKAAEEEAKRKAEEEDKVKEKPAEQPQPAPAPKAE
KPAPAPKPNPAPAEQPKAEKPADQQAEEDYARRSEEEYNRLTQQQPKTEKPA
QPSTPKTGWKQENGWYFYNTDGSMATGWLQNNGSWYYLNSNGAMATG
15 WLQNNNGSWYYLNANGSMATGWLQNNNGSWYYLNANGSMATGWLQYNGS
WYYLNANGSMATGWLQYNGSWYYLNANGDMATGWLQYNGS
GAMKASQWFKVSDKWYYNGSGALAVNTTVDGYGVNANGEWVN. (SEQ ID
NO 2)

20 DNA sequence encoding the amino acid sequence of the CbpA of serotype 4:
GAGAACGAGGGAGCTACCCAAGTACCCACTTCTTCTAATAGGGCAAATGA
AAGTCAGGCAGAACAGGAGAACACCTAAAAACTCGATTAGAACGAA
GATAAGGCAAGGAAAGAGGTCGAGGAATATGAAAAAAAAATAGTGGGTG
AGAGCTATGCAAAATCAACTAAAAAGCGACATACAATTACTGTAGCTCA
25 GTTAACGAGTTGAACAACATTAAGAACGGAGTATTGAATAAAATAGTTGA
ATCAACCTCAGAAAGCCAACACTACAGATACTGATGATGGAGAGTCGATCAA
AAGTAGATGAAGCTGTCTAAGTTGAAAAGGACTCATCTTCTCGTCAA
GTTCAGACTCTTCCACTAAACCGGAAGCTTCAGATACAGCGAAGCCAAAC
AAGCCGACAGAACCAACAGGAGAAAAGTAGCAGAACGTAAGAAGAAGGTG
30 AAGAAGCTGAGAAAAAAAGCCAAGGATCAAAAGAAGAAGATCGTCGTAAC
CTACCCAACCATTACTTACAAACGCTGAACTGAAATTGCTGAGTCCG

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ATGTGGAAGTTAAAAAGCGGAGCTGAACTAGTAAAAGTGAAGCTAA
CGAACCTCGAGACGAGCAAAAAATTAAAGCAAGCAGAAGCGGAAGTTGAG
AGTAAACAAGCTGAGGCTACAAGGTTAAAAAAATCAAGACAGATCGTG
AAGAAGCAGAAGAAGAAGCTAACGAAGAGCAGATGCTAAAGAGCAAG
5 GTAAACCAAAGGGCGGGCAAAACGAGGAGTTCTGGAGAGCTAGCAAC
ACCTGATAAAAAAGAAAATGATGCGAAGTCTCAGATTCTAGCGTAGGTG
AAGAAACTCTCCAAGCCCATCCCTGAAACCAGAAAAAAAGGTAGCAGA
AGCTGAGAAGAAGGTTGAAGAAGCTAACGAAAGGCCGAGGATCAAAAA
GAAGAAGATGCCGTAACTACCCACCAACTTACAAAACGCTTGAAC
10 TGAAATTGCTGAGTCCGATGTGGAAGTTAAAAAGCGGAGGCTTGAAC
GTAAAAGAGGAAGCTAACGAAACCTCGAAACGAGGAAAAAGTTAAGCAAG
CAAAAGCGGAAGTTGAGAGTAAAAAGCTGAGGCTACAAGGTTAGAAAA
AATCAAGACAGATCGTAAAAAGCAGAAGAAGCTAACGAAAGCA
GCAGAAGAAGATAAAAGTTAAAGAAAACCAGCTGAACAAACCAAC
15 CGCCGGCTCCAAAAGCAGAAAAACAGCTCCAGCTCCAAAACCAGAGAA
TCCAGCTGAACAACCAAAAGCAGAAAAACAGCTGATCAACAAGCTGAA
GAAGACTATGCTCGTAGATCAGAAGAAGATAATCGCTTGA
ACTCAAC
GCAACCGCCAAAACGCTAACGCAACCATCTACTCCAAAACA
GGCTGGAAACAAGAAAACGGTATGTGGTACTTCTACA
AACTGATGGTTC
20 AATGGCGACAGGTGGCTCCAAAACAAAGGCTCATGGTAC
TACCTCAACAG
CAATGGCGCTATGGCGACAGGATGGCTCCAAAACAATGGTTCATGGTACT
ATCTAAACGCTAATGGTCAATGGCAACAGGATGGCTCCAAAACAATGGT
TCATGGTACTACCTAACGCTAATGGTCAATGGCGACAGGATGGCTCCA
ATACAATGGCTCATGGTACTACCTAACGCTAATGGTCAATGGCGACAG
25 GATGGCTCCAATACAATGGCTCATGGTACTACCTAACGCTAATGGT
GAT
ATGGCGACAGGTGGTGAAGAGATGGAGATACCTGGTACTATCTTGAAGC
ATCAGGTGCTATGAAAGCAAGCCAATGGTCAAAGTATCAGATAAATGGT
ACTATGTCAATGGCTCAGGTGCCCTGCAGTCAACACAACTGTAGATGGC
TATGGAGTCAATGCCAATGGTGAATGGTAAACTAA (SEQ ID NO 13).

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"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 :

ENEGATQVPTSSNRANESQAEGEQPKKLDSERDKARKEVEEYVKKIVGESY
AKSTKKRHTITVALVNELNNIKNEYLNKIVESTSESQQLQILMMESRSKVDEAV
SKFEKDSSSSSDSSTKPEASDTAKPNKPTEPGEKVAEAKKKVEEAEKKAKD
QKEEDRRNYPTITYKTLELEIAESDVEVKKAELELVKVKANEPRDEQKIKQAE
AEVESKQAEATRLKKIKTDREEAEEEAKRRADAKEQGKPKGRAKRGVPGEL
ATPDKKENDAKSSDSSVGEETL (SEQ ID NO 3).

10

The DNA sequence which encodes polypeptide R1 is:

GAGAACCGAGGGAGCTACCCAAGTACCCACTTCTCTAATAGGGCAATGA
AAGTCAGGCAGAACAGGAGAACACCTAAAAACTCGATTAGAACGAG
GATAAGGCAGGAAAGAGGTCAGGAATATGTAAAAAAATAGTGGGTG
AGAGCTATGCAAAATCAACTAAAAGCGACATACAATTACTGTAGCTCTA
GTTAACGAGTTAACACATTAAGAACGAGTATTGAAATAAAATAGTGTG
ATCAACCTCAGAAAGCCAACACTACAGATACTGATGATGGAGAGTCGATCAA
AAGTAGATGAAGCTGTCTAAGTTGAAAGGACTCATCTCTCGTCAA
GTTCAAGACTCTCCACTAAACCGGAAGCTCAGATACAGCGAAGCCAAAC
AAGCCGACAGAACCAAGGAGAAAGGTAGCAGAACGTAAGAAGAAGTTG
AAGAACGCTGAGAAAAAGCCAAGGATCAAAAAGAACGAGATCGTCGAA
CTACCCAACCATTACTTACAAAACGCTTGAACCTGAAATTGCTGAGTCCG
ATGTGGAAGTAAAAAGCGGAGCTGAACTAGTAAAGTGAAGCTAA
CGAACCTCGAGACGAGCAAAAATTAGCAAGCAGAACGCGGAAGTTGAG
AGTAAACAAGCTGAGGCTACAAGGTTAAAAAAATCAAGACAGATCGTG
AAGAACGAGAACGAGAACGCTAAACGAAGAGCAGATGCTAAAGAGCAAG
GTAAACCAAAGGGGCGGGCAAAACGAGGAGTCTCTGGAGAGCTAGCAAC
ACCTGATAAAAAGAAAATGATGCGAAGTCTTCAGATTCTAGCGTAGGTG
AAGAAACTCTTC (SEQ ID NO 14).

30

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"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:

5 KPEKKVAEAEKKVEEAKKKAEDQKEEDRRNYPNTYKTLLEIAESDVEVK
KAELELVKEEAKEPRNEEKVKQAKAEVESKKAATRLEKIKTDRKKAAEEEAK
RKA (SEQ ID NO 4).

The DNA sequence of polypeptide C/R2

10 AAACCAGAAAAAAAGGTAGCAGAAGCTGAGAAGAAGGTTGAAGAAGCTA
AGAAAAAAAGCCGAGGATCAAAAAGAAGAAGATGCCGTAACTACCCAAC
CAATACTTACAAAACGCTGAACTGAAATTGCTGAGTCCGATGTGGAAG
TTAAAAAAAGCGGAGCTTGAACTAGTAAAAGAGGAAGCTAAGGAACCTCG
AAACGAGGAAAAAGTTAACAGCAAGCAAAAGCGGAAGTTGAGAGTAAAAAA
15 GCTGAGGCTACAAAGGTTAGAAAAAAATCAAGACAGATCGTAAAAAAGCAG
AAGAAGAAGCTAACGAAAAGCA (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 20 269 of the N-terminal of choline binding protein A (CbpA) serotype type 4 which has the following sequence:

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

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

ACAGAACCAAGGAGAAAAGGTAGCAGAAGCTAACAGAAGAAGGTTGAAGAA
GCTGAGAAAAAAAGCCAAGGATCAAAAAGAAGAAGATCGCTGTAACTACC
30 CAACCATTACTTACAAAACGCTTGAACCTGAAATTGCTGAGTCCGATGTG
GAAGTTAAAAAAGCGGAGCTTGAACTAGTAAAAGCTAACGAAC

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CTCGAGACGAGCAAAAATTAAGCAAGCAGAAGCGGAAGTTGAGAGTAA
ACAAGCTGAGGCTACAAGGTTAAAAAAATCAAGACAGATCGTGAAGAA
GCAGAAGAAGAAGCTAACGAAGAGCAGATGCT (SEQ ID NO 16).

5 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

10 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

15 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

20 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

25 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

30 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

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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 5 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 10 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 15 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:
ENEGSTQAATSSNMAKTEHRKAAKQVVDEYIEKMLREIQLDRRKHTQNAL
NIKLSAIKTKYLRELNVLEEKSKDELPSEIKAKLDAAFEKFKDTLKPGKVA
EAKKKVEEAKKKAEDQKEEDRRNYPTNTYKTLELEIAEFDVKVKEAELELVK
20 EEAKEYSNEGTTIKQAKEKVESKKAETRLENKTDKKAAEEEAKRKADAKLK
EANVATSDQGKPKGRAKRGVPGELETDPDKENDAKSSDSSVGEETLPSSSLK
SGKKVAAEAKKVEEAEKKAKDQKEEDRRNYPTNTYKTLDLEIAESDVVKVE
AELELVKEEAKEPRDEEKIKQAKAKVESKKAETRLENKTDKKAAEEEAKR
KAAEEDKVKEKPA (SEQ ID NO 7)

25

The DNA sequence which encodes polypeptide R2 serotype R6x:

GAAAACGAAGGAAGTACCCAAGCAGCCACTTCTTCTAATATGGCAAAGAC
AGAACATAGAAAAGCTGCTAACACAAGTCGTCGATGAATATATAGAAAAA
ATGTTGAGGGAGATTCAACTAGATAGAAGAAAACATACCCAAAATGTCGC
30 CTTAAACATAAAGTTGAGCGCAATTAAAACGAAGTATTTGCGTGAATTAA
ATGTTTAGAAGAGAAGATCGAAAGATGAGTTGCCGTAGAAATAAAAGCA

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AAGTTAGACGAGCTTTGAGAAGTTAAAAAGATACATTGAAACCAGG
AGAAAAGGTAGCAGAAGCTAAGAAGAAGGTTGAAGAAGCTAAGAAAAAA
GCCGAGGATCAAAAGAAGAAGATCGCTAACTACCCAACCAATACTTA
CAAAACGCTTGAACCTGAAATTGCTGAGTCGATGTGAAAGTTAAAGAAG
5 CGGAGCTGAACTAGTAAAAGAGGAAGCTAAAGAA~~t~~CTCGAAACGAGGGC
ACAATTAAGCAAGCAAAGAGAAAGTTGAGAGTAAAAAGCTGAGGCTA
CAAGGTTAGAAAAC~~t~~CAAGACAGA~~t~~CGTAAAAAGCAGAAGAAGCT
AAACGAAAAGCAGATGCTAAGTTGAAGGAAGCTAATGTAGCGACTTCAG
~~t~~CAAGGTAAACCAAGGGCGGGCAAAACGAGGAGTTCTGGAGAGCTA
10 GCAACACCTGATAAAAAGAAAATGATGCGAAGTCTCAGATTCTAGCGT
AGGTGAAGAAACTCTCCAAGCTCATCCCTGAAATCAGGAAAAAGGTAG
CAGAAGCTGAGAAGAAGGTTGAAGAAGCTGAGGAAAAAGCCAAGGATCA
AAAAGAAGAAGATGCCGTAACTACCCAACCAATACTTACAAACGCTTG
ACCTGAAATTGCTGAGTCGATGTGAAAGTTAAAGAAGCGGAGCTTGAA
15 CTAGTAAAAGAGGAAGCTAAGGAACCTCGAGACGAGGAAAAAATTAAGC
AAGCAAAGCGAAAGTTGAGAGTAAAAAGCTGAGGCTACAAGGTTAGA
AAACATCAAGACAGATCGTAAAAAGCAGAAGAAGCTAAACGAAAA
GCAGCAGAAGAAGATAAAGTTAAAGAAAAACCAGCTG (SEQ ID NO 17)

20 Amino acid sequence of CbpA of serotype R6x:
ENEGSTQAATSSNMAKTEHRKAQKVDEYIEKMLREIQLDRRKHTQVAL
NIKLSAIKTKYLRELNVLEEKSKDELPSEIKAKLDAAFEFKKDTLKPGEKVA
EAKKKVEAKKKAEDQKEEDRRNYPTNTYKTLLEIAEFDVKVKEAELELVK
EEAKESRNEGTIKQAKEKVESKKAETRLENIKTDRKKAEEEAKRKADAKLK
25 EANVATSDQGKPKGRAKRGVPGELETSDKKENDAKSSDSSVGEETLPSSLK
SGKKVAEAEKVVEAEKKAKDQKEEDRRNYPTNTYKTLDEIAESDVKVKE
AELELVKEEAKPRDEEKIKQAKAKVESKKAETRLENIKTDRKKAEEEAKR
KAAEEDKVKEKPAEQPQPATQPEKPKPAEOPKAETDDQQAEEDY
ARRSEEEYNRLTQQQPKTEKPAQPSTPKTGWKQENGMWYFYNTDGSMAT
30 GWLQNNGSWYYLNANGAMATGWLQNNGSWYYLNANGSMATGWLQNNG
SWYYLNANGAMATGWLQYNGSWYYLNSNGAMATGWLQYNGSWYYLNA

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NGDMATGWLQNNGSWYYLNANGDMATGWLQYNGSWYYLNANGDMATG
WVKDGDTWYYLEASGAMKASQWFKVSDKWYYVNGSGALAVNTTVDGYG
VNANGEWVN (SEQ ID NO 8).

5 DNA sequence encoding the amino acid sequence of the CbpA of serotype R6x:
GAAAACGAAGGAAGTACCCAAGCAGCCACTTCTCTAATATGGCAAAGAC
AGAACATAGGAAAGCTGCTAAACAAGTCGTCGATGAATATATAGAAAAAA
ATGTTGAGGGAGATTCAACTAGATAGAAGAAAACATACCCAAATGTCGC
CTTAAACATAAAGTTGAGCGCAATTAAAACGAAGTATTGCGTGAATTAA
10 ATGTTTAGAAGAGAAGTCGAAAGATGAGTTGCCGTAGAAATAAAAGCA
AAGTTAGACGCAGCTTGAGAAGTTAAAAAGATACATTGAAACCAGG
AGAAAAGGTAGCAGAAGCTAAGAAGAAGGTTGAAGAAGCTAAGAAAAAA
GCCGAGGATCAAAAAGAAGAAGATGTCGTAACTACCCAACCAATCTTA
CAAAACGCTTGAACCTGAAATTGCTGAGTCGATGTGAAAGTTAAAGAAG
15 CGGAGCTGAACTAGTAAAAGAGGAAGCTAAAGAACTCGAAACGAGGGC
ACAATTAAGCAAGCAAAAGAGAAAGTTGAGAGTAAAAAGCTGAGGCTA
CAAGGTTAGAAAACAACAGACAGATCGTAAAGGAAAGCTAATGTAGCGACT
AAACGAAAAGCAGATGCTAAGTTGAAGGAAAGCTAATGTAGCGACT
tCAAGGTAAACCAAGGGGGGGCAAAACGAGGAGTTCTGGAGAGCTAG
20 CAACACCTGATAAAAAAGAAAATGATGCGAAGTCTTCAGATTCTAGCGTA
GGTGAAGAAACTCTCCAAGCTCATCCCTGAAATCAGGAAAAAGGTAGC
AGAAGCTGAGAAGAAGGTTGAAGAAGCTGAGAAAAAGCCAAGGATCAA
AAAGAAGAAGATGCCGTAACTACCCAACCAATACTTACAAAACGCTTGA
CCTTGAAATTGCTGAGTCGATGTGAAAGTTAAAGAAGCGGAGCTTGAAC
25 TAGAAAAGAGGAAGCTAAGGAACCTCGAGACGAGGAAAAAATTAAGCA
AGCAAAAGCGAAAGTTGAGAGTAAAAAGCTGAGGCTACAAGGTTAGAA
AACATCAAGACAGATCGTAAAAGCAGAAGAAGAAGCTAAACGAAAAG
CAGCAGAAGAAGATAAAGTTAAAGAAAAACAGCTGAACAAACCACACC
AGCGCCGGCTACTCAACCGAAAAACAGCTCCAAAACAGAGAAGGCCA
30 GCTGAACAACCAAAAGCAGAAAAACAGATGATCAACAAGCTGAAGAAG
ACTATGCTCGTAGATCAGAAGAAGATATAATCGCTGACTCAACAGCAA

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CCGCCAAAAACTGAAAAACCAGCACACCCTACTCCAAAACAGGCT
GGAAACAAGAAAACGGTATGGTACTTCTACAATACTGATGGTCAATG
GCAACAGGATGGCTAAAACAACGGTCATGGTACTATCTAAACGCTAA
TGGTCTATGGCAGAGGATGGCTAAAACAATGGTCAATGGTACTATC
5 TAAACGCTAATGGTCAATGGCAACAGGATGGCTAAAACAATGGTCA
TGGTACTACCTAAACGCTAATGGTCTATGGCAGAGGATGGCTCAATA
CAATGGTCAATGGTACTACCTAAACAGCAATGGCCTATGGCAGAGGAT
GGCTCAATACAATGGCTATGGTACTACCTCAACGCTAATGGTATATG
GCGACAGGATGGCTAAAACAACGGTCATGGTACTACCTCAACGCTAA
10 TGGTGTATGGCAGAGGATGGCTCAATACAACGGTCATGGTATTACC
TCAACGCTAATGGTGTATGGCAGAGGTTGGTCAAAGATGGAGATACC
TGGTACTATCTGAAGCATCAGGTCTATGAAAGCAAGCCAATGGTCAA
AGTATCAGATAAAATGGTACTATGTCATGGCTCAGGTGCCCTGCAGTCA
ACACAACGTAGATGGCTATGGAGTCATGCCAATGGTGAATGGTAAAC
15 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 :

20 ENEGSTQAATSSNMAKTEHRKAQKVDEYIEKMLREIQLDRRKHTQNV
NIKLSAIKTKYLRELNVLEEKSKDELPSEIKAKLDAAFEKFKDTLPGEKVA
EAKKKVEEAKKAEDQKEEDRRNYPTNTYKTLLEIAEFDVKVKEAEELVK
EEAKESRNEGTIKQAKEKVESKKAETRLENIKTDRKKAEEAKRKADAKLK
EANVATSDQGKPKGRAKRGVPGEATPDKKENDAKSSDSSVGEETL (SEQ ID
25 NO 9).

The DNA sequence which encodes polypeptide R1 is:

30 GAAAACGAAGGAAGTACCCAAGCAGCCACTTCTTCTAATATGGCAAAGAC
AGAACATAGGAAAGCTGCTAACAAAGTCGTCGATGAATATATAGAAAAA
ATGTTGAGGGAGATTCAACTAGATAGAAGAAAACATACCCAAAATGTCGC
CTTAAACATAAAGTTGAGCGCAATTAAAACGAAGTATTGCGTGAATTAA

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ATGTTTACAAGAGAAGTCGAAAGATGAGTTGCCGTAGAAATAAAAGCA
AAGTTAGACGCAGCTTTGAGAAGTTAAAAAGATACATTGAAACCAGG
AGAAAAGGTAGCAGAAGCTAAGAAGAAGGTTGAAGAAGCTAAGAAAAAA
GCGAGGATCAAAAAGAAGAAGATCGCTAACTACCCAACCAACTTA
5 CAAACGCTTGAACCTGAAATTGCTGAGTCGATGTGAAAGTTAAAGAAG
CGGAGCTTGAACTAGTAAAGAGGAAGCTAAAGAATCTCGAACCGAGGG
CACAATTAAGCAAGCAAAAGAGAAAGTTGAGAGTAAAAAGCTGAGGCT
ACAAGGTTAGAAAACtCAAGACAGATCGAAAAAGCAGAAGAAGAAG
CTAAACGAAAAGCAGATGCTAAGTTGAAGGAAGCTAATGTAGCGACTTCA
10 GATCAAGGTAACCAAAGGGCGGGCAAACGAGGAGTTCTGGAGAGC
TAGCAACACCTGATAAAAAGAAAATGATGCGAAGTCTTCAGATTCTAGC
GTAGGTGAAGAAACTCTTC (SEQ ID NO 19).

"Polypeptide C/R2 serotype R6x" means an polypeptide comprising a repeat region C
15 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:
KSGKKVAEAEKKVEEAEKKAKDQKEEDRRNYPTNTYKTLDEIAESDVVKV
EAELELVKEEAKEPRDEEKIKQAKAKVESKKAETRLENIKTDRKKAEAEAK
20 RKA (SEQ ID NO 10)

The DNA sequence of polypeptide C/R2 serotype R6x:

AAATCAGGAAAAAAAGGTAGCAGAAGCTGAGAAGAAGGTTGAAGAAGCTG
AGAAAAAAAGCCAAGGATCAAAAAGAAGAAGATGCCGTAACTACCCAAC
25 CAATACTTACAAAACGCTTGCACCTTGAATTGCTGAGTCGATGTGAAAG
TTAAAGAAGCGGAGCTGAACTAGTAAAGAGGAAGCTAAGGAACCTCG
AGACGAGGAAAAAAATTAAAGCAAGCAAAAGCGAAAGTTGAGAGTAAAAAA
GCTGAGGCTACAAGGTTAGAAAACATCAAGACAGATCGAAAAAGCAG
AAGAAGAAGCTAACGAAAAGCA (SEQ ID NO 20).

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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:

5 PGEKVAEAKKKVEEAKKKAEDQKEEDRRNYPNTNTYKTLELEIAEFDVKVKE
AELEVKEEAKESRNEGTIKQAKEKVESKKAEATRLENIKTDRKKAEAAA
KADA (SEQ ID NO 11)

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

10 CCAGGAGAAAAGGTAGCAGAAGCTAAGAAGAAGGTTGAAGAAGCTAAGA
AAAAAGCCGAGGATCAAAAGAAGAAGATCGTCGTAACCTACCCACCAA
TACTTACAAAACGCTTGAACCTGAAATTGCTGAGTTGATGTGAAAGTTA
AAGAACGGAGCTTGAACACTAGTAAAAGAGGAAGCTAAAGAACTCGAAC
GAGGGCACAATTAGCAAGCAAAGAGAAAGTTGAGAGTAAAAAGCTG
15 AGGCTACAAGGTTAGAAAACtCAAGACAGATCGTAAAAAAGCAGAAGA
AGAAGCTAACCGAAAAGCAGATGCT (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
20 fragments, mutants, variants, or analogs, or derivatives, thereof.
SPSLKPEKKVAEAEKKVEEAKKKAEDQKEEDRRNYPNTNTYKTLELEIAESDV
EVKKAEELVKEEAKEPNNEEKVKQAKAEVESKKAEATRLEKIKTDRKKAE
EAKRKAEEEDKVKEKPA (SEQ ID NO 22; serotype 4; position 323-434); or
PSSSLKSGKKVAEAEKKVEEAKKAKDQKEEDRRNYPNTNTYKTLDEIAESD
25 VKVKEAELELVKEEAKEPDEEKIKQAKAKVESKKAEATRLENIKTDRKKAE
EEAKRKAEEEDKVKEKRA (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
30 (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

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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.

5 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 15 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 20 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 25 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.

30 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

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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 5 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 10 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 15 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.

20 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 25 afforded by the conjugation of PEG is that of effectively reducing the immunogenicity 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 30 immune response against the compound or against cells which may produce the

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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. NH₂ 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^a-amino protected N^a-t-butyloxycarbonyl) amino acid resin with the

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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 Carmino and Han (1972, J. Org. Chem. 37:3403-3409). Thus, polypeptide
5 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, C^α-methyl amino acids, and N^α-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
10 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 CO₂H or CONH₂ side chain to simulate a free
15 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.

20 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
25 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
30 particular N-terminal group is present in 50% of the peptides, would readily be

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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., $R_1\text{-CH}_2\text{-NH-}R_2$, 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, cyclize 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); α -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

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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
5 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
10 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
15 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 -
(CH₂)_nCH₃ 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-
20 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
25 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
30 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

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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

5 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, 10 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.

15 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 NH₂ can be maintained.

20

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.

25

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"

30 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-

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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, 5 "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

10 N-terminal pyroglutamate, 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*.
15 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.
20 *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.
25 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.

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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

5 (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propylene glycol homopolymers, propylene oxide/ethylene oxide co- polymers, polyoxyethylated polyols and polyvinyl alcohol. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water.

10

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 15 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).

20 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 polyethyleneglycols). The proportion of polymer molecules to component or components molecules will vary, 25 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.

30

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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

5 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
10 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. Sulfhydryl 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.

15

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
20 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.

25

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

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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 5 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 10 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 15 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 20 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 25 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. 30

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

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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.

5

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 E1, 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 2 μ 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, R1.1, B-W and L-M cells, African Green Monkey kidney

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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 5 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 10 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 15 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 20 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 25 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 30 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.

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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 5 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 10 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., 15 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 20 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- 25 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 30 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.*

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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.

5 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,

10 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

15 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.

20 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

25 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

30 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.

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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
5 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,
10 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
15 may be selected from ^3H , ^{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
20 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, β -glucuronidase, β -D-glucosidase, β -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
25 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
30 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,

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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 5 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
- 10 (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 15 antibody. Also, agents which competitively block or inhibit pneumococcal bacterium are contemplated 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.

20 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 25 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 30 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,

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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
5 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
10 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, 15 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).

20 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, 25 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 30 infection with a Gram positive bacterium, preferably streptococcal, and more preferably pneumococcal, 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

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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

5 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.

10

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

15 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.

20 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.

25 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.

30 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.

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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.

5 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
10 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
15 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
20 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.,
25 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
30 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,

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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 5 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.

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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 15 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 20 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.

25 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 30 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

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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.

- 5 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
- 10 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 be 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.

- 30 *Dosages.* The sufficient amount may include but is not limited to from about 1 μ g/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.

5

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, pulmonarily, 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

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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

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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,

5 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

10 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

15 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

30 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

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Intravenous Formulation I

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

10 Intravenous Formulation II

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

Intravenous Formulation III

	<u>Ingredient</u>	<u>mg/ml</u>
20	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

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Intravenous Formulation IV

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

Intravenous Formulation V

<u>Ingredient</u>	<u>mg/ml</u>
Polypeptide antagonist	5.0
sodium bisulfite USP	3.2
5 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 10 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 15 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) 20 (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) (α 1-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) 25 (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.

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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

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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
5 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.

- 10 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.
- 15 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,
- 20 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
25 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
30 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

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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 5 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 10 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 15 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 20 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 25 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 30 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

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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 5 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 10 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 15 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 20 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 25 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.

30 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.

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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 jejunum, 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

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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.

5 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
10 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
15 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
20 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
25 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
30 diluents could include carbohydrates, especially mannitol, α -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

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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.

25

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.

30 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 benzethonium

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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 5 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.

10

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 15 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 20 systemic effect is described in U.S. Patent No. 5,451,569, issued September 19, 1995 to 25 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 30 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

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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
5 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
10 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.

15 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
20 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.

25 *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.

30 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)**

5 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
10 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 Sall restriction site for cloning purposes, and the natural stop codon from CbpA, and is based on both type 4 and R6x sequence.

15 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 Sall restriction enzymes and cloned into the pQE30 expression vector (Qiagen, Inc.) digested
20 with BamHI, XbaI, and SmaI restriction enzymes.

Polypeptide R2:

25 The naturally occurring 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
30 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
5 region of CbpA, namely the A region of polypeptide R1. Here the naturally occurring
XmnI site between the two amino repeats (nucleic acid 856 of the Type 4 sequence) was
utilized. cbpA full length clone PM1580 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
10 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
15 specific antibodies.

Purification of R1 and R2 :

To induce production of and purify recombinant proteins from *E. coli* a single colony was
20 selected from plated bacteria containing the recombinant plasmid and grown overnight
at 37° in 6.0 mls LB buffer with 50 μ g/ml kanamycin and 100 μ g/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
25 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 NaH₂PO₄, 10mM Tris, 6M GuC1,
300mM NaCl, pH 8.0 (Buffer A). This mixture was rotated at room temperature for 30
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

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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 NaH₂PO₄, 8M Urea, 20mM MES, pH6.0 (Buffer B) as the final target buffer was run 5 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 10 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).

15

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 20 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 25 (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).

30 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

-60-

washing, fluorescein 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
5 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,
10 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
15 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
20 lectin activity or that they are both lectin with slightly different specificities (\pm sialic
acid).

Table 1
Inhibition of Binding of R6 pneumococci to purified glycoconjugate
by soluble forms of CbpA

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

N=3 experiments LNnt each 3 wells

N=2 experiments SiL each 3 wells

15

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

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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.

5

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

15 **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

20 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.

25

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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)
10 Preimmune Antibody	198 (64); 88 (4)	100%
Antibody to Truncate R2	56 (11); 9 (2)	28%; 10%

5 $5 \mu\text{l}$ of rabbit antibody undiluted + $5 \mu\text{l}$ 2×10^7 R6x Preincubate, 6 at RT x 30 min, then add to LNnT coated wells for adherence assay. Two independent experiments are shown.

15 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 ($5 \mu\text{l}$) plus $5 \mu\text{l}$ of 2×10^7 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 20 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 preimmune versus anti-CbpA R2 antibody for inhibition of binding of pneumococci R6x to the model receptor LNnT. Greater than 25 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

-64-

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.

5 **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 10 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 15 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) 20 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.

25 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 1×10^8 CbpA defective - bacteria for 1 hour at R1. The other choline binding proteins on this mutant absorb out anti-CBD antibodies which are 30 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

-65-

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

5 **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%)	
10 Anti CbpA antiserum absorbed to remove CBD antibodies	175 ± 38 (31%)	

15 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.

20 Pre-incubation of pneumococci 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.

25

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

-66-

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:

5 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 10 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 15 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.

20 **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 25 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 30 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

-67-

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 5 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 10 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**

15 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.

20 Rats were treated intranasally with 10 μ l of PBS containing 0.8 μ g of polypeptide R2 or R1 or no protein. 15 min later Type 3 pneumococci (Strain SIII) (10 μ l containing 1 x 10^5 cfu) 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 25 of 4 animals per group. Rats receiving SIII alone displayed 2200, 6500, 6900 and 8700 (mean 6075) colonies per 10 μ l. Animals treated with truncate R2 showed the greatest decrease (3600, 3500, 2500, 2100) to mean 2925 bacteria 10 μ l (48% of control). Animals treated with truncate R1 also showed decreased colonization (5000, 4800, 3500, 1600) to mean 3725 (61% of control).

30

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

5 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

10 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.

15 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

20 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

25 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.

Throughout the description and claims of the specification the word "comprise" and variations of the word, such as "comprising" and "comprises", is not intended to exclude other additives, components, integers or steps.

EDITORIAL NOTE

APPLICATION NUMBER - 34797/99

**The following Sequence Listing pages 1 to 75 are part of the
description. The claims pages follow on pages 69 to 82.**

- 1 -

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

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<140> 09/056,019

<141> 1998-04-07

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<211> 284
<212> PRT
<213> *Streptococcus pneumoniae*

<400> 3
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

- 10 -

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 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

- 11 -

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

275

280

<210> 4

<211> 106

<212> PRT

<213> *Streptococcus pneumoniae*

<400> 4

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

1

5

10

15

Lys Lys Lys Ala Glu Asp Gln Lys Glu Glu Asp Arg Arg Asn Tyr Pro

20

25

30

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

65

70

75

80

Lys Lys Ala Glu Ala Thr Arg Leu Glu Lys Ile Lys Thr Asp Arg Lys

- 12 -

85

90

95

Lys Ala Glu Glu Glu Ala Lys Arg Lys Ala

100

105

<210> 5

<211> 109

<212> PRT

<213> Streptococcus pneumoniae

<400> 5

Thr Glu Pro Gly Glu Lys Val Ala Glu Ala Lys Lys Lys Val Glu Glu

1

5

10

15

Ala Glu Lys Lys Ala Lys Asp Gln Lys Glu Glu Asp Arg Arg Asn Tyr

20

25

30

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

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

65

70

75

80

Ser Lys Gln Ala Glu Ala Thr Arg Leu Lys Lys Ile Lys Thr Asp Arg

85

90

95

- 13 -

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

<210> 6

<211> 4

<212> PRT

<213> Streptococcus pneumoniae

<400> 6

Lys Xaa Xaa Glu

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<210> 7

<211> 376

<212> PRT

<213> Streptococcus pneumoniae

<400> 7

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

- 14 -

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 Ala Lys Arg Lys Ala Asp Ala Lys Leu Lys

- 15 -

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

- 16 -

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

370

375

<210> 8

<211> 663

<212> PRT

<213> Streptococcus pneumoniae

<400> 8

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

- 17 -

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 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

- 18 -

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 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

- 20 -

545 550 555 560

Gly Asp Met Ala Thr Gly Trp Leu Gln Asn Asn Gly Ser Trp Tyr Tyr
565 570 575Leu Asn Ala Asn Gly Asp Met Ala Thr Gly Trp Leu Gln Tyr Asn Gly
580 585 590Ser Trp Tyr Tyr Leu Asn Ala Asn Gly Asp Met Ala Thr Gly Trp Val
595 600 605Lys Asp Gly Asp Thr Trp Tyr Tyr Leu Glu Ala Ser Gly Ala Met Lys
610 615 620Ala Ser Gln Trp Phe Lys Val Ser Asp Lys Trp Tyr Val Asn Gly
625 630 635 640Ser Gly Ala Leu Ala Val Asn Thr Thr Val Asp Gly Tyr Gly Val Asn
645 650 655Ala Asn Gly Glu Trp Val Asn
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<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 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

- 22 -

145 150 155 160

Glu Ser Arg Asn Glu Gly Thr Ile Lys Gln Ala Lys Glu Lys Val Glu
165 170 175Ser Lys Lys Ala Glu Ala Thr Arg Leu Glu Asn Ile Lys Thr Asp Arg
180 185 190Lys Lys Ala Glu Glu Ala Lys Arg Lys Ala Asp Ala Lys Leu Lys
195 200 205Glu Ala Asn Val Ala Thr Ser Asp Gln Gly Lys Pro Lys Gly Arg Ala
210 215 220Lys Arg Gly Val Pro Gly Glu Leu Ala Thr Pro Asp Lys Lys Glu Asn
225 230 235 240Asp 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

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105

<210> 11

<211> 107

<212> PRT

<213> *Streptococcus pneumoniae*

<400> 11

Pro Gly Glu Lys Val Ala Glu Ala Lys Lys Val Glu Glu Ala Lys

1

5

10

15

- 24 -

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

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90

95

Ala Glu Glu Glu Ala Lys Arg Lys Ala Asp Ala

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105

<210> 12

<211> 1219

<212> DNA

<213> *Streptococcus pneumoniae*

<400> 12

gagaacgagg gagctaccca agtacccact tcttctaata gggcaaatga aagtcaggca 60

gaacaaggag aacaaccta aaaaactcgat tcagaacgag ataaggcaag gaaagaggtc 120

gaggaatatg taaaaaaaaat agtgggtgag agctatgcaa aatcaactaa aaagcgacat 180

acaattactg tagctctagt taacgagttt aacaacattha agaacgagta tttgaataaa 240

atagttgaat caacctcaga aagccaaacta cagatactga tgatggagag tcgatcaaaa 300
gtagatgaag ctgtgtctaa gtttggaaag gactcatctt cttcgtaag ttcagactct 360
tccactaaac cggaaagcttc agatacagcg aagccaaaca agccgacaga accaggagaa 420
aaggtagcg aagctaaagaa gaagggtgaa gaagctgaga aaaaagccaa ggatcaaaaa 480
gaagaagatc gtcgttaacta cccaaaccatt acttacaaaa cgcttgaact tgaaattgct 540
gagtccgatg tggaaagttaa aaaagcggag cttgaactag taaaatgtaa agctaacgaa 600
cctcgagacg agcaaaaaat taagcaagca gaagcggag ttgagagtaa acaagctgag 660
gctacaaggat taaaaaaaaat caagacagat cgtgaagaag cagaagaaga agctaaacga 720
agagcagatg ctaaagagca aggttaaacc aaggggcggg caaaacgagg agttcctgga 780
gagctagcaa cacctgataa aaaagaaaaat gatgcgaagt cttcagatcc tagcgttagt 840
gaagaaactc ttccaagccc atccctgaaa ccagaaaaaa aggttagcaga agctgagaag 900
aaggttgaag aagctaaagaa aaaagccgag gatcaaaaaag aagaagatcg ccgtaaactac 960
ccaaccaata cttacaaaaac gcttgaacctt gaaattgctg agtccgatgt ggaagttaaa 1020
aaagcggagc ttgaaactagt aaaagaggaa gctaaggaac ctcgaaacga gggaaaagtt 1080
aagcaagcaa aagcggaaatg tgagagtaaa aaagctgagg ctacaaggatc agaaaaaatac 1140
aagacagatc gtaaaaaaaaa agaagaagaa gctaaacgaa aagcagcaga agaagataaa 1200
gtttaaagaaa aaccagctg 1219

<210> 13

<211> 1969

<212> DNA

<213> *Streptococcus pneumoniae*

<400> 13

gagaacgagg gagctaccca agtacccact tcttctaata gggcaaataa aagtcaggca 60
gaacaaggag aacaacctaa aaaactcgat tcagaacgag ataaggcaag gaaagaggtc 120
gaggaatatg taaaaaaaaat agtgggtgag agctatgcaaa aatcaactaa aaagcgacat 180
acaattactg tagctctatg taacgagttt aacaacatta agaacgagta tttgaataaa 240
atagttgaat caacctcaga aagccaaacta cagatactga tgatggagag tcgatcaaaa 300

- 26 -

gtagatgaag ctgtgtctaa gtttggaaaag gactcatttt cttcgtaaag ttccagactct 360
tccactaaac cggaaagcttc agatacagcg aagccaaaca agccgacaga accaggagaa 420
aaggttagcag aagctaagaa gaagggtgaa gaagctgaga aaaaagccaa ggtcaaaaa 480
gaagaagatc gtcgttaacta cccaaaccatt acttacaaaaa cgcttgaact tggaaattgct 540
gagtccgatg tggaaagttaa aaaagcggag cttgaactag taaaagtgaa agctaacgaa 600
cctcgagacg agcaaaaaat taagcaagca gaagcggaaag ttgagagatg acaagctgag 660
gctacaaggtaaaaaaaat caagacagat cgtgaagaag cagaagaaga agctaaacga 720
agagcagatg ctaaagagca aggttaaacca aaggggcggg caaaacgagg agttcctgga 780
gagctgcaaa cacctgataaa aaaagaaaaat gatgcgaatg cttcgatcc tagcgttaggt 840
gaagaaaactc ttccaagccc atccctgaaa ccagaaaaaa aggttagcaga agctgagaag 900
aagggttgaag aagctaagaa aaaagccgag gatcaaaaaa aagaagatcg ccgtaaactac 960
ccaaaccaata cttacaaaaac gcttgaactt gaaattgtcg agtccgatgt ggaagttaaa 1020
aaagcggagg cttgaactag taaaagagga agctaaggaa cctcgaaacg agaaaaaaagt 1080
taagcaagca aaagcggaaag ttgagagatg aaaaagctgag gctacaaggtaaaaaat 1140
caagacagat cgtaaaaaaag cagaagaaga agctaaacga aaagcagcag aagaagataa 1200
agtttaaagaa aaaccagctg aacaaccaca accagcggcc gctccaaaaag cagaaaaacc 1260
agctccagct cccaaacccag agaattccagc tgaacaacca aaagcagaaaa aaccagctga 1320
tcaacaagct gaagaagact atgctcgtag atcagaagaa gaataataatc gcttgcacta 1380
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ccaaaaacaat ggctcatggt actacctcaa cagcaatggc gctatggcga caggatggct 1560
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gaaagatgga gataccttgt actatcttga agcatcagggt gctatgaaag caagccatg 1860
gttcaaagta tcagataaat ggtactatgt caatggctca ggtgccttg cagtcaacac 1920
aactgttagat ggctatggag tcaatgccaa tggtaatgg gtaaaactaa 1969

- 27 -

<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
gagggatatg taaaaaaaaat agtgggtgag agtctatgca aatcaactaa aaagcgacat 180
acaattactg tagctctagt taacgagttg aacaacatta agaacgagta tttgaataaa 240
atagttgaat caacctcaga aagccaaacta cagatactga tcatggagag tcgatcaaaa 300
gttagatgaag ctgtgtctaa gtttggaaag gactcatctt ctgcgtcaag ttccagactct 360
tccactaaac cggaagcttc agatacagcg aagccaaaca agccgacaga accaggagaa 420
aaggtagcag aagctaagaa gaaggttgaa gaagctgaga aaaaagccaa ggatcaaaaa 480
gaagaagatc gtcgttaacta cccaaaccatt acttacaaaaa cgcttgaact tggaaattgct 540
gagtccgatg tggaaagttaa aaaagcgagg cttgaacttag taaaagtgaa agctaacgaa 600
cctcgagacg agcaaaaaat taagcaagca gaagcgaaag ttgagagtaa acaagctgag 660
gctacaaggat taaaaaaaaat caagcagat cgtgaagaag cagaagaaga agctaaacgaa 720
agagcagatg ctaaagagca aggttaaacc aaggggcggg caaaacgagg agttcctgga 780
gagctagcaa cacctgataa aaaagaaaaat gatgcgaagt ctgcgtatcc tagcgttaggt 840
gaagaaaactc ttc

853

<210> 15

<211> 318

<212> DNA

<213> *Streptococcus pneumoniae*

<400> 15

aaaccagaaaa aaaaggttagc agaagctgag aagaaggttg aagaagctaa gaaaaaagcc 60

- 28 -

gaggatcaa aagaagaaga tcgcgtaac tacccaacc aatacttacaa aacgcttgaa 120
cttggaaattt ctgagtcgcg tttggaaagt aaaaaagcg agcttgaact agtaaaagag 180
gaagcttcaagg aacctcgaaa cgaggaaaaa gttttagcag caaaaaggcg agttgagagt 240
aaaaaaagctg aggctacaag gtttagaaaaa atcaagacag atcgtaaaaa agcagaagaa 300
gaagctttaac gaaaagca 318

3.18

<210> 16

<211> 327

<212> DNA

<213> *Streptococcus pneumoniae*

<400> 16

acagaaccag gagaaaagggt agcagaagct aagaagaagg ttgaagaagc tgagaaaaaa 60
gc当地
gaacttgaaa ttgctgagtc cgatgtggaa gttaaaaaaag cggagcttga actagtaaaa 180
gtgaaaagcta acgaacctcg agacgagcaa aaaattaagc aagcagaagc ggaagtttag 240
agtaaacaag ctgaggctac aaggtaaaaaaaatcaaga cagatgtga agaagcagaa 300
gaagaagcta aacgaagagc agatgct 327

327

<210> 17

<211> 1129

<212> DNA

<213> *Streptococcus pneumoniae*

<400> 17

aaaaaccaag gaagtaccca agcagccact tcttctaata tggcaaagac agaacatagg 60
aaagctgcta aacaagtgcgt cgatgaatat atagaaaaaa tgttgaggga gattcaacta 120
gatagaagaa aacataaccca aaatgtgcgc ttaaacataa agttgagcgc aattaaaaacg 180
aagtatttgc gtgaattaaa tggtttagaa gagaagtcga aagatgtactt gccgtcagaa 240

- 29 -

ataaaaagcaa agtttagacgc agctttttag aagtttaaaa aagatacatt gaaaccagga 300
gaaaaggttag cagaagctaa gaagaagggtt gaagaagcta agaaaaaagc cgaggatcaa 360
aaagaagaag atcgtcgtaa ctacccaacc aataacttaca aaacgcttga acttgaaatt 420
gctgagttcg atgtgaaagt taaagaagcg gagcttgaac tagttaaaaga ggaagctaaa 480
gaatctcgaa acgagggcac aattaagcaa gcaaagaga aagttgagag taaaaaagct 540
gaggctacaa gtttagaaaa catcaagaca gatcgtaaaa aagcagaaga agaagctaaa 600
cgaaaaagcag atgctaagtt gaaggaagct aatgttagcga cttcagatca aggttaacca 660
aaggggcgaaa caaaacgagg agttccttgg aagcttagaa cacctgataa aaaagaaaaat 720
gatgcgaagt cttcagatc tagcgtaggt gaagaaactc ttccaaagctc atccctgaaa 780
tcaggaaaaa aggttagcaga agctgagaag aaggttgaag aagctgagaa aaaagccaag 840
gatcaaaaaa aagaagatcg ccgttaactac ccaaccaata cttacaaaac gcttgacctt 900
gaaattgctg agtccgatgt gaaagttaaa gaagcggagc ttgaactagt aaaagaggaa 960
gctaaggaac ctgcgagacga ggaaaaaatt aagcaagcaa aagcgaagt tgagagtaaa 1020
aaagctgagg ctacaagggtt agaaaacatc aagacagatc gtaaaaaagc agaagaagaa 1080
gcttaaacgaa aagcagcaga agaagataaa gttaaagaaa aaccagctg 1129

<210> 18

<211> 1992

<212> DNA

<213> *Streptococcus pneumoniae*

<400> 18

gaaaacgaag gaagtaccca agcagccact tcttctaata tggcaaagac agaacatagg 60
aaagctgcta aacaagtctt cgtatgaatat atagaaaaaa tggagggtt gattcaacta 120
gatagaagaa aacataccca aatgtcgcc ttaaacatcaa agttgagcgc aataaaaacg 180
aagtatttgc gtgaattaaa tggggtagaa gagaagtctt aagatgagtt ggcgtcagaa 240
ataaaaagcaa agtttagacgc agctttttag aagtttaaaa aagatacatt gaaaccagga 300
gaaaaggttag cagaagctaa gaagaagggtt gaagaagcta agaaaaaagc cgaggatcaa 360
aaagaagaag atcgtcgtaa ctacccaacc aataacttaca aaacgcttga acttgaaatt 420

- 30 -

gctgagttcg atgtgaaagt taaagaagcg gagcttgaac tagtaaaaga ggaagctaaa 480
gaatctcgaa acgagggcac aattaagcaa gcaaaagaga aagttgagag taaaaaagct 540
gaggctacaa ggttagaaaa catcaagaca gatcgtaaaa aagcagaaga agaagctaaa 600
cgaaaagcag atgctaagt gaaggaagct aatgttagcga cttcagatca aggttaaacca 660
aaggggcggg caaaacgagg agttcctgga gagctagcaa cacctgataa aaaagaaaaat 720
gatgcgaagt cttcagatcc tagcttaggt gaagaaactc ttccaagctc atccctgaaa 780
tcaggaaaaa aggttagcaga agctgagaag aaggttgaag aagctgagaa aaaagccaag 840
gatcaaaaag aagaagatcg ccgttaactac ccaaccaata cttacaaaac gcttgaccc 900
gaaattgctg agtcccgatgt gaaagttaaa gaagcggagc ttgactagt aaaagagggaa 960
gctaaggaac ctcgagacga ggaaaaatt aagcaagcaa aagcgaaagt tgagagtaaa 1020
aaagctgagg ctacaagggtt agaaaacatc aagacagatc gtaaaaaagc agaagaagaa 1080
gctaaacgaa aagcagcaga agaagataa gttaaagaaa aaccagctga acaaccacaa 1140
ccagcgccgg ctactcaacc agaaaaacca gctccaaaac cagagaagcc agctgaacca 1200
ccaaaacgag aaaaaacaga tcatcaacaa gctgaagaag actatgctg tagatcagaa 1260
gaagaatata atcgcttgac tcaacagcaa ccgcacaaaaa ctgaaaaacc agcacaacca 1320
tctactccaa aaacaggctg gaaacaagaa aacggtatgt ggtacttcta caatactgtat 1380
ggtcaatgg caacaggatg gctccaaaac aacggttcat ggtactatct aaacgctaat 1440
ggtgctatgg cgacaggatg gctccaaaac aatggttcat ggtactatct aaacgctaat 1500
ggtcaatgg caacaggatg gctccaaaac aatggttcat ggtactatct aaacgctaat 1560
ggtgctatgg cgacaggatg gctccaaatac aatggttcat ggtactatct aaacagcaat 1620
ggcgctatgg cgacaggatg gctccaaatac aatggctcat ggtactatct caacgctaat 1680
ggtgatatgg cgacaggatg gctccaaaac aacggttcat ggtactatct caacgctaat 1740
ggtgatatgg cgacaggatg gctccaaatac aacggttcat ggtattatct caacgctaat 1800
ggtgatatgg cgacaggatg ggtgaaagat ggagatactt ggtactatct tgaagcatca 1860
ggtgctatga aagcaagcca atggttcaa gtatcagata aatggtacta tgtcaatggc 1920
tcaggtgccc ttgcagtcaa cacaactgta gatggctatg gagtcaatgc caatggtaa 1980
tgggtaaaact aa

1992

<210> 19

<211> 763

<212> DNA

<213> *Streptococcus pneumoniae*

<400> 19

aaaaacgaaag gaagtaccca agcagccact tcttctaata tggcaaagac agaacatagg 60
aaagctgcta aacaagtgcgt cgatgaatat atagaaaaaa tggtgaggga gattcaacta 120
gatagaagaa aacataccca aaatgtgcgc ttaaacataa agttgagcgc aattaaaacg 180
aagtatggc gtgaattaaa tggttttagaa gagaagtcga aagatgagtt gccgtcagaa 240
ataaaagcaa agttagacgc agctttttag aagttaaaaa aagatacatt gaaaccaggaa 300
aaaaaggtag cagaagctaa gaagaaggaa gaagaagcta agaaaaaagc cgaggatcaa 360
aaagaagaag atcgtcgtaa ctacccaacc aataacttaca aaacgcttga acttggaaatt 420
gctgagttcg atgtgaaagt taaagaagcg gagcttgaac tagttaaaaga ggaagctaa 480
gaatctcgaa acgggggcac aatthaagcaa gcaaaagaga aagttgagag taaaaaagct 540
gaggctacaa ggttagaaaa catcaagaca gatcgaaaaa aagcagaaga agaagctaa 600
cgaaaagcag atgctaagtt gaaggaaagct aatgttagcga cttagatca aggttaaacca 660
aaggggcggg caaaaacgagg agttctggg gagcttagaa cacctgataa aaaagaaaaat 720
gatgcgaagt cttagatcc tagcgtaggt gaagaaaactc ttc 763

763

<210> 20

<211> 318

<212> DNA

<213> *Streptococcus pneumoniae*

<400> 20

aaatcaggaa aaaaggtacg agaagctgag aagaaggttg aagaagctga gaaaaaaagcc 60
aaggatcaaa aagaagaaga tcgccgtac tacccaaacca atacttacaa aacgcttgac 120
cttggaaattt ctgagttccga tgtgaaagtt aaagaagcgg agcttgaact agtaaaaagag 180
gaagctaaagg aacctcgaga cgaggaaaaaa attaagcaag caaaagcgaa agttggagat 240

- 32 -

aaaaaaagctg aggctacaag gtttagaaaaac atcaagacag atcgtaaaaa agcagaagaa 300
gaagctaaac gaaaagca 318

<210> 21
<211> 321
<212> DNA
<213> *Streptococcus pneumoniae*

<400> 21
ccaggagaaa aggttagcaga agctaagaag aaggttgaag aagctaagaa aaaagccgag 60
gatcaaaaag aagaagatcg tcgttaactac ccaaccaata cttacaaaaac gcttgaactt 120
gaaattgctg agttcgatgt gaaagttaa gaagcggagc ttgaactagt aaaagaggaa 180
gctaaagaat ctcgaaacga gggcacaatt aagcaagcaa aagagaaaagt 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 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

- 33 -

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

- 35 -

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 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 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

- 37 -

325 330 335

Val Glu Val 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 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

- 38 -

<210> 26

<211> 33

<212> DNA

<213> *Streptococcus pneumoniae*

<400> 26

gcccgtcgact 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 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 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

- 40 -

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

- 41 -

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 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

- 42 -

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 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

- 43 -

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 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

- 45 -

<213> Streptococcus pneumoniae

<400> 30

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

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 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

- 47 -

290 295 300

Arg Asn Tyr Pro Thr Asn Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala
305 310 315 320Glu Ser Asp Val Lys Val Lys Glu Ala Glu Leu Glu Leu Val Lys Glu
325 330 335Glu Ala Lys Glu Ser Arg Asn Glu Glu Lys Val Asn Gln Ala Lys Ala
340 345 350Lys Val Glu Ser Lys Lys Ala Glu Ala Thr Arg Leu Glu Lys Ile Lys
355 360 365Thr Asp Arg Lys Lys Ala Glu Glu Ala Lys Arg Lys Ala Ala Glu
370 375 380Glu Asp Lys Val Lys Glu Lys Pro Ala Glu Gln Pro Gln Pro Ala Pro
385 390 395 400Ala Pro Gln Pro Glu Lys Pro Thr Glu Glu Pro Glu Asn Pro Ala Pro
405 410 415Ala Pro Lys Pro Glu Lys Pro Ala Glu Gln Pro Lys Ala Glu Lys Thr
420 425 430Asp 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 140Thr Tyr Lys Thr Leu Glu Leu Glu Ile Ala Glu Ser Asp Val Glu Val
145 150 155 160Lys Lys Ala Glu Leu Glu Leu Val Lys Val Lys Ala Asn Glu Pro Arg
165 170 175Asp Glu Glu Lys Ile Lys Gln Ala Glu Ala Glu Val Glu Ser Lys Lys
180 185 190Ala Glu Ala Thr Arg Leu Lys Lys Ile Lys Thr Asp Arg Glu Lys Ala
195 200 205Glu Glu Glu Ala Lys Arg Arg Val Asp Ala Lys Glu Gln Asp Glu Ser
210 215 220Ser Lys Arg Arg Lys Ser Arg Val Lys Arg Gly Asp Val Gly Glu Gln
225 230 235 240Ala Thr Pro Asp Lys Glu Asn Asp Ala Lys Ser Ser Asp Ser Ser
245 250 255Val 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 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

- 52 -

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 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 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

- 54 -

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 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 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 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

- 56 -

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

- 57 -

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

- 58 -

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	

- 59 -

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

- 60 -

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
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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

- 61 -

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 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 Ala Glu Ala Thr Arg Leu Glu Asn Ile Lys Thr

340 345 350

Asp Arg Lys Lys Ala 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

- 63 -

370 375 380

Thr Gln Pro Glu Lys Pro Ala Pro Lys Pro Glu Lys Pro Ala Glu Gln
385 390 395 400Pro 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 15Arg Ser Glu Asn Asn Pro Thr Val Thr Ser Ser Gly Gln Asp Ile Ser
20 25 30Lys Lys Tyr Ala Asp Glu Val Lys Ser His Leu Glu Lys Ile Leu Ser
35 40 45Glu Ile Gln Thr Asn Leu Asp Arg Ser Lys His Ile Lys Thr Val Asn
50 55 60Leu 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

- 65 -

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

- 66 -

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 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

- 67 -

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

- 68 -

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 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 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> 450

<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

- 70 -

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 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 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

- 72 -

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

435 440 445

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

450 455 460

<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

1 5 10 15

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 Ala 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

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

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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

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

435

440

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Ala Glu Lys Pro Ala Asp Gln Gln Ala Glu Glu

450

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THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. An isolated polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 1, 3, or 24, wherein said polypeptide does not bind to choline.

5

2. The isolated polypeptide of claim 1, wherein said amino acid sequence comprises up to 475 amino acids.

3. The isolated polypeptide of claim 1, wherein said amino acid sequence

10 comprises up to 460 amino acids.

4. An isolated polypeptide comprising a variant of an amino acid sequence set forth in SEQ ID NO: 1, 3, or 24, wherein said amino acid sequence comprises at least one to 57 amino acid substitutions of SEQ ID NO: 1, 3, or 24,

15 does not bind to choline and is immunogenic.

5. An isolated polypeptide comprising an analog or a derivative of an amino acid sequence set forth in SEQ ID NO: 1, 3, or 24, wherein said polypeptide does not bind to choline, and is immunogenic.

20

6. An isolated polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 5, wherein said polypeptide does not bind to choline.

25

7. The isolated polypeptide of claim 6, wherein said amino acid sequence comprises up to 475 amino acids.

8. The isolated polypeptide of claim 6, wherein said amino acid sequence comprises up to 460 amino acids.

30

9. An isolated polypeptide comprising a variant of an amino acid sequence set forth in SEQ ID NO: 5, wherein said variant comprises at least one to 57 amino acid substitutions of SEQ ID NO: 5, comprises up to 398 amino acids, does not bind to choline, and is immunogenic.

10. An isolated polypeptide comprising an analog or a derivative of the amino acid sequence set forth in SEQ ID NO: 5, wherein said polypeptide does not bind to choline and is immunogenic.

5 11. An isolated polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 4 or 22, wherein said polypeptide does not bind to choline.

12. The isolated polypeptide of claim 11, wherein said amino acid sequence comprises up to 475 amino acids.

10

13. The isolated polypeptide of claim 11, wherein said amino acid sequence comprises up to 460 amino acids.

15

14. An isolated polypeptide comprising a variant of an amino acid sequence set forth in SEQ ID NO: 4 or 22, wherein said variant comprises at least one to 57 amino acid substitutions of SEQ ID NO: 4 or 22, comprises up to 398 amino acids, does not bind to choline, and is immunogenic.

20

15. An isolated polypeptide comprising an analog or derivative of an amino acid sequence set forth in SEQ ID NO: 4 or 22, wherein said polypeptide does not bind to choline and is immunogenic.

25

16. The isolated polypeptide of claim 1, 2, 3, 4, or 5, wherein said polypeptide interacts with an antibody, wherein said antibody is capable of interacting with a full-length CpbA polypeptide.

25

17. The isolated polypeptide of claim 6, 7, 8, 9, or 10, wherein said polypeptide interacts with an antibody, wherein said antibody is capable of interacting with a full-length CpbA polypeptide.

30

18. The isolated polypeptide of claim 11, 12, 13, 14, or 15, wherein said polypeptide interacts with an antibody, wherein said antibody is capable of interacting with a full-length CpbA polypeptide.

19. An isolated polypeptide comprising a fragment of SEQ ID NO: 24, wherein said fragment comprises at least 138 consecutive amino acids of SEQ ID NO: 24, wherein said polypeptide does not bind to choline.

5 20. An isolated polypeptide consisting of an amino acid sequence set forth in SEQ ID NO: 1, 3, 4, 5, 22, or 24.

21. An isolated polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 7 or 9, wherein said polypeptide does not bind to choline, and
10 comprises up to 376 amino acids.

22. An isolated polypeptide comprising a variant of an amino acid sequence set forth in SEQ ID NO: 7 or 9, wherein said variant comprises at least one to 57 amino acid substitutions of SEQ ID NO: 7 or 9, does not bind to choline and is
15 immunogenic.

23. An isolated polypeptide comprising an analog or a derivative of an amino acid sequence set forth in SEQ ID NO: 7 or 9, wherein said polypeptide does not bind to choline, comprises up to 376 amino acids, and is immunogenic.

20
24. An isolated polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 10, 11, or 23, wherein said polypeptide does not bind to choline, and comprises up to 328 amino acids.

25
25. An isolated polypeptide comprising a variant of an amino acid sequence set forth in SEQ ID NO: 10, 11, or 23, wherein said variant comprises at least one to 57 amino acid substitutions of SEQ ID NO: 10, 11, or 23, does not bind to choline, and is immunogenic.

30
26. An isolated polypeptide comprising an analog or a derivative of an amino acid sequence set forth in SEQ ID NO: 10, 11, or 23, wherein said polypeptide does not bind to choline, comprises up to 328 amino acids, and is immunogenic.

27. An isolated polypeptide consisting of an amino acid sequence set forth in SEQ ID NO: 7, 9, 10, 11, or 23.

28. The isolated polypeptide of claim 21, 22, 23, 24, 25, or 26, wherein said polypeptide interacts with an antibody, wherein said antibody is capable of interacting with a full-length CbpA polypeptide.

29. The isolated polypeptide of claim 1, 2, 3, 6, 7, 8, 11, 12, 13, 21, or 24, wherein said polypeptide is immunogenic.

10 30. The isolated polypeptide of claim 4, 9, 14, or 22, wherein said amino acid substitutions comprise conservative amino acid substitutions.

15 31. The isolated polypeptide of any of claims 1-15, and 19-27, wherein said polypeptide has lectin activity.

20 32. The isolated polypeptide of any of claims 1-5, 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.

25 33. The isolated polypeptide of claim 5, 10, 15, 23, or 26, wherein the analog comprises an N-terminal methionine or an N-terminal polyhistidine.

30 34. A pharmaceutical composition comprising an isolated antibody that specifically binds to a polypeptide comprising an amino acid sequence of an N-terminal choline binding protein A truncate, wherein said isolated antibody does not bind to a choline binding domain and said isolated antibody is substantially free of antibodies that bind to the choline binding domain.

35. The isolated antibody of claim 34, wherein said amino acid sequence comprises SEQ ID NO: 7, 9, 10, 11, or 23.

36. The isolated antibody of claim 34, wherein said amino acid sequence comprises SEQ ID NO: 1, 3, 4, 5, 22, or 24.

37. The isolated antibody of claim 34, 35, or 36, wherein the antibody is a
5 monoclonal antibody.

38. The isolated antibody of claim 34, 35, or 36, wherein the antibody is a polyclonal antibody.

10 39. The isolated antibody of claim 34, 35, or 36, wherein the antibody is a chimeric (bispecific) antibody.

40. A pharmaceutical composition comprising the polypeptide of any of claims 1-15 or 19-27, and a pharmaceutically acceptable carrier or diluent.

15 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 a pharmaceutical composition comprising a polypeptide having an amino acid sequence of an N-terminal
20 choline binding protein A truncate which does not bind to choline thereby inducing an immune response.

42. The method of claim 41, wherein said amino acid sequence comprises SEQ ID NO: 7, 9, 10, 11, or 23.

25 43. The method of claim 41, wherein said amino acid sequence comprises the polypeptide of claim 21 or 24.

44. The method of claim 41, wherein said amino acid sequence comprises
30 the polypeptide of claim 22 or 25.

45. The method of claim 41, wherein said amino acid sequence comprises the polypeptide of claim 23 or 26.

46. The method of claim 41, wherein said amino acid sequence comprises SEQ ID NO: 1, 3, 4, 5, 22, or 24.

47. The method of claim 41, wherein said amino acid sequence comprises a 5 polypeptide of claim 2, 3, 7, 8, 12, 13, or 19.

48. The method of claim 41, wherein said amino acid sequence comprises a polypeptide of claim 4, 9, or 14.

10 49. The method of claim 41, wherein said amino acid sequence comprises a polypeptide of claim 5, 10, or 15.

50. The method of claim 41, 42, or 46, wherein a protective immune response is induced.

15 51. A method for preventing infection by a *pneumococcal* bacterium in a subject comprising administering to the subject an amount of a pharmaceutical composition effective to prevent *pneumococcal* bacterium attachment, thereby preventing infection by the *pneumococcal* bacterium, wherein said 20 pharmaceutical composition comprises a polypeptide having an amino acid sequence of an N-terminal choline binding protein A truncate which does not bind to choline.

52. The method of claim 51, wherein said amino acid sequence comprises 25 SEQ ID NO: 7, 9, 10, 11, or 23.

53. The method of claim 51, wherein said amino acid sequence comprises the polypeptide of claim 21 or 24.

30 54. The method of claim 51, wherein said amino acid sequence comprises the polypeptide of claim 22 or 25.

55. The method of claim 51, wherein said amino acid sequence comprises the polypeptide of claim 23 or 26.

56. The method of claim 51, wherein said amino acid sequence comprises
5 SEQ ID NO: 1, 3, 4, 5, 22, or 24.

57. The method of claim 51, wherein said amino acid sequence comprises the polypeptide of claim 2, 3, 7, 8, 12, 13, or 19.

10 58. The method of claim 51, wherein said amino acid sequence comprises the polypeptide of claim 4, 9 or 14.

59. The method of claim 51, wherein said amino acid sequence comprises the polypeptide of claim 5, 10, or 15.

15 60. A method for preventing infection by a *pneumococcal* bacterium in a subject comprising administering to the subject an amount of a pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent and an antibody capable of binding to a polypeptide comprising an amino acid
20 sequence of an N-terminal choline binding protein A truncate wherein said antibody does not bind to a choline binding domain and said pharmaceutical composition is substantially free of antibodies that bind the choline binding domain, thereby preventing infection by a *pneumococcal* bacterium.

25 61. The method of claim 60, wherein said pharmaceutical composition is delivered to the respiratory tract or the nasopharynx.

62. The method of claim 60, wherein said antibody is capable of binding to the polypeptide having the amino acid sequence comprising SEQ ID NO: 7, 9,
30 10, 11, or 23.

63. The method of claim 60, wherein said antibody is capable of binding to the polypeptide of claim 21 or 24.

64. The method of claim 60, wherein said antibody is capable of binding to the polypeptide of claim 22 or 25.

65. The method of claim 60, wherein said antibody is capable of binding to 5 the polypeptide of claim 23 or 26.

66. The method of claim 60, wherein said antibody is capable of binding to the polypeptide having the amino acid sequence comprising SEQ ID NO: 1, 3, 4, 5, 22, or 24.

10

67. The method of claim 60, wherein said antibody is capable of binding to the polypeptide of claim 2, 3, 7, 8, 12, 13, or 19.

15

68. The method of claim 60, wherein said antibody is capable of binding to the polypeptide of claim 4, 9 or 14.

69. The method of claim 60, wherein said antibody is capable of binding to the polypeptide of claim 5, 10, or 15.

20

70. A vaccine comprising a polypeptide and a pharmaceutically acceptable adjuvant or carrier, wherein said polypeptide comprises an amino acid sequence of an N-terminal choline binding protein A truncate which does not bind to choline.

25

71. The vaccine of claim 70, wherein the polypeptide comprises the amino acid sequence as set forth in any of SEQ ID NO: 7, 9, 10, 11, or 23.

30

72. The vaccine of claim 70, wherein the polypeptide comprises the amino acid sequence of claim 21 or 24.

35

73. The vaccine of claim 70, wherein the polypeptide comprises the amino acid sequence of claim 22 or 25.

74. The vaccine of claim 70, wherein the polypeptide comprises the amino acid sequence of claim 23 or 26.

5 75. The vaccine of claim 70, wherein the polypeptide comprises the amino acid sequence as set forth in any of SEQ ID NO: 1, 3, 4, 5, 22, or 24.

76. The vaccine of claim 70, wherein the polypeptide comprises the amino acid sequence as set forth in claim 2, 3, 7, 8, 12, 13, or 19.

10 77. The vaccine of claim 70, wherein the polypeptide comprises the amino acid sequence as set forth in claim 4, 9 or 14.

78. The vaccine of claim 70, wherein the polypeptide comprises the amino acid sequence as set forth in claim 5, 10 or 15.

15 79. The vaccine of claim 70, wherein the polypeptide comprises the amino acid sequence of a N-terminal choline binding protein A truncate as set forth in Figure 2.

80. A vaccine comprising a polypeptide having an amino acid sequence which comprises a conserved region as set forth in Figure 2 and a pharmaceutically acceptable adjuvant or carrier wherein said polypeptide does not bind to choline.

25 81. The vaccine of claim 80, wherein the conserved region is selected from the group consisting of 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.

82. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide set forth in SEQ ID NO: 1, 3, or 24, wherein said polypeptide does not bind to choline.

83. The isolated nucleic acid molecule of claim 82, wherein said nucleotide sequence comprises SEQ ID NO: 12 or 14.

84. An isolated nucleic acid molecule comprising a nucleotide sequence 5 encoding a polypeptide comprising a variant of SEQ ID NO: 1, 3, or 24, wherein said variant comprises at least one to 57 amino acid substitutions of SEQ ID NO: 1, 3, or 24, does not bind choline, and is immunogenic.

85. An isolated nucleic acid molecule comprising a nucleotide sequence 10 encoding a polypeptide set forth in SEQ ID NO: 22 or 4, wherein said polypeptide does not bind to choline.

86. The isolated nucleic acid molecule of claim 85, wherein said nucleotide sequence comprises SEQ ID NO: 15.

15 87. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide comprising a variant of SEQ ID NO: 22 or 4, wherein said variant comprises at least one to 57 amino acid substitutions of SEQ ID NO: 22 or 4, does not bind to choline and is immunogenic.

20 88. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide set forth in SEQ ID NO: 5, wherein said polypeptide does not bind to choline.

25 89. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide comprising a variant of SEQ ID NO: 5, wherein said variant comprises at least one to 57 amino acid substitutions of SEQ ID NO: 5, does not bind to choline, is immunogenic, and comprises up to 398 amino acids.

30 90. The isolated nucleic acid molecule of claim 89, wherein said nucleotide sequence comprises SEQ ID NO: 16.

91. A nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide having at least 138 consecutive amino acids of SEQ ID NO: 24, wherein said polypeptide does not bind to choline.

5 92. A nucleic acid molecule comprising a nucleotide sequence having at least 318 consecutive nucleotides of SEQ ID NO: 12, wherein said nucleotide sequence encodes a polypeptide that does not bind choline.

10 93. An isolated nucleic acid molecule consisting of SEQ ID NO: 12, 14, 15, or 16.

94. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide set forth in SEQ ID NO: 7 or 9, wherein said polypeptide comprises up to 376 amino acids and does not bind to choline.

15 95. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide comprising a variant of SEQ ID NO: 7 or 9, wherein said variant comprises at least one to 57 amino acid substitutions of SEQ ID NO: 7 or 9, comprises up to 376 amino acids, does not bind choline, and is 20 immunogenic.

96. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide set forth in SEQ ID NO: 10, 11, or 23, wherein said polypeptide comprises up to 328 amino acids and does not bind to choline.

25 97. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide comprising a variant of SEQ ID NO: 10, 11, or 23, wherein said variant comprises at least one to 57 amino acid substitutions of SEQ ID NO: 10, 11, or 23, comprises up to 328 amino acids, does not bind 30 choline, and is immunogenic.

98. An isolated nucleic acid molecule comprising the nucleotide sequence set forth in SEQ ID NO: 17 or 19, wherein said nucleotide sequence encodes a polypeptide that does not bind to choline.

5 99. An isolated nucleic acid molecule consisting of SEQ ID NO: 17, 19, 20, or 21.

100. An isolated nucleic acid molecule comprising a nucleotide sequence set forth in SEQ ID NO: 20 or 21, wherein said nucleotide sequence encodes a 10 polypeptide having up to 328 amino acids and wherein said polypeptide does not bind to choline.

101. The isolated nucleic acid molecule of any of claims 82-100, wherein the nucleic acid molecule is DNA.

15

102. The isolated nucleic acid molecule of any of claims 82-100, wherein the nucleic acid molecule is cDNA.

20

103. The isolated nucleic acid molecule of any of claims 82-100, wherein the nucleic acid molecule is genomic DNA.

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104. The isolated nucleic acid molecule of any of claims 82-100, wherein the nucleic acid molecule is RNA.

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105. An isolated nucleic acid molecule of any of claims 82-100, wherein said nucleic acid molecule is operatively linked to a promoter.

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.....

106. The isolated nucleic acid molecule of claim 105, wherein said promoter is a promoter of RNA transcription.

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107. A vector comprising the nucleic acid molecule of any of claims 82-100.

108. The vector of claim 107, wherein said vector further comprises a promoter.

109. The vector of claim 107, wherein the vector is a plasmid, a cosmid, a 5 yeast artificial chromosome (YAC), a bacteriophage or a eukaryotic viral DNA.

110. A host vector system for the production of a polypeptide which comprises the vector of claim 107 in a suitable host cell.

10 111. The host vector system of claim 110, wherein the suitable host cell comprises a prokaryotic or a eukaryotic cell.

112. A cell comprising the vector of claim 107.

15 113. A method of obtaining a polypeptide in purified form comprising:

- a) introducing the vector of claim 107 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).

20

114. A vaccine comprising an isolated nucleic acid molecule encoding a polypeptide and a pharmaceutically acceptable adjuvant or carrier, wherein said polypeptide comprises an amino acid sequence of an N-terminal choline binding protein A truncate that does not bind to choline.

25

115. The vaccine of claim 114, wherein said nucleic acid molecule comprises the nucleotide sequence set forth in SEQ ID NO: 12, 14, 15, or 16.

30

116. The vaccine of claim 114, wherein said nucleic acid molecule comprises the nucleotide sequence of claim 82, 85, 88, or 92.

117. The vaccine of claim 114, wherein said nucleic acid molecule comprises the nucleotide sequence of claim 84, 87, or 89.

118. The vaccine of claim 114, wherein said nucleic acid molecule comprises the nucleotide sequence set forth in SEQ ID NO: 17, 19, 20, or 21.

119. The vaccine of claim 114, wherein said nucleic acid molecule comprises 5 the nucleotide sequence set forth in any of claim 94, 96, or 99.

120. The vaccine of claim 114, wherein said nucleic acid molecule comprises the nucleotide sequence of claim 95 or 97.

10 121. A vaccine comprising the vector of claim 107 and a pharmaceutically acceptable adjuvant or carrier.

15 122. 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 70-81 or 114-121, thereby treating the subject.

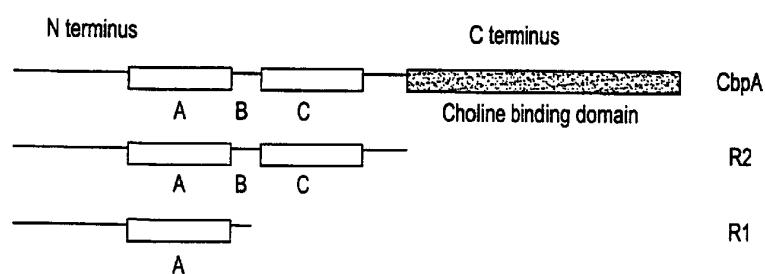
19 123. An isolated polypeptide according to claim 1, 4, 5, 6, 9, 10, 11, 14, 15, 19, 20, 21, 22, 23, 24, 25, 26 or 27, substantially as hereinbefore described.

20 124. A method according to claim 41, 51 or 60, substantially as hereinbefore described.

25 125. An isolated nucleic acid according to claim 82, 84, 85, 87, 88, 89, 91, 92, 95, 96, 97, 98 or 100, substantially as hereinbefore described.

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30 Attorneys for:
ST JUDE CHILDREN'S RESEARCH HOSPITAL and MEDIMMUNE, INC.

FIG. 1



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FIG. 2A-1

SPB328 (23F)	Cbpa	- AVASLVMGSVVHATENEYITTOVATSSNRANESTEHRAAKO	- - - - - VDEYIKKKL - - - - -	53
SPB365 (23F)	Cbpa	- AVASLVMGSVVHATEKEVTTTOVATSSNRANKSOTEHMKAAKO	- - - - - VDEYIKKKL - - - - -	53
SPB105 (6B)	Cbpa	- AVASLVMGSVVHATEKEVTTTOVATSSNRANESOAGHRKAEEQF	- - - - - VDEYIKTM - - - - -	54
SPSJ12 (19A)	Cbpa	- AVASLVMGSVVHATENEYITTOAPTSSNRNGESOAEHMKAAKO	- - - - - VDEYIEKLM - - - - -	53
SPB331 (14)	Cbpa	- - - - - VASLVMGSVVHATEKEVTTTOVATSSNRANKSOTEHMKAAKO	- - - - - VDEYIEKLM - - - - -	52
SPR332 (9V)	Cbpa	- CIVASLVMGSVVHATENERTTQVPTSSNRKG -- PERRKAEEQF	- - - - - VDEYIEKMKI - - - - -	51
ATCC2 Cbpa	trunc	- CIVASLVMGSVVHATEKEVTTQVPTSSNRKG -- TEHRAAKO --	- - - - - VDEYIEKMLRE I0 - - -	55
R6X (2)	Cbpa	- - - - - ENEGSTQATSSNMAK -- TEHRAAKO --	- - - - - VDEYIEKMLRE I0 - - -	40
SPSJ9 (14)	Cbpa	t Y - ASLFLGGVVAE -- GVRSGNNPTVTSQODI SKKYADE --	- - - - - VKSHELIUSIE1QTNL	54
ATCC6B Cbpa	trunc	- ASLFLGGVVAE	- - - - - VESHLQSIKDVKNKL	52
NType4 Cbpa	trunc	CIVASLVMGSVVHATENEGA TOVPTSSNRANESOAEQGEOPKLD	- - - - - SERDKARKEVEEYKK	70
ATCC4 Cbpa	trunc	- IVASLVMGSVVHATENEGATQVPTSSNRANESOAEQGEOPKLD	- - - - - SERDKARKEVEEYKK	69
SPB328 (23F)	Cbpa	DRRKHTONVALNTLSAITEYLNGSVLEEKSEALPSE IKA	- - - - - KLDAAFEQFKKDTL	11
SPB365 (23F)	Cbpa	DRRKHTONVALNTLSAITEYLNGSVLEEKSEALPSE IKA	- - - - - KLDAAFEQFKKDTL	11
SPB105 (6B)	Cbpa	DRRKHTONVALNLSR1KTEYRKLNVLEEKSKAELPSETIK	- - - - - E1DAAEFOFKKDTN	11
SPSJ12 (19A)	Cbpa	DRRKHTONVALNLSR1KTEYRKLNVLEEKSKAELPSETIK	- - - - - KLTAAFKQFKKDTL	11
SPS331 (14)	Cbpa	DRRKHTONVALNLSR1KTEYRKLNVLEEKSKAELPSETIK	- - - - - KLTAAFKQFKKDTL	11
SPR332 (9V)	Cbpa	DRRKHTONVALNLSR1KTEYRKLNVLEEKSKAELPSETIK	- - - - - KLTAAFKQFKKDTL	11
ATCC2 Cbpa	trunc	DRRKHTONVALNLSR1KTEYRKLNVLEEKSKAELPSETIK	- - - - - KLTAAFKQFKKDTL	10
R6X (2)	Cbpa	trunc DRRKHTONVALNLSR1KTEYRKLNVLEEKSKAELPSETIK	- - - - - KLTAAFEQFKKDTL	10
SPSJ9 (14)	Cbpa	t DRSKHTKTVNLINKLSK IKTLYELNLEEKSKAELPSETIK	- - - - - KLTAAFEQFKKDTL	98
ATCC6B Cbpa	trunc	TKKHTONADFKNLSK IKTLYELNLEEKSKAELPSETIK	- - - - - KLTAAFEQFKKDTL	11
NType4 Cbpa	trunc	TKKHTITVALNENNNKINEYN - KIWESTSESOQIILMERSKVKD	- - - - - AVSKFEKQDSSSSSSDSTK	13
ATCC4 Cbpa	trunc	TKKHTITVALNENNNKINEYN - KIWESTSESOQIILMERSKVKD	- - - - - AVSKFEKQDSSSSSSDSTK	13

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FIG. 2A-2

SPB328 (23F) CbPA ----- PTEPGKKVVAEAKKVEEAKKK ----- AEDQKEEDRRNYPNTITYKTLLEIAESDVEV 150
 SPB365 (23F) CbPA ----- PTEPGKKVVAEAKKVEEAKKK ----- AEDQKEEDRRNYPNTITYKTLLEIAESDVEV 162
 SPB105 (6B) CbPA ----- KVAEAKKVEEAKKK ----- AKAKEEDDRNYPNTITYKTLLEIAESDVEV 162
 SP SJ12 (19A) CbPA ----- TK ----- AKAKEEDDRNYPNTITYKTLLEIAESDVEV 161
 SPB331 (14) CbPA ----- KPEKKVVAEAKKVEEAKKK ----- AEDQKEEDDRNYPNTITYKTLLEIAESDVEV 160
 SPR332 (9V) CbPA ----- PTEPGKKVVAEAKKVEEAKKK ----- AEDQKEEDDRNYPNTITYKTLLEIAESDVEV 161
 ATCC2 CbPA trunc ----- PTEPEKVVVAEAKKVEEAKKK ----- AKAKEEDDRNYPNTITYKTLLEIAESDVEV 164
 R6X(2) CbPA trunc ----- KLGEKVVVAEAKKVEEAKKK ----- AKAKEEDDRNYPNTITYKTLLEIAESDVEV 159
 SPSJ9 (14) CbPA t ----- KGEKVVVAEAKKVEEAKKK ----- AEDQKEEDDRNYPNTITYKTLLEIAESDVEV 147
 ATCC6B CbPA trunc ----- PTEPGKKVVAEAKKVEEAKKK ----- AKAKEEDDRNYPNTITYKTLLEIAESDVEV 163
 Ntype4 CbPA trunc PEASDTAKPNKPTEPGEKVAEAKKVEEAKKK ----- AEDQKEEDDRNYPNTITYKTLLEIAESDVEV 164
 ATCC4 CbPA trunc PEASDTAKPNKPTEPGEKVAEAKKVEEAKKK ----- AKDQKEEDDRNYPNTITYKTLLEIAESDVEV 201
 160 170 180 190 200 210

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FIG. 2B-1

KKAEELVKEEAKESRDEEK INQAKAVESKKAETRLKKIKTDRKAEEE-AKRRADAKLQEA---NVA 220
 SPB365 (23F) Cbpa KKAELVKEEAKESRDEEK INQAKAVENKKAAETRLKKIKTDRKAEEE-AKRRADAKLQEA---NVA 227
 SPB105 (6B) Cbpa KKAELVKEEAKESRDEEK INQAKAVENKKAAETRLKKIKTDRKAEEE-AKRRADAKLQEA---NVA 227
 SPSJ12 (19A) Cbpa KKAELVKEEAKESRDEEK IKOAEEVESKKAETRLKKIKTDRKAEEE-AKRRADAKLQEA---NVA 230
 SPB331 (14) Cbpa KKAELVKEEAKESRDEEK IKOAEEVESKKAETRLKKIKTDRKAEEE-AKRRADAKLQEA---NVA 224
 SPR332 (9V) Cbpa KKAELVKEEAKESRDEEK INQAKAVENKKAAETRLKKIKTDRKAEEE-AKRRADAKLQEA---NVA 226
 ATCC2 Cbpa trunc KKAELVKEEAKESRDEEK INQAKAVENKKAAETRLKKIKTDRKAEEE-AKRRADAKLQEA---NVA 229
 R6X12 Cbpa trunc KKAELVKEEAKESRDEEK INQAKAVENKKAAETRLKKIKTDRKAEEE-AKRRADAKLQEA---NVA 222
 SPSJ9 (14) Cbpa trunc KKAELVKEEAKESRDEEK IKOAEEVESKKAETRLKKIKTDRKAEEE-AKRRADAKLQEA---NVA 113
 ATCC6B Cbpa trunc KKAELVKEEAKESRDEEK IKOAEEVESQAE INQAKAVESQAE-AKRRADAKLQEA---NVA 227
 Ntype4 Cbpa trunc KKAELVKEEAKESRDEEK IKOAEEVESQAE ATRLKKIKTDRQAEATRLNKKTDRQAEATRLNKKTDRQAEEEAKVK 234
 ATCC4 Cbpa trunc KKAELVKEEAKESRDEEK IKOAEEVESQAE ATRLKKIKTDRQAEATRLNKKTDRQAEEE-AKRRADAKLQEA---NVA 261
 -SEODKPKRAKRGIPGELATPDKKENDAKSSDSSVGEETLPSPLKPEKKVAEEAKKVAEEAKKAEDOK 260

KKAEELVKEEAKESRDEEK INQAKAVESKKAETRLKKIKTDRKAEEE-AKRRADAKLQEA---NVA 220
 SPB365 (23F) Cbpa TSEODKSKRRAKREYXGELATPDKKENDAKSSDSSVGEETLPSPLKPEKKVAEEAKKVAEEAKKAEDOK 297
 SPB105 (6B) Cbpa TSEODKSKRRAKREYXGELATPDKKENDAKSSDSSVGEETLPSPLKPEKKVAEEAKKVAEEAKKAEDOK 297
 SPSJ12 (19A) Cbpa -S-SKRRSRXKRGRDGEQATPDKKENDAKSSDSSVGEETLPSPLKPEKKVAEEAKKVAEEAKKAQK 300
 SPB331 (14) Cbpa TSEODKSKRRAKREYXGELATPDKKENDAKSSDSSVGEETLPSPLKPEKKVAEEAKKVAEEAKKAQK 291
 SPR332 (9V) Cbpa ---SKRRSRXKRGRDGEATPDKKENDAKSSDSSVGEETLPSPLKPEKKVAEEAKKVAEEAKKAQK 296
 ATCC2 Cbpa trunc ---DLKRRTRKAVPGPEATPDKKENDAKSSDSSVGEETLPSPLKPEKKVAEEAKKVAEEAKKAQK 295
 R6X12 Cbpa trunc TSDOKPKGRKAVPGPEATPDKKENDAKSSDSSVGEETLPSPLKPEKKVAEEAKKVAEEAKKAQK 288
 SPSJ9 (14) Cbpa t -S--KRRSRVKGDFGEATPDKKENDAKSSDSSVGEETLPSPLKPEKKVAEEAKKVAEEAKKAQK 283
 ATCC6B Cbpa trunc ---DEPKRTKAVPGPEATPDKKENDAKSSDSSVGEETLPSPLKPEKKVAEEAKKVAEEAKKAQK 293
 Ntype4 Cbpa trunc --EQGKPKGRKAVPGPEATPDKKENDAKSSDSSVGEETLPSPLKPEKKVAEEAKKVAEEAKKAQK 300
 ATCC4 Cbpa trunc --EQGKPKGRKAVPGPEATPDKKENDAKSSDSSVGEETLPSPLKPEKKVAEEAKKVAEEAKKAQK 328

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FIG. 2B-2

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439
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FIG. 2B-3

EE
EE
SPB328 (23F) Cbpa EE
SPB365 (23F) Cbpa
SPB105 (6B1) Cbpa, EE
SPSJ12 (19A) Cbpa
SPB331 (14) Cbpa, E
SPR332 (9V) Cbpa, E
ATCC2 Cbpa trunc EE
R6X (2) Cbpa trunc EE
SPSJ9 (14) Cbpa t EE
ATCC68 Cbpa trunc EE
Ntype4 Cbpa trunc EE
ATCC4 Cbpa trunc EE

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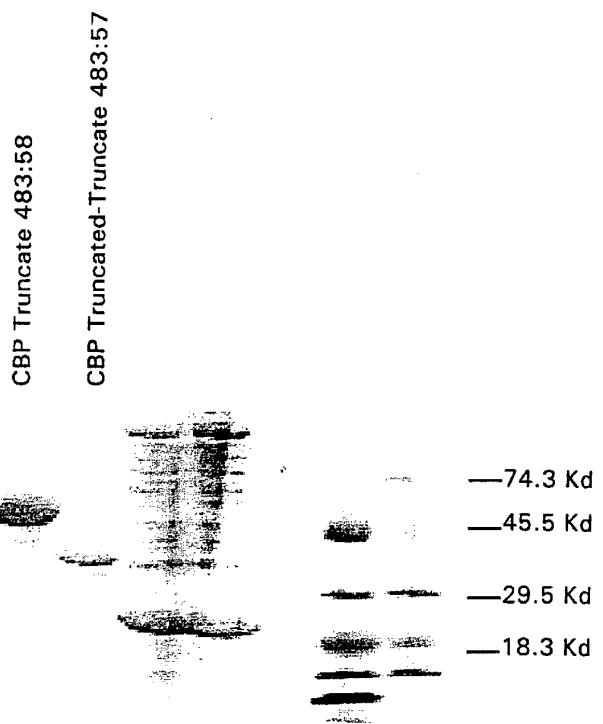
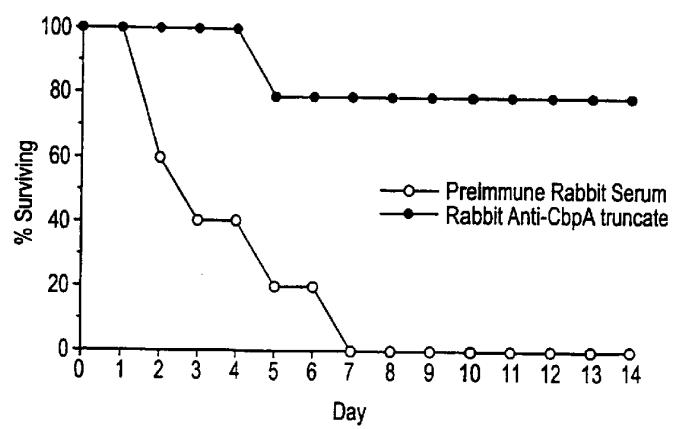


FIG. 3

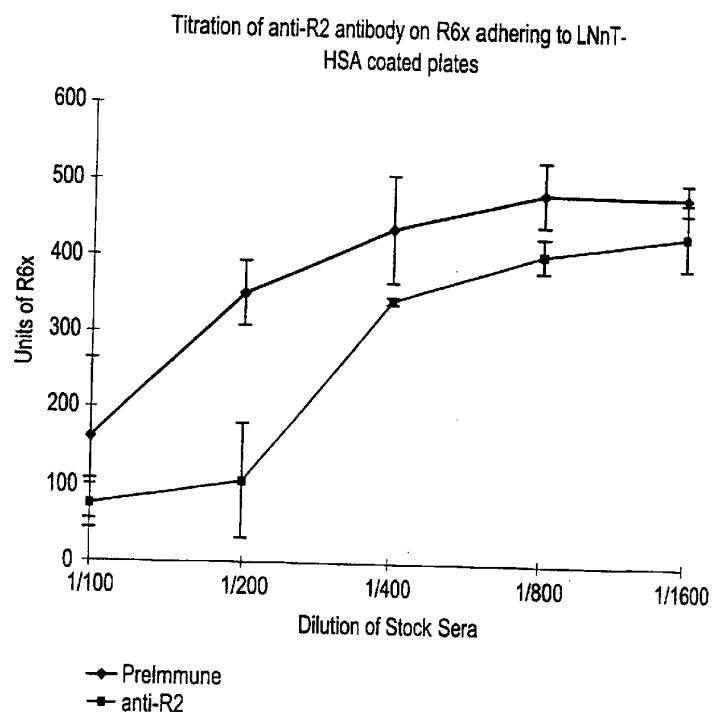
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FIG. 4



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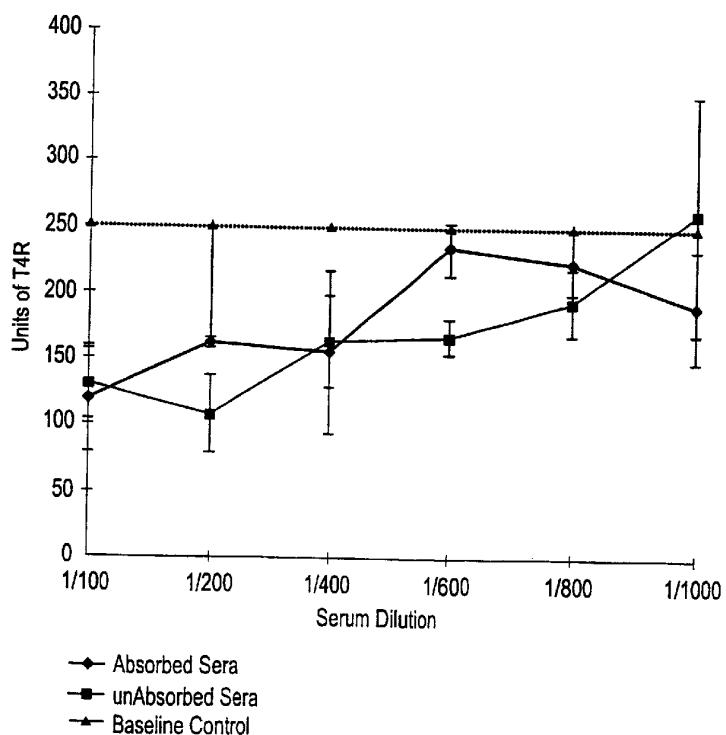
FIG. 5



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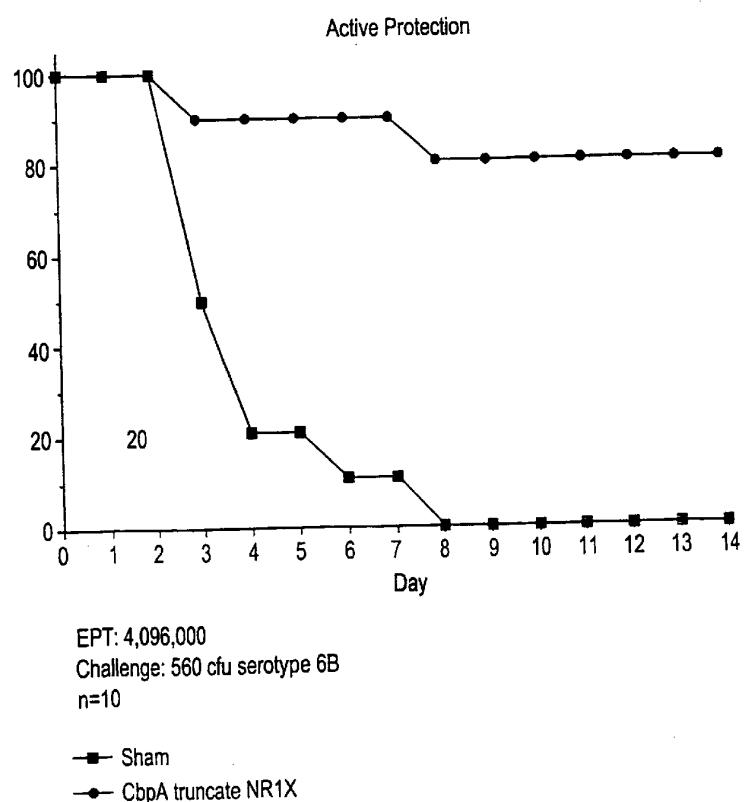
FIG. 6

T4R Pretreated with anti-CbpA Antibody Prior to
Adhesion to LNnT-HSA Coated Plates



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FIG. 7



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