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(54) **MULTICOMPONENT VACCINES**

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- (52) **U.S. Cl.** **424/243.1**; 424/184.1;
424/190.1; 424/193.1; 424/203.1; 424/234.1;
424/244.1; 530/350
- (58) **Field of Search** 424/184.1, 190.1,
424/193.1, 197.11, 203.1, 234.1, 243.1,
244.1; 530/350

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(57) **ABSTRACT**

Multicomponent vaccines are provided which aid in the prevention and treatment of staphylococcal infections and which include certain selected combinations of bacterial binding proteins or fragments thereof, or antibodies to those proteins or fragments. By careful selection of the proteins, fragments, or antibodies, a vaccine is provided that imparts protection against a broad spectrum of Staphylococcus bacterial strains and against proteins that are expressed at different stages of the logarithmic growth curve. In one embodiment of the invention, a composition is provided that includes at least a collagen binding protein or peptide (or an appropriate site directed mutated sequence thereof) such as CNA, or a protein or fragment with sufficiently high homology thereto, in combination with a fibrinogen binding protein, preferably Clumping factor A ("ClfA") or Clumping factor B ("ClfB"), or a useful fragment thereof or a protein or fragment with sufficiently high homology thereto. The vaccines and products of the present invention are advantageous in that they respond to the urgent need of the medical community for a substitute for small molecule antibiotics, which are rapidly losing effectiveness and provide effective combinations of the large number of known bacterial surface adhesins which can impart effective protection against a broad spectrum of bacterial infections.

8 Claims, 12 Drawing Sheets

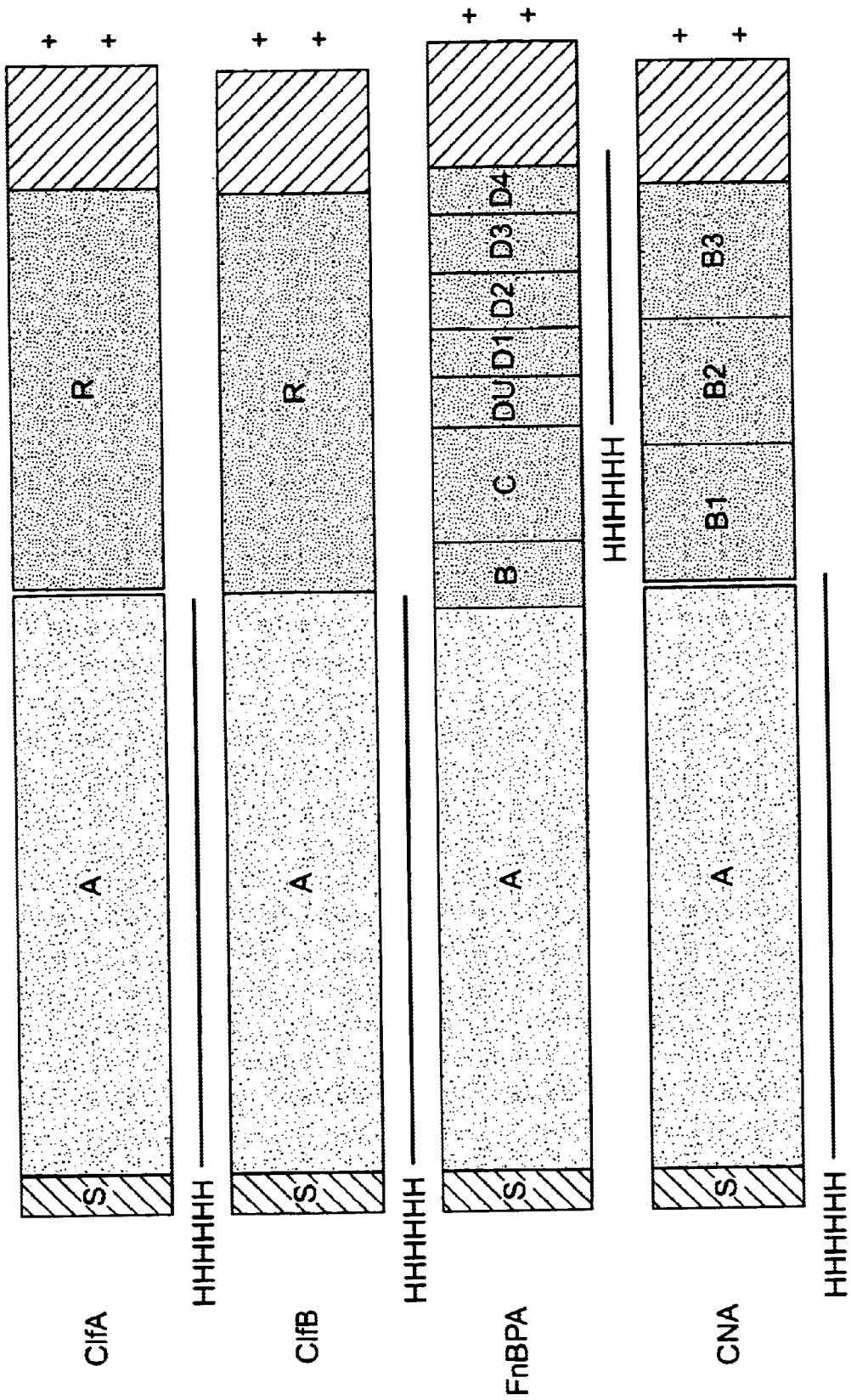


FIG. 1

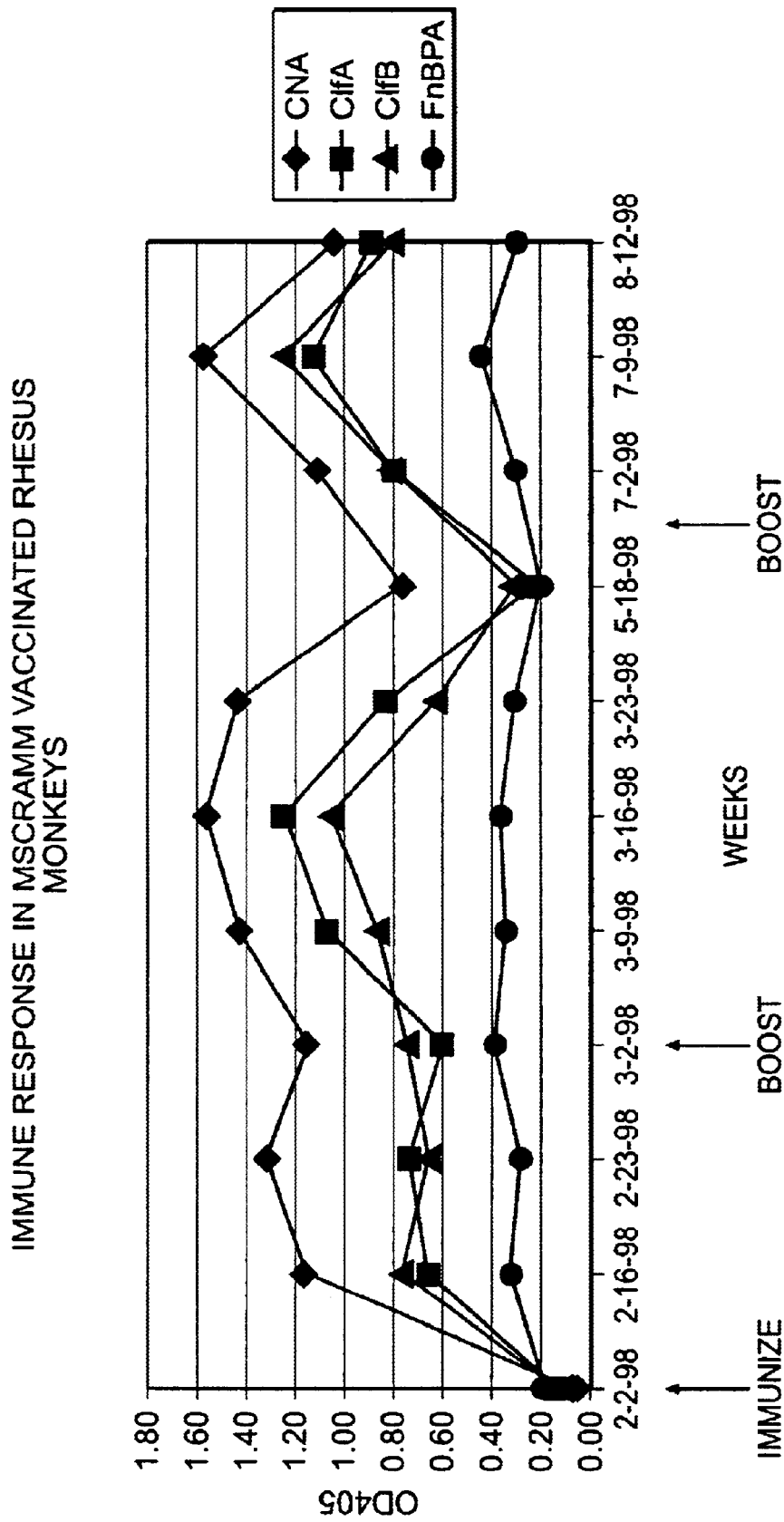


FIG. 2

CODING SEQUENCE FOR SdrF - INCLUDES FLANKING SEQUENCES

tattggataaattatgcttataaaagtatttacataaaaatgtaaattgcaatttacaagta
Y W I N Y A Y K V F T - K C K C N L Q V
aatattcaaattatttccttgtaaaaatatttattttaactggagggtatagtatgaaaaag
N I Q I I S L - N I Y F N W R Y S M K K
agaagacaaggaccaattaacaagagagtggattttctatccaacaaggtaacaagtac
R R Q G P I N K R V D F L S N K V N K Y
tcgattaggaagttcacagtaggtacagcttcaatactcgtgggtgctacgttaatgttt
S I R K F T V G T A S I L V G A T L M F
ggtgccgcagacaatgaggctaaagcggctgaagacaatcaattagaatcagcttcaaaa
G A A D N E A K A A E D N Q L E S A S K
gaagaacagaaaggtagtcgtgataatgaaaactcaaaacttaatcaagtcgatttagac
E E Q K G S R D N E N S K L N Q V D L D
aacggatcacatagttctgagaaaacaacaatgtaaacaatgcaactgaagtaaaaaaa
N G S H S S E K T T N V N N A T E V K K
gttgaagcaccacgacaagtgacgtatctaagcctaaagctaatgaagcagtagtgacg
V E A P T T S D V S K P K A N E A V V T
aatgagtcactaaacccaaaacaacagaagcaccaactgttaatgaggaatcaatagct
N E S T K P K T T E A P T V N E E S I A
gaaacacccaaaacctcaactacacaacaagattcgactgagaagaataatccatcttta
E T P K T S T T Q Q D S T E K N N P S L
aaagataatttaaattcatcctcaacgacatctaagaaaagtaaaacagacgaacattct
K D N L N S S S T T S K E S K T D E H S
actaagcaagctcaaatgtctactaataaatcaaatttagacacaaatgactctccaact
T K Q A Q M S T N K S N L D T N D S P T
caaagtgagaaaacttcatcacaagcaataacgacagtacagataatcagtcagcacct
Q S E K T S S Q A N N D S T D N Q S A P
tctaaacaattagattcaaaccatcagaacaaaagtatataaaacaaaatttaatgat
S K Q L D S K P S E Q K V Y K T K F N D
gaacctactcaagatggtgaacacacgacaactaaattaaaacaccttctgtttcaaca
E P T Q D V E H T T T K L K T P S V S T
gatagttcagtcfaatgataagcaagattacacacgaagtgcgtgtagctagtttaggtgtt
D S S V N D K Q D Y T R S A V A S L G V
gattctaattgaacagaaagcaattacaaatgcagttagagacaatttagatttaaaagct
D S N E T E A I T N A V R D N L D L K A
gcatttagagaacaaatcaatgaagcaatcattgctgaagcactaaaaaagacttttct
A S R E Q I N E A I I A E A L K K D F S

FIG. 3

aaccctgattatgggtgctcgatagccattagctctaacagatctcaatcaaaaaattca
N P D Y G V D T P L A L N R S Q S K N S
ccacataagagtgcaggtccacgcatgaatttaatgagtttagctgctgagcctaataagt
P H K S A S P R M N L M S L A A E P N S
ggtaaaaatgtgaatgataaagttaaaatcaciaaacctacgctttcacttaataagagt
G K N V N D K V K I T N P T L S L N K S
aataatcacgctaataacgtaatatggccaacaagtaacgaacaatttaatttaaagca
N N H A N N V I W P T S N E Q F N L K A
aattatgaattagatgacagcataaaagaggagatacttttactattaagtatggtcag
N Y E L D D S I K E G D T F T I K Y G Q
tatattagaccgggtggtttagaacttctgcaataaaaactcaactacgtagtaaggat
Y I R P G G L E L P A I K T Q L R S K D
ggctctattgtagctaatgggtgtatatgataaaaactacaaatacagcagacttatacattt
G S I V A N G V Y D K T T N T T T Y T F
actaactatgttgatcaatatcaaaatattacaggtagttttgatttaattgacgacgct
T N Y V D Q Y Q N I T G S F D L I A T P
aagagggaaacagcaattaaggataatcagaattatcctatggaagtgcagattgctaac
K R E T A I K D N Q N Y P M E V T I A N
gaagtagtcaaaaaagacttcattgtggattatggtaataaaaaggacaatacaactaca
E V V K K D F I V D Y G N K K D N T T T
gcagcggtagcaaatgtggataatgtaaataataaacataacgaagttgtttatctaaac
A A V A N V D N V N N K H N E V V Y L N
caaaataaccaaaccctaaatagctaaatatttctcaacagtaaaaaatgggtgaattt
Q N N Q N P K Y A K Y F S T V K N G E F
ataccaggtgaagtgaagtttacgaagtgcggataccaatgcatggttagatagcttc
I P G E V K V Y E V T D T N A M V D S F
aatcctgattttaaatagttctaatgtaaagatgtgacaagtcaatttgcacctaaagta
N P D L N S S N V K D V T S Q F A P K V
agtgcagatggtagctagagttgatatcaattttgctagaagtatggcaaattggtaaaaag
S A D G T R V D I N F A R S M A N G K K
tatattgtaactcaagcagtgagaccaacgggaactggaaatgtttataccgaatattgg
Y I V T Q A V R P T G T G N V Y T E Y W
ttaacaagagatggtagctaccaatacaaatgatttttaccgtggaacgaagtctacaacg
L T R D G T T N T N D F Y R G T K S T T
gtgacttatctcaatggttcttcaacagcacaggggataatcctacatatagtctaggt
V T Y L N G S S T A Q G D N P T Y S L G
gactatgtatgggttagataaaaataaaaacgggtgttcaagatgatgatgagaaaggttta
D Y V W L D K N K N G V Q D D D E K G L

FIG. 3 (CONT'D 1)

gcaggtgtttatgttactcttaaagacagtaacaatagagaattacaacgtgtaactact
A G V Y V T L K D S N N R E L Q R V T T
gatcaatctggacattatcaatcttgataatttacaaaatggaacgtacacagtcgagttt
D Q S G H Y Q F D N L Q N G T Y T V E F
gcgattcctgataattatacgccatctcccgaaaataattctacaaatgatgcaatagat
A I P D N Y T P S P A N N S T N D A I D
tcagatggtgaacgtgatggtacacgtaaagtagttggtgccaaggaacaattaataat
S D G E R D G T R K V V V A K G T I N N
gctgataatgatgactgtagatactggcttttatcttaactcctaaatacaatgtcggagat
A D N M T V D T G F Y L T P K Y N V G D
tatgtatgggaagatacaaaataaagatggtatccaagatgacaatgaaaaggaatttct
Y V W E D T N K D G I Q D D N E K G I S
ggtgttaaagtaacgttaaaaaataaaaatggagatactattggcacaacgacaacagat
G V K V T L K N K N G D T I G T T T T D
tcaaatggtaaatatgaattcacaggttagagaacggggattacacaatagaatttgag
S N G K Y E F T G L E N G D Y T I E F E
acgccggaaggctacacaccgactaaacaaaactcgggaagtgacgaaggtaaagattca
T P E G Y T P T K Q N S G S D E G K D S
aacggtacgaaaacaacagtcacagtcaaagatgcagataataaaacaatagactcaggt
N G T K T T V T V K D A D N K T I D S G
ttctacaagccaacataataacttaggtgactatgtagtgggaagatacaaaataaagatggt
F Y K P T Y N L G D Y V W E D T N K D G
attcaagacgacagtgaaaagggatttctggggtaaagtgacgttaaagataaaaat
I Q D D S E K G I S G V K V T L K D K N
ggaaatgccattgggacaacgacaacagacgcaagtggtcattatcaatttaaaggatta
G N A I G T T T T D A S G H Y Q F K G L
gaaaatggaagctacacagttgagtttgagacaccatcaggttatacaccgacaaaagcg
E N G S Y T V E F E T P S G Y T P T K A
aattcaggtcaagatataactgtagattccaacggtataacaacaacaggtatcattaac
N S G Q D I T V D S N G I T T T G I I N
ggagctgataatctcacaattgatagtggtttctacaaaacacccaaaatagtggtcgga
G A D N L T I D S G F Y K T P K Y S V G
gattatgtagtgggaagatacaaaataaagatggtatccaagatgacaatgaaaaggaatt
D Y V W E D T N K D G I Q D D N E K G I
tctggtgtaaagtaacgttaaaggatgaaaaggaatataattagcactacaacaact
S G V K V T L K D E K G N I I S T T T T
gatgaaaatgggaagtatcaatcttgataatttagatagtggttaattacattattcattt
D E N G K Y Q F D N L D S G N Y I I H F

FIG. 3 (CONT'D 2)

gagaaaccggaaggcatgactcaaactacagcaaattctggaaatgatgatgaaaaagat
E K P E G M T Q T T A N S G N D D E K D
gctgatggggaagatgttcgtgttacgattactgatcatgatgactttagtatagataat
A D G E D V R V T I T D H D D F S I D N
ggttattttgacgatgattcagacagtgactcagacgcagatagtgattcagactcagac
G Y F D D D S D S D S D A D S D S D S D
agtgactcggacgcagacagcgattctgacgcagacagtgactcagacgcagatagtgat
S D S D A D S D S D A D S D S D A D S D
tctgactcagacagcgactcagacgcagatagtgattccgattcagacagcgactcggat
S D S D S D S D A D S D S D S D S D S D
tcagatagtgattcggatgcagacagcgactcggattctgacagtgattctgacgcagac
S D S D S D A D S D S D S D S D S D A D
agtgactcagattcagacagtgactcggattcagacagcgattccgattccgattcagac
S D S D S D S D S D S D S D S D S D S D
agtgactcggattcagacagtgactcagactccgacagtgattccgattcagatagcgac
S D S D S D S D S D S D S D S D S D S D
tccgacgcagatagtgattcggacgcagacagtgactcagattcagacagtgattcggac
S D A D S D S D A D S D S D S D S D S D
gcagacagtgactcggactcagatagtgattcagatgcagacagcgattcagactcagat
A D S D S D S D S D S D A D S D S D S D
agcgactcggattcagacagcgactccgacgcagacagcgactcggattcagatagtgat
S D S D S D S D S D A D S D S D S D S D
tctgactcagacagtgactcagattccgatagtgattcggattcagatagtgattccgac
S D S D S D S D S D S D S D S D S D S D
gcagacagcgattcggattccgatagcgattcagactcagacagcgattcagattcagac
A D S D S D S D S D S D S D S D S D S D
agcgactcagattcagatagtgattccgacgcagacagcgatgcagacagcgactcagac
S D S D S D S D S D A D S D A D S D S D
gcagacagtgattcagatgcagacagcgattctgactcagatagtgactcagacgcagat
A D S D S D A D S D S D S D S D S D A D
agtgattccgattccgatagcgattcagattctgatagtgactcagactcagacagtgac
S D S D S D S D S D S D S D S D S D S D
tcagattccgatagcgactcggattcagatagtgattccgacgcagacagtgactcagac
S D S D S D S D S D S D S D S D S D S D
tcagatagtgactcggattccgatagtgattccgacgcagacagcgattctgactcagat
S D S D S D S D S D S D A D S D S D S D
agtgactcagacgcagatagtgattccgattccgatagcgattcggatgcagacagcgac
S D S D A D S D S D S D S D S D A D S D

FIG. 3 (CONT'D 3)

tcggattcagatagtgattccgacgcagacagtgactcagactcagatagtgactcggat
S D S D S D S D A D S D S D S D S D S D
tccgatatgattccgacgcagacagcgattccgattccgatatgattcagactccgac
S D S D S D A D S D S D S D S D S D S D
agcgattcagattcagacagcgactcagattccgatatgattccgattcagacagtgac
S D S D S D S D S D S D S D S D S D S D
tcggattccgatatgactcagactcagacagtgactcagattcagatatgactcagat
S D S D S D S D S D S D S D S D S D S D
tcagacagtgattccgactcagatatgactccgattcagacagtgattccgattccgac
S D S D S D S D S D S D S D S D S D S D
agcgattccgattccgatatgactcggattcagacagtgattccgactcagacagcgac
S D S D S D S D S D S D S D S D S D S D
tccgattcagatagtgattccgactcagacagcgattccgattccgatatgactcggat
S D S D S D S D S D S D S D S D S D S D
tcagacagtgattccgactcagacagcgactccgattcagatatgattccgacgcagac
S D S D S D S D S D S D S D S D S D A D
agcgactccgattcagatatgattccgacgcagacagcgattccgatatgactcggat
S D S D S D S D S D A D S D S D S D S D
tcagacagtgattccgactcagacagcgattccgattcagacagtgactccgactcagat
S D S D S D S D S D S D S D S D S D S D
agcgactccgattcagacagtgactccgactcagatatgactccgattcagacagcgac
S D S D S D S D S D S D S D S D S D S D
tcggattctgataaaaatgcaaaagataaattacctgatacaggagcaaatgaagatcat
S D S D K N A K D K L P D T G A N E D H
gattctaaaggcacattacttggactttatttgcaggtttaggagcattattattagga
D S K G T L L G T L F A G L G A L L L G
agacgtcgtaaaaaagataataaagaaaaatagcactattgattcattcataagttattt
R R R K K D N K E K * H Y - F I H K L F
caagccaggtctatatggcctggttgaatcatattaattgaaaggagaaaaagatga
Q A R S I W P G L K S Y - I E R R K R -
gtatg
V W

FIG. 3 (CONT'D 4)

SdrG coding and flanking sequences.

atattgcaaaaaagacttatatactatattgtatcttactctagaaacgatttttacttgaa
I A K K T Y I L Y C I L L - K R F L L E
aattacattgaaaatagtc aaagataaggagtttttatgattaaaaaaaaataatttacta
N Y I E I V K D K E F L - L K K N N L L
actaaaagaaacctatagcaaaataaatccaataaatatgcaattagaaaattcacagta
T K K K P I A N K S N K Y A I R K F T V
ggtagcagcgtctattgtaataggtgcagcattattgtttggttaggtcataatgaggcc
G T A S I V I G A A L L F G L G H N E A
aaagctgaggagaatacagtacaagacgttaagattcgaatatggatgatgaattatca
K A E E N T V Q D V K D S N M D D E L S
gatagcaatgatcagtcagtaatgaagaaaagaatgatgtaatcaataatagtcagtc
D S N D Q S S N E E K N D V I N N S Q S
ataaacaccgatgatgataaccaataaaaaagaagaaacgaatagcaacgatgccata
I N T D D D N Q I K K E E T N S N D A I
gaaaatcgctctaaagatatacacagtcacaacaaatgtagatgaaaacgaagcaaca
E N R S K D I T Q S T T N V D E N E A T
ttttacaaaagaccctcaagataatactcagcttaaagaagaagtggtaaaagaacc
F L Q K T P Q D N T Q L K E E V V K E P
tcacagtcgaatcctcaaattcatcaatggatactgcccacaaccatctcatacaaca
S S V E S S N S S M D T A Q Q P S H T T
ataaatagtgaagcatctattcaacaagtgataatgaagaaaattcccgcgtatcagat
I N S E A S I Q T S D N E E N S R V S D
tttgctaactctaaaataatagagagtaacactgaatccaataaagaagagaatactata
F A N S K I I E S N T E S N K E E N T I
gagcaacctaaacaagtaagagaagattcaataacaagtcaaccgtctagctataaaaat
E Q P N K V R E D S I T S Q P S S Y K N
atagatgaaaaaatttcaaatacaagatgagttattaatttaccataaatgaatatgaa
I D E K I S N Q D E L L N L P I N E Y E
aataagggttagaccgttatctacaacatctgcccaccatcgagtaagcgtgtaaccgta
N K V R P L S T T S A Q P S S K R V T V
aatcaattagcggcagaacaagggttcgaatgtaatcatttaattaaagtactgatcaa
N Q L A A E Q G S N V N H L I K V T D Q
agtattactgaaggatgatgatgtagtggtattattaagcacatgatgctgaaaac
S I T E G Y D D S D G I I K A H D A E N
ttaatctatgatgtaacttttgaagtagatgataagggtgaaatctgggtgatacgatgaca
L I Y D V T F E V D D K V K S G D T M T

FIG. 4

gtgaatatagataagaatacagttccatcagatttaaccgatagttttgcaataccaaaa
V N I D K N T V P S D L T D S F A I P K
ataaaagataattctggagaaatcatcgctacaggtacttatgacaacacaaataaacia
I K D N S G E I I A T G T Y D N T N K Q
attacctacacttttacagattatgtagataaataatgaaaatattaaagcgcaccttaa
I T Y T F T D Y V D K Y E N I K A H L K
ttaacatcatacattgataaatcaaaggttccaaataataacactaagttagatgtagaa
L T S Y I D K S K V P N N N T K L D V E
tataagacggccctttcatcagtaataaaacaattacgggttgaaatcaaaaacctaac
Y K T A L S S V N K T I T V E Y Q K P N
gaaaatcggactgctaaccttcaaagtatgttcacaacatagatacgaanaaccataca
E N R T A N L Q S M F T N I D T K N H T
gttgagcaaacgatttatattaacctcttcggttattcagccaaagaaacaaatgtaaat
V E Q T I Y I N P L R Y S A K E T N V N
atctcaggggaatggcgatgaaggttcaacaattatcgacgatagtacaatcattaaagtt
I S G N G D E G S T I I D D S T I I K V
tataaggttgagataatcaaaatttaccagatagtaacagaatttatgattacagtgaa
Y K V G D N Q N L P D S N R I Y D Y S E
tatgaagatgtcacaatgatgattatgcccaattaggaaataataatgacgtgaatatt
Y E D V T N D D Y A Q L G N N N D V N I
aattttggtaatatagattcaccatataattattaagttattagtaaatatgaccctaat
N F G N I D S P Y I I K V I S K Y D P N
aaggacgattacacgacgatacagcaactgtgacaatgcaaacgactataaatgagtat
K D D Y T T I Q Q T V T M Q T T I N E Y
actggtgagtttagaacagcatcctatgataatacaattgctttctctacaagttcaggt
T G E F R T A S Y D N T I A F S T S S G
caaggacaaggtgacttgcctcctgaaaaaacttataaaatcggagattacgtatgggaa
Q G Q G D L P P E K T Y K I G D Y V W E
gatgtagataaagatggtattcaaaatacaaatgataatgaaaaccgcttagtaatgta
D V D K D G I Q N T N D N E K P L S N V
ttggtaaccttgacgtatcctgatggaacttcaaatcagtcagaacagatgaagagggg
L V T L T Y P D G T S K S V R T D E E G
aaatatcaatttgatgggttaaaaaacggattgacttataaaattacattcgaaacaccg
K Y Q F D G L K N G L T Y K I T F E T P
gaaggatatacggcgcgcttaaacattcaggaacaaatcctgcactagactcagaagggc
E G Y T P T L K H S G T N P A L D S E G
aattctgtatgggtaactattaacggacaagacgatatgactattgatagcggattttat
N S V W V T I N G Q D D M T I D S G F Y

FIG. 4 (CONT'D 1)

caaacacctaataatagcttagggaactatgtatgggtatgacactaataaagatgggtatt
Q T P K Y S L G N Y V W Y D T N K D G I
caaggtgatgatgaaaaggaatctctggagtaaaagtgacgttaaaagatgaaaacgga
Q G D D E K G I S G V K V T L K D E N G
aatatcattagtagacaacaactgatgaaaatggaaagtatcaatttgataatttaaat
N I I S T T T T D E N G K Y Q F D N L N
agtggtaattatattgttcattttgataaaccttcaggtatgactcaaacaacaacagat
S G N Y I V H F D K P S G M T Q T T T D
tctgggtgatgatgacgaacaggatgctgatggggaagaagtccatgtaacaattactgat
S G D D D E Q D A D G E E V H V T I T D
catgatgacttttagtatagataacggatactatgatgacgactcagattcagatagtgat
H D D F S I D N G Y Y D D D S D S D S D
tcagactcagatagcgcgactcagactccgatagcgattccgactcagacagcgactca
S D S D S D D S D S D S D S D S D S D S
gattccgatagtgattcagattcagacagtgactcagactcagatagtgattcagattca
D S D S D S D S D S D S D S D S D S D S
gacagcgattccgactcagacagtgactcaggattagacaatagctcagataagaataca
D S D S D S D S D S G L D N S S D K N T
aaagataaattaccggatacaggagctaatgaagatcatgattctaaaggcacattactt
K D K L P D T G A N E D H D S K G T L L
ggagctttatttgcaggtttaggagcggttattattaggggaagcgctcgcaaaaatagaaaa
G A L F A G L G A L L L G K R R K N R K
aataaaaattaaattattcaaataaattagtgaaagaagcagatacgcacatttgaatag
N K N * I I Q M K L V K E A D T T F E -
aaagtatatttagtccaacaataataaggtgttg
K V Y L V Q Q I - G V

FIG. 4 (CONT'D 2)

SdrH coding region

atgaaaagtttaacattaacattcatttatgcttacgggctttgctttcatggtaact
M K K F N I K H S F M L T G F A F M V T
acatcattattcagtcaccaagcacatgctgaaggtaatcatcctattgacattaat
T S L F S H Q A H A E G N H P I D I N F
tctaaagatcaaattgataaaaatacagctaagagcaatattatcaatcgagtgaatgac
S K D Q I D R N T A K S N I I N R V N D
actagtcgcacaggaattagtagtaattcggataatgatttagatacagatatcgtttca
T S R T G I S M N S D N D L D T D I V S
aatagtgactcagaaaatgacacatattagatagtgattcagattcagacagtgactca
N S D S E N D T Y L D S D S D S D S D S
gattcagatagtgactcagattcagatagtgactcagattcagatagtgactcagattca
D S D S D S D S D S D S D S D S D S D S D S
gacagtgattcagactcagatagtgactcagattcagacagtgattcagactcagatagt
D S D S D S D S D S D S D S D S D S D S D S
gattcagattcagacagtgattcagattcagacagtgactcagactcagacagtgattca
D S D S D S D S D S D S D S D S D S D S D S
gattcagatagtgattcagattcagatagtgattcagattcagatagtgattcagattca
D S D S D S D S D S D S D S D S D S D S D S
gacagtgactcagactcagacagtgattcagattcagatagtgattcagactcagatagt
D S D S D S D S D S D S D S D S D S D S D S
gactcagattcagatagtgattcagactctggtacaagttcaggtaagggttcacatacc
D S D S D S D S D S G T S S G K G S H T
ggaaaaaacctggtaaccctaaaggaaatacaaatagaccttctcaaagacatacgaat
G K K P G N P K G N T N R P S Q R H T N
caaccccaaaggcctaaatacaatcaaaccaatcaaaacaatataaacaatataaacat
Q P Q R P K Y N Q T N Q N N I N N I N H
aatattaatcatacacgtactagtgagatggtgcgcttttaaacgtcaacaaaatatt
N I N H T R T S G D G A P F K R Q Q N I
attaattctaattcaggtcatagaaatcaaaataatataaatcaatttatatggaacaaa
I N S N S G H R N Q N N I N Q F I W N K
aatggcttttttaaatctcaaaataataccgaacatagaatgaatagtagcgataatacc
N G F F K S Q N N T E H R M N S S D N T
aattcattaattagcagattcagacaattagccacgggtgcttataagtacaatccgttt
N S L I S R F R Q L A T G A Y K Y N P F
ttgattaatcaagtaaaaaatttgaatcaattagatggaagggtgacagatagtgacatt
L I N Q V K N L N Q L D G K V T D S D I

FIG. 5

tatagcttgtttagaaagcaatcatttagaggaaatgaatatttaaattcattacaaaa
Y S L F R K Q S F R G N E Y L N S L Q K
gggacaagctatttcagatttcaatattttaatccacttaattctagtaaatactatgaa
G T S Y F R F Q Y F N P L N S S K Y Y E
aatttagatgatcaggtttttagctttaattacaggagaaatcggctcaatgccagaactt
N L D D Q V L A L I T G E I G S M P E L
aaaaaacctacggataaagaagataaaaaatcatagcgccttcaaaaaccatagtgcat
K K P T D K E D K N H S A F K N H S A D
gagataacaacaataatgatggacactccaaagattatgataagaaaaagaaaatacat
E I T T N N D G H S K D Y D K K K K I H
cgaagtcttttatcgттаagtattgcaataattggaatttttctaggagtcactggacta
R S L L S L S I A I I G I F L G V T G L
tatatctttagaagaaaaaagtaa
Y I F R R K K *

FIG. 5 (CONT'D)

MULTICOMPONENT VACCINES

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims the benefit of U.S. provisional application Ser. No. 60/098,439, filed Aug. 31, 1998.

The present invention was made in part from work supported by grant no. 97-35204-5046 from the United States Department of Agriculture. The U.S. government has certain rights in this invention.

The invention is in the field of biological products for the treatment and diagnosis of bacterial infections.

BACKGROUND OF THE INVENTION

Staphylococci are Gram-positive spherical cells, usually arranged in grape-like irregular clusters. Some are members of the normal flora of the skin and mucous membranes of humans, others cause suppuration, abscess formation, a variety of pyogenic infections, and even fatal septicemia. Pathogenic staphylococci often hemolyze blood, coagulate plasma, and produce a variety of extracellular enzymes and toxins. The most common type of food poisoning is caused by a heat-stable staphylococci enterotoxin.

The genus *Staphylococcus* has at least 30 species. The three main species of clinical importance are *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Staphylococcus saprophyticus*. *Staphylococcus aureus* is coagulase-positive, which differentiates it from the other species. *S. aureus* is a major pathogen for humans. Almost every person has some type of *S. aureus* infection during a lifetime, ranging in severity from food poisoning or minor skin infections to severe life-threatening infections. The coagulase-negative staphylococci are normal human flora which sometimes cause infection, often associated with implanted devices, especially in very young, old and immunocompromised patients. Approximately 75% of the infections caused by coagulase-negative staphylococci are due to *S. epidermidis*. Infections due to *Staphylococcus warneri*, *Staphylococcus hominis*, and other species are less common. *S. saprophyticus* is a relatively common cause of urinary tract infections in young women. The staphylococci produce catalase, which differentiates them from the streptococci.

S. aureus colonization of the articular cartilage, of which collagen is a major component, within the joint space appears to be an important factor contributing to the development of septic arthritis. Hematogenously acquired bacterial arthritis remains a serious medical problem. This rapidly progressive and highly destructive joint disease is difficult to eradicate. Typically, less than 50% of the infected patients fail to recover without serious joint damage. *S. aureus* is the predominant pathogen isolated from adult patients with hematogenous and secondary osteomyelitis.

In hospitalized patients, *Staphylococcus* bacteria such as *S. aureus* are a major cause of infection. Initial localized infections of wounds or indwelling medical devices can lead to more serious invasive infections such as septicemia, osteomyelitis, mastitis and endocarditis. In infections associated with medical devices, plastic and metal surfaces become coated with host plasma and matrix proteins such as fibrinogen and fibronectin shortly after implantation. The ability of *S. aureus* and other staphylococcal bacteria to adhere to these proteins is essential to the initiation of infection. Vascular grafts, intravenous catheters, artificial heart valves, and cardiac assist devices are thrombogenic and prone to bacterial colonization. Of the staphylococcal

bacteria, *S. aureus* is generally the most damaging pathogen of such infections.

A significant increase in *S. aureus* isolates that exhibit resistance to most of the antibiotics currently available to treat infections has been observed in hospitals throughout the world. The development of penicillin to combat *S. aureus* was a major advance in infection control and treatment. Unfortunately, penicillin-resistant organisms quickly emerged and the need for new antibiotics was paramount. With the introduction of every new antibiotic, *S. aureus* has been able to counter with β -lactamases, altered penicillin-binding proteins, and mutated cell membrane proteins allowing the bacterium to persist. Consequently, methicillin-resistant *S. aureus* (MRSA) and multidrug resistant organisms have emerged and established major footholds in hospitals and nursing homes around the world. (Chambers, H. F., *Clin Microbiol Rev*, 1:173, 1988; and Mulligan, M. E., et al., *Am J Med*, 94:313, 1993) Today, almost half of the staphylococcal strains causing nosocomial infections are resistant to all antibiotics except vancomycin, and it appears to be only a matter of time before vancomycin will become ineffective as well.

There is a strong and rapidly growing need for therapeutics to treat infections from staphylococci such as *S. aureus* which are effective against antibiotic resistant strains of the bacteria. The U.S. National Institutes for Health has recently indicated that this goal is now a national priority.

MSCRAMMs

Bacterial adherence to host tissue occurs when specific microbial surface adhesins termed MSCRAMMs (Microbial Surface Components Recognizing Adhesive Matrix Molecules) specifically recognize and bind to extracellular matrix (ECM) components, such as fibronectin, fibrinogen, collagen, and elastin. Many pathogenic bacteria have been shown to specifically recognize and bind to various components of the ECM in an interaction which appears to represent a host tissue colonization mechanism. This adherence involves a group of bacterial proteins termed MSCRAMMs (Patti, J., et al., *Ann Rev Microbiol*, 48:585-617, 1994; Patti, J. and Hook, M., *Cur Opin Cell Biol*, 6:752-758, 1994).

MSCRAMMs on the bacterial cell surface and ligands within the host tissue interact in a lock and key fashion resulting in the adherence of bacteria to the host. Adhesion is often required for bacterial survival and helps bacteria evade host defense mechanisms and antibiotic challenges. Once the bacteria have successfully adhered and colonized host tissues, their physiology is dramatically altered and damaging components such as toxins and enzymes are secreted. Moreover, the adherent bacteria often produce a biofilm and quickly become resistant to the killing effect of most antibiotics.

A bacterium can express MSCRAMMs that recognize a variety of matrix proteins. Ligand-binding sites in MSCRAMMs appear to be defined by relatively short contiguous stretches of amino acid sequences (motifs). Because a similar motif can be found in several different species of bacteria, it appears as though these functional motifs are subjected to interspecies transfer (Patti and Hook, *Curr Opin Cell Biol*, 6:752-758, 1994). In addition, a single MSCRAMM can sometimes bind several ECM ligands.

Vaccination Studies

Historically, studies on bacterial adherence have focused primarily on Gram-negative bacteria, which express a wide

variety of fimbrial adhesive proteins (designated adhesins) on their cell surface (Falkow, S., *Cell*, 65:1099–1102, 1991). These adhesins recognize specific glycoconjugates exposed on the surface of host cells (particularly epithelial layers). Employing the lectin-like structures in attachment allows the microorganism to efficiently colonize the epithelial surfaces. This provides the bacteria an excellent location for replication and also the opportunity to disseminate to neighboring host tissues. It has been demonstrated that immunization with pilus adhesins can elicit protection against microbial challenge, such as in *Hemophilus influenzae* induced otitis media in a chinchilla model (Sirakova et al., *Infect Immun*, 62(5):2002–2020, 1994), *Moraxella bovis* in experimentally induced infectious bovine keratoconjunctivitis (Lepper et al., *Vet Microbiol*, 45(2–3):129–138, 1995), and *E. coli* induced diarrhea in rabbits (McQueen et al., *Vaccine*, 11:201–206, 1993). In most cases, immunization with adhesins leads to the production of immune antibodies that prevent infection by inhibiting bacterial attachment and colonization, as well as enhancing bacterial opsonophagocytosis and antibody-dependent complement-mediated killing.

The use of molecules that mediate the adhesion of pathogenic microbes to host tissue components as vaccine components is emerging as an important step in the development of future vaccines. Because bacterial adherence is the critical first step in the development of most infections, it is an attractive target for the development of novel vaccines. An increased understanding of the interactions between MSCRAMMs and host tissue components at the molecular level coupled with new techniques in recombinant DNA technology have laid the foundation for a new generation of subunit vaccines. Entire or specific domains of MSCRAMMs, either in their native or site-specifically altered forms, can now be produced. Moreover, the ability to mix and match MSCRAMMs from different microorganisms creates the possibility of designing a single vaccine that will protect against multiple bacteria.

Recent clinical trials with a new subunit vaccine against whooping cough, consisting of the purified *Bordetella pertussis* MSCRAMMs filamentous hemagglutinin and pertactin, in addition to an inactivated pertussis toxin, are a prime example of the success of this type of approach. Several versions of the new acellular vaccine were shown to be safe and more efficacious than the old vaccine that contained whole bacterial cells (Greco et al., *N Eng J Med*, 334:341–348, 1996; Gustaffson et al., *N Eng J Med*, 334:349–355, 1996).

Natural immunity to *S. aureus* infections remains poorly understood. Typically, healthy humans and animals exhibit a high degree of innate resistance to *S. aureus* infections. Protection is attributed to intact epithelial and mucosal barriers and normal cellular and humoral responses. Titers of antibodies to *S. aureus* components are elevated after severe infections (Ryding et al., *J Med Microbiol*, 43(5):328–334, 1995), however to date there is no serological evidence of a correlation between antibody titers and human immunity.

Over the past several decades live, heat-killed, and formalin fixed preparations of *S. aureus* cells have been tested as vaccines to prevent staphylococcal infections. A multicenter clinical trial was designed to study the effects of a commercial vaccine, consisting of a staphylococcus toxoid and whole killed staphylococci, on the incidence of peritonitis, exit site infection, and *S. aureus* nasal carriage among continuous peritoneal dialysis patients (Poole-Warren et al., *Clin Nephrol*, 35:198–206, 1991). Although immunization with the vaccine elicited an increase in the

level of specific antibodies to *S. aureus*, the incidence of peritonitis was unaffected. Similarly, immunization of rabbits with whole cells of *S. aureus* could not prevent or modify any stage in the development of experimental endocarditis, reduce the incidence of renal abscess, or lower the bacterial load in infected kidneys (Greenberg, D. P., et al., *Infect Immun*, 55:3030–3034, 1987).

Currently there is no FDA approved vaccine for the prevention of *S. aureus* infections. However, a *S. aureus* vaccine (StaphVAX), based on capsular polysaccharide, is currently being developed by NABI (North American Biologicals Inc.). This vaccine consists of type 5 or type 8 capsular polysaccharides conjugated to *Pseudomonas aeruginosa* exotoxin A (rEPA). The vaccine is designed to induce type-specific opsonic antibodies and enhance opsonophagocytosis (Karakawa et al., *Infect Immun*, 56:1090–1095, 1988). Using a refined lethal challenge mouse model (Fattom et al., *Infect Immun*, 61:1023–1032, 1993) it has been shown that intraperitoneal infusion of type 5 capsular polysaccharide specific IgG reduces the mortality of mice inoculated intraperitoneally with *S. aureus*. The type 5 capsular polysaccharide-rEPA vaccine has also been used to vaccinate seventeen patients with end-stage renal disease (Welch et al., *J Amer Soc Nephrol*, 7(2):247–253, 1996). Geometric mean (GM) IgG antibody levels to the type 5 conjugate increased between 13 and 17-fold after the first immunization, however no additional increases could be detected after additional injections. Interestingly, the GM IgM levels of the vaccinated patients were significantly lower than control individuals. Supported by the animal studies, the vaccine has recently completed a Phase II trial in continuous ambulatory peritoneal dialysis patients. The clinical trial showed the vaccine to be safe but ineffective in preventing staphylococcal infections (NABI SEC FORM 10-K405, Dec. 31, 1995). Two possible explanations for the inability of Staph VAX to prevent infections related to peritoneal dialysis in vaccinated patients are that the immunogenicity of the vaccine was too low due to suboptimal vaccine dosing or that antibodies in the bloodstream are unable to affect infection in certain anatomic areas, such as the peritoneum.

Gram-positive bacteria related sepsis is on the increase. In fact between one-third and one-half of all cases of sepsis are caused by Gram-positive bacteria, particularly *S. aureus* and *S. epidermidis*. In the United States, it can be estimated that over 200,000 patients will develop Gram-positive related sepsis this year.

Using a mouse model (Bremell et al., *Infect Immun*, 59(8):2615–2623, 1991), it has been clearly demonstrated that active immunization with M55 domain of the Col-binding MSCRAMM can protect mice against sepsis induced death. Mice were immunized subcutaneously with either M55 or a control antigen (bovine serum albumin) and then challenged intravenously with *S. aureus*. Eighty-three percent (35/42) of the mice immunized with M55 survived compared to only 27% of the BSA immunized mice (12/45). This a compilation of 3 separate studies.

Schennings, et al, demonstrated that immunization with fibronectin binding protein from *S. aureus* protects against experimental endocarditis in rats (*Micro Pathog*, 15:227–236, 1993). Rats were immunized with a fusion protein (gal-FnBP) encompassing beta-galactosidase and the domains of fibronectin binding protein from *S. aureus* responsible for binding to fibronectin. Antibodies against fusion protein gal-FnBP were shown to block the binding of *S. aureus* to immobilized fibronectin in vitro. Endocarditis in immunized and non-immunized control rats was induced by

catheterization via the right carotid artery, resulting in damaged aortic heart valves which became covered by fibrinogen and fibronectin. The catheterized rats were then infected intravenously with 1×10^5 cells of *S. aureus*. The number of bacteria associated with aortic valves was determined 11/2 days after the challenge infection and a significant difference in bacterial numbers between immunized and non-immunized groups was then observed.

A mouse mastitis model was used by Mamo, et al., (*Vaccine*, 12:988-992, 1994) to study the effect of vaccination with fibrinogen binding proteins (especially FnBP-A) and collagen binding protein from *S. aureus* against challenge infection with *S. aureus*. The mice vaccinated with fibrinogen binding proteins showed reduced rates of mastitis compared with controls. Gross examination of challenged mammary glands of mice showed that the glands of mice immunized with fibrinogen binding proteins developed mild intramammary infection or had no pathological changes compared with glands from control mice. A significantly reduced number of bacteria could be recovered in the glands from mice immunized with fibrinogen binding proteins as compared with controls. Mamo then found that vaccination with FnBP-A combined with staphylococcal alpha toxoid did not improve the protection (Mamo, et al., *Vaccine*, 12:988-992, 1994). Next, Mamo, et al., immunized mice with only collagen binding protein, which did not induce protection against the challenge infection with *S. aureus*.

Whole killed staphylococci were included in a vaccine study in humans undergoing peritoneal dialysis (Poole-Warren et al., *Clin. Nephrol.*, 35:198-206, 1991). In this clinical trial, a commercially available vaccine of alpha-hemolysin toxoid combined with a suspension of whole killed bacteria) was administered intramuscularly ten times over 12 months, with control patients receiving saline injections. Vaccination elicited significant increases in the levels of antibodies to *S. aureus* cells in the peritoneal fluid and to alpha-hemolysin in the serum. However, immunization did not reduce the incidences of peritonitis, catheter-related infections or nasal colonization among vaccine recipients. The lack of protective efficacy in this trial were attributed to a suboptimal vaccine formulation.

Secreted proteins have been explored as components of subcellular vaccines. The alpha toxin is among the most potent staphylococcal exotoxins; it has cytolytic activity, induces tissue necrosis and kills laboratory animals. Immunization with formaldehyde-detoxified alpha toxin does not protect animals from systemic or localized infections, although it may reduce the clinical severity of the infections (Ekstedt, R. D., in *The Staphylococci*, 385-418, 1972).

One study has evaluated the protective efficacy of antibodies to the *S. aureus* microcapsule in an experimental model of staphylococcal infection (Nemeth, J. and Lee, J. C., *Infect. Immun.* 63:375-380, 1995). Rats were actively immunized with killed, microencapsulated bacteria or passively immunized with high-titer rabbit antiserum specific for the capsular polysaccharide. Control animals were injected with saline or passively immunized with normal rabbit serum. Protection against catheter-induced endocarditis resulting from intravenous challenge with the same strain was then evaluated. Despite having elevated levels of anti-capsular antibodies, the immunized animals were susceptible to staphylococcal endocarditis and immunized and control animals had similar numbers of bacteria in the blood.

As described in the Detailed Description of the Invention hereinbelow, a number of patents and patent applications describe the gene sequences for fibronectin, fibrinogen,

collagen, elastin, and MHC II analogous type binding proteins. These patents and patent applications are incorporated by reference in their entirety. These documents teach that the proteins, fragments, or antibodies immunoreactive with those proteins or fragments can be used in vaccinations for the treatment of *S. aureus* infections. PCT/US97/087210 discloses the vaccination of mice with a combination of a collagen binding protein (M55 fragment), a fibronectin binding peptide (formulin treated FnBP-A (D1-D3)) and a fibrinogen binding peptide (ClfA).

The lack of adequate protection against staphylococcal infection that has been seen to date from the vaccines described above is likely the result of the failure to generate the proper immune response, perhaps along with improper immunization scheduling or an improper immunization route. Additional factors that also contribute to the poor performance of past vaccines can be reflected in the fact that staphylococcal bacteria such as *S. aureus* have been observed to temporally regulate the expression of most of its virulence factors via regulatory genes loci *agr* and *sar*. For example, *S. aureus* contains two genes that encode cell surface fibrinogen binding proteins, ClfA and ClfB. Interestingly, ClfA is predominately expressed in early exponential growth, while ClfB is expressed later in the growth phase. Accordingly, the antigens that the invading organism presents to the host in vivo may not be the same as those used in the vaccine. In addition, not every *S. aureus* antigen is expressed on every isolate. For example, only about 50% of *S. aureus* clinical isolates express the gene *cna*, which encodes for the collagen binding MSCRAMM. To generate an effective immunotherapeutic against *S. aureus*, the vaccine must be multi-component and contain antigens that span the growth cycle as well as include antigens that are expressed by a majority of *S. aureus* isolates.

Despite the advances in the art of compositions for the treatment of infections from staphylococcal bacteria such as *S. aureus*, there remains a need to provide a more effective product, and preferably one that exhibits a broad spectrum immunization against staphylococcal bacteria of various strains, and to particular proteins which may be expressed at different stages of the bacterial growth phase.

Therefore, it is an object of the invention to provide a new therapeutic composition for immunization against infections from staphylococcal bacteria such as *S. aureus* and *S. epidermidis*.

It is another object of the present invention to provide a vaccine that will provide protection against mastitis, arthritis, endocarditis, septicemia, and osteomyelitis, furunculosis, cellulitis, pyemia, pneumonia, pyoderma, supuration of wounds, food poisoning, bladder infections and other infectious diseases.

It is another object of the present invention to provide a therapeutic composition that immunizes against staphylococcal infection, enhances the amount of intracellular killing of staphylococcal bacteria, and increases the rate of phagocytosis of staphylococcal bacteria.

It is still another object of the present invention to provide a composition that will further protect the host by neutralizing exotoxins.

SUMMARY OF THE INVENTION

It has been discovered that the treatment of staphylococcal infections can be significantly enhanced by immunization with certain selected combinations of bacterial binding proteins or fragments thereof, or antibodies to those proteins

or fragments. The proteins or fragments can be used in active vaccines, and the antibodies in passive vaccines. Alternatively, the combinations can be used to select donor blood pools for the preparation of purified blood products for passive immunization. By careful selection of the proteins, fragments, or antibodies, a vaccine is provided that imparts protection against a broad spectrum of Staphylococcus bacterial strains and against proteins that are expressed at different stages of the logarithmic growth curve.

The vaccine and products described herein respond to the urgent need of the medical community for a substitute for small molecule antibiotics, which are rapidly losing effectiveness. The vaccines are a significant improvement over the prior art, which while generally teaching the use of MSCRAMMs to impart immunization, did not teach which combinations of the large number of known MSCRAMMs should be used to impart superior protection.

In one embodiment of the invention, a composition is provided that includes at least a collagen binding protein or peptide (or an appropriate site directed mutated sequence thereof) such as CNA, or a protein or fragment with sufficiently high homology thereto, in combination with a fibrinogen binding protein, preferably Clumping factor A ("ClfA") or Clumping factor B ("ClfB"), or a useful fragment thereof or a protein or fragment with sufficiently high homology thereto.

In another embodiment of the invention, a composition is provided that includes at least a fibronectin binding protein or peptide (or an appropriate site directed mutated sequence thereof), or a protein or fragment with sufficiently high homology thereto, in combination with the fibrinogen binding protein, preferably A or B (ClfA or ClfB, respectively), or a useful fragment thereof or a protein or fragment with sufficiently high homology thereto.

In a third embodiment, a composition is provided that includes at least the fibrinogen binding protein A (ClfA) and the fibrinogen binding protein B (ClfB), or useful fragments thereof or a protein or fragment with sufficiently high homology thereto.

In a fourth embodiment, a composition is provided that includes at least a fibronectin binding protein or peptide (or an appropriate site directed mutated sequence thereof), or a protein or fragment with sufficiently high homology thereto, in combination with (i) the fibrinogen binding protein A and B (ClfA and ClfB), or a useful fragment thereof or a protein or fragment with sufficiently high homology thereto; and (ii) a collagen binding protein or useful fragment thereof.

In an additional embodiment, a composition is provided that includes the components of the prior embodiments in combination with an elastin binding protein or peptide or a protein or fragment with sufficiently high homology thereto.

In another embodiment, a composition is provided that includes the components of the prior embodiments in combination with a MHC II analogous protein or peptide or a protein or fragment with sufficiently high homology thereto.

In another embodiment, a composition is provided that includes the components of any of the prior combinations in combination with a bacterial component to increase the rate of phagocytosis of the staphylococcal bacteria. In a one such embodiment, the bacterial component comprises a capsular polysaccharide, such as capsular polysaccharide type 5 or type 8.

In an additional embodiment, a composition is provided that includes any of the prior combinations in combination with the extracellular matrix-binding proteins SdrC, SdrD,

SdrE or a consensus or variable sequence amino acid motif, or useful fragments thereof or proteins or fragments with sufficiently high homology thereto.

In an additional embodiment, a composition is provided that includes and of the prior combinations in combination with the extracellular matrix-binding proteins SdrF, SdrG, or SdrH, or a consensus or variable sequence amino acid motif, or useful fragments thereof or proteins or fragments with sufficiently high homology thereto. This embodiment is particularly effective in developing vaccines that can be useful with regard to both coagulase-positive and coagulase-negative staphylococcal bacteria.

In another embodiment, a composition is provided that includes at least the extracellular matrix-binding proteins SdrC, SdrD and SdrE or useful fragments thereof, such as the consensus or variable sequence amino acid motif, or a protein or fragment with sufficiently high homology thereto.

Alternatively, compositions are provided that include monoclonal or polyclonal antibodies which are immunoreactive to the selected combination of described components. These compositions can be used in vaccinations to treat patients infected with Staphylococcus infections.

In other embodiments of the invention, the combinations of proteins, fragments or antibodies as described are used in diagnostic kits.

As described below, proteins and peptides to be used in the composition which bind to fibronectin, fibrinogen, collagen, and elastin are known. Alternatively, one can identify new fibronectin, fibrinogen, collagen, and elastin binding proteins, or the epitopes thereof for use in the composition. Methods of identifying a peptide of a binding domain of a binding protein that binds to the ligand of choice are known. For example, one can contact a candidate protein or peptide with the ligand under conditions effective to allow binding of the ligand to the binding domain of a binding protein, and identify a positive candidate peptide that binds to the ligand.

Antibodies that bind to the binding domains of the composition proteins or peptides can be generated by administering to an animal a pharmaceutical composition comprising an immunologically effective amount of the combination of proteins or peptides, even though the peptide does not specifically bind to the ECM.

The combination of the isolated, recombinant or synthetic MSCRAMM proteins, or active fragments thereof or fusion proteins thereof, are also useful as scientific research tools to identify staphylococcal binding sites on the host ECM molecules, thereby promoting an understanding of the mechanisms of bacterial pathology and the development of antibacterial therapies. Furthermore, the isolated, recombinant or synthetic proteins, or antigenic portions thereof (including epitope-bearing fragments), or fusion proteins thereof can be administered to animals as immunogens or antigens, alone or in combination with an adjuvant, for the production of antisera reactive with MSCRAMM proteins. In addition, the proteins can be used to screen antisera for hyperimmune patients from whom can be derived antibodies having a very high affinity for the proteins. Antibodies isolated from the antisera are useful for the specific detection of staphylococcal bacteria or binding proteins, as research tools, or as therapeutic treatments against staphylococcal infection.

The proteins, or active fragments thereof, and antibodies to the proteins are useful for the treatment of infections from staphylococcal infections from bacteria such as *S. aureus* as described above; for the development of anti-

Staphylococcus vaccines for active or passive immunization; and, when administered as pharmaceutical composition to a wound or used to coat medical devices or polymeric biomaterials in vitro and in vivo, both the proteins and the antibodies are useful as blocking agents to prevent or inhibit the binding of staphylococcal bacteria to the wound site or biomaterials.

Preferably, animal derived antibody is modified so that it is less immunogenic in the patient to whom it is administered. For example, if the patient is a human, the antibody may be "humanized" by transplanting the complementarily determining regions of the hybridoma-derived antibody into a human monoclonal antibody as described by Jones et al. (*Nature* 321:522-525 (1986)) or Tempest et al. (*Biotechnology* 9:266-273 (1991)).

Kits are also provided that are useful as a diagnostic agent for the detection of staphylococcal infections. According to yet another embodiment, anti-MSCRAMM antibodies as well as the MSCRAMM polypeptides of this invention, are useful as diagnostic agents for detecting infection by staphylococcal bacteria, because the polypeptides are capable of binding to antibody molecules produced in animals, including humans that are infected with staphylococcal bacteria such as *S. aureus*, and the antibodies are capable of binding to particular staphylococcal bacteria or antigens thereof.

Diagnostic agents may be included in a kit which can also include instructions for use and other appropriate reagents. The kit can also contain a means to evaluate the product of the assay, for example, a color chart, or numerical reference chart. The polypeptide or antibody may be labeled with a detection means that allows for the detection of the MSCRAMM polypeptide when it is bound to an antibody, or for the detection of the anti-MSCRAMM polypeptide antibody when it is bound to Staphylococcus bacteria.

The detection means may be a fluorescent labeling agent such as fluorescein isocyanate (FIC), fluorescein isothiocyanate (FITC), and the like, an enzyme, such as horseradish peroxidase (HRP), glucose oxidase or the like, a radioactive element such as ¹²⁵I or ⁵¹Cr that produces gamma ray emissions, or a radioactive element that emits positrons which produce gamma rays upon encounters with electrons present in the test solution, such as ¹¹C, ¹⁵O, or ¹⁵N. The linking of the detection means is well known in the art. For instance, monoclonal anti-MSCRAMM polypeptide antibody molecules produced by a hybridoma can be metabolically labeled by incorporation of radioisotope-containing amino acids in the culture medium, or polypeptides may be conjugated or coupled to a detection means through activated functional groups.

The diagnostic kits of the present invention may be used to detect the presence of a quantity of Staphylococcus bacteria or anti-Staphylococcus antibodies in a body fluid sample such as serum, plasma or urine. Thus, in preferred embodiments, an MSCRAMM polypeptide or anti-MSCRAMM polypeptide antibody composition of the present invention is bound to a solid support typically by adsorption from an aqueous medium. Useful solid matrices are well known in the art, and include crosslinked dextran; agarose; polystyrene; polyvinylchloride; cross-linked polyacrylamide; nitrocellulose or nylon-based materials; tubes, plates or the wells of microtiter plates. The polypeptides or antibodies of the present invention may be used as diagnostic agents in solution form or as a substantially dry powder, e.g., in lyophilized form.

BRIEF DESCRIPTION OF THE DRAWING FIGURES

FIG. 1 is a schematic representation of the peptides used in illustrative vaccine, MSCRAMM IV. This drawing illus-

trates the essential features of the collagen binding MSCRAMM CNA, fibrinogen binding MSCRAMM ClfA, fibrinogen binding MSCRAMM ClfB and fibronectin binding MSCRAMM FnBPA proteins. The MSCRAMMs are shown with regions denoted that were expressed as recombinant proteins and used to generate antibodies in rabbits immunized with MSCRAMM IV. All proteins were designed with an amino terminal histidine tag to facilitate purification by metal chelating chromatography.

FIG. 2 is a time course graph of the immune response in MSCRAMM vaccinated Rhesus Monkeys as shown by changes in antibody titers against the MSCRAMMs CNA, ClfA, ClfB and FnBPA, respectively. The titers were analyzed by ELISA and measured as changes in absorbance (quantified at 405 nm) during each week over the course of a six-month period of treatment following the original immunization with the antigen.

FIG. 3 shows the nucleic acid sequence coding for the *sdrF* gene from *S. epidermidis*, identified as SEQ ID NO: 1 and the amino acid sequence coded thereby, identified as SEQ ID NOS: 2-6.

FIG. 4 shows the nucleic acid sequence coding for the *sdrG* gene from *S. epidermidis*, identified as SEQ ID NO: 7 and the amino acid sequence coded thereby, identified as SEQ ID NOS: 8-12.

FIG. 5 shows the nucleic acid sequence coding for the *sdrH* gene from *S. epidermidis*, identified as SEQ ID NO: 13 and the amino acid sequence coded thereby, identified as SEQ ID NO: 14.

DETAILED DESCRIPTION OF THE INVENTION

Compositions suitable for use as vaccines are provided that include at least:

- (i) A collagen binding protein, peptide or domain (or an appropriate site directed mutated sequence thereof) such as CNA, or a protein, fragment or domain with sufficiently high homology thereto, in combination with a fibrinogen binding protein, preferably Clumping factor A ("ClfA") or Clumping factor B ("ClfB"), or a useful fragment thereof or a protein or fragment with sufficiently high homology thereto;
- (ii) a fibronectin binding protein or peptide (or an appropriate site directed mutated sequence thereof), or a protein or fragment with sufficiently high homology thereto, in combination with the fibrinogen binding proteins A and B (ClfA and ClfB), or useful fragments thereof or proteins or fragments with sufficiently high homology thereto; or
- (iii) the fibrinogen binding protein A (ClfA) and the fibrinogen binding protein B (ClfB), or useful fragments thereof or a protein or fragment with sufficiently high homology thereto; or
- (iv) fibronectin binding protein or peptide (or an appropriate site directed mutated sequence thereof), or a protein or fragment with sufficiently high homology thereto, in combination with the fibrinogen binding protein A and B (ClfA and ClfB), or a useful fragment thereof or a protein or fragment with sufficiently high homology thereto; and a collagen binding protein or useful fragment thereof, or a protein or fragment with sufficiently high homology thereto;
- (v) components of any of the above embodiments in combination with an elastin binding protein or peptide or a protein or fragment with sufficiently high homology thereto; or

- (vi) components of any of the above embodiments in combination with a MHC II analogous type binding protein or peptide, protein or fragment with sufficiently high homology thereto; or
- (vi) components of any of the above embodiments in combination with a bacterial component to increase the rate of phagocytosis of a staphylococcal bacteria such as *S. aureus*; or
- (vii) components of any of the above embodiments in combination with the extracellular matrix-binding proteins SdrC, SdrD or SdrE, or useful fragments thereof, such as a consensus or variable sequence amino acid motif, or proteins or fragments with sufficiently high homology thereto; or
- (viii) components of any of the above embodiments in combination with the extracellular matrix-binding proteins SdrF, SdrG or SdrH, or useful fragments thereof, such as a consensus or variable sequence amino acid motif, or proteins or fragments with sufficiently high homology thereto, such that a vaccine created from said components will also be useful to immunize a patient against infection from coagulase-negative bacteria such as *S. epidermidis* as well as coagulase positive bacteria such as *S. aureus*; or
- (ix) the extracellular matrix-binding proteins SdrC, SdrD and SdrE or useful fragments thereof, such as a consensus or variable sequence amino acid motif, or a protein or fragment with sufficiently high homology thereto.

Isolated protein fragments from wild-type or naturally occurring variants or synthetic or recombinant peptides corresponding to wild-type, naturally occurring variants or introduced mutations that do not correspond to a naturally occurring binding domain of a binding protein can be used in these embodiments.

The isolated peptides should be of a sufficient length to allow for the generation of an antibody that binds both to the isolated peptide and the binding domain, and blocks the binding of the binding protein to its ligand. In certain aspects, peptides comprising at least about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 22, about 24, about 25, about 30, about 35, about 40, about 45 or about 50 contiguous amino acids are preferred. In other preferred aspects of the invention, the isolated peptide comprises at least about 6 contiguous amino acids from the wild type sequence of the binding domain.

In one aspect of the invention, the isolated peptide or antibody compositions are used to generate an immunological response in an animal. In this aspect, the compositions preferably further comprise an adjuvant. Many adjuvants are known for use in vaccinations and are readily adapted to this composition. The isolated peptide or protein composition is preferably dispersed in a pharmaceutically acceptable excipient.

The isolated peptide can be linked to a selected amino acid sequence to make a fusion protein. As a nonlimiting example, a fusion protein can be made that comprises at least a first peptide of a binding domain of a binding protein operatively linked to a selected amino acid sequence. In one embodiment, if the peptide is a fibronectin binding domain, the first peptide does not specifically bind to fibronectin. In preferred aspects, the first peptide is linked to a selected carrier molecule or amino acid sequence, including, but not limited to, keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA).

Immunological compositions, including vaccine, and other pharmaceutical compositions containing the selected

MSCRAMM proteins or the DNA encoding such MSCRAMM proteins are included within the scope of the present invention. The combination of binding proteins, or active or antigenic fragments thereof, or fusion proteins thereof can be formulated and packaged, alone or in combination with other antigens, using methods and materials known to those skilled in the art for vaccines. The immunological response may be used therapeutically or prophylactically and may provide antibody immunity or cellular immunity such as that produced by T lymphocytes such as cytotoxic T lymphocytes or CD4+ T lymphocytes.

Vaccines can be prepared for use in both active and passive immunizations. Preferably the antigenic material is extensively dialyzed to remove undesired small molecular weight molecules and/or lyophilized for more ready formulation into a desired vehicle.

I. Definitions

The terms FnBP-A protein, FnBP-B protein, ClfA protein, ClfB protein, SdrC protein, SdrD protein, SdrE protein, SdrF protein, SdrG protein, SdrH protein, CNA protein, EbpS protein and MHCII protein are defined herein to include FnBP-A, FnBP-B, ClfA, ClfB, SdrC, SdrD, SdrE, SdrF, SdrG, SdrH, CNA, EbpS; and MHCII subdomains, respectively, active or antigenic fragments of FnBP-A, FnBP-B, ClfA, ClfB, SdrC, SdrD, SdrE, SdrF, SdrG, SdrH, CNA, EbpS and MHCII proteins, and proteins or fragments that have sufficiently high homology therewith. Active fragments of FnBP-A, FnBP-B, ClfA, ClfB, SdrC, SdrD, SdrE, SdrF, SdrG, SdrH, CNA, EbpS and MHCII proteins are defined herein as peptides or polypeptides capable of blocking the binding of *Staphylococcus* bacteria to host ECM. Antigenic fragments of FnBP-A, FnBP-B, ClfA, ClfB, SdrC, SdrD, SdrE, SdrF, SdrG, SdrH, CNA, EbpS and MHCII proteins are defined herein as peptides or polypeptides capable of producing an immunological response.

The term "adhesin" as used herein includes naturally occurring and synthetic or recombinant proteins and peptides which can bind to extracellular matrix proteins and/or mediate adherence to host cells.

The term "amino acid" as used herein includes naturally occurring and synthetic amino acids and includes, but is not limited to, alanine, valine, leucine, isoleucine, proline, phenylalanine, tryptophan, methionine, glycine, serine, threonine, cysteine, tyrosine, asparagine, glutamate, aspartic acid, glutamic acid, lysine, arginine, and histidine.

An "antibody" is any immunoglobulin, including antibodies and fragments thereof, that binds a specific epitope. The term as used herein includes monoclonal antibodies, polyclonal, chimeric, single chain, bispecific, simianized, and humanized-antibodies as well as Fab fragments, including the products of an Fab immunoglobulin expression library.

The phrase "antibody molecule" in its various grammatical forms as used herein contemplates both an intact immunoglobulin molecule and an immunologically active portion of an immunoglobulin molecule.

As used herein, an "antigenically functional equivalent" protein or peptide is one that incorporates an epitope that is immunologically cross-reactive with one or more epitopes either derived from any of the particular MSCRAMM proteins disclosed (e.g., FnB-B, FnB-A, FnBP-B and FnBP-A) or derived from any of the particular bacterial components disclosed (e.g., teichoic acids, alpha toxin and capsular polysaccharide type 5). Antigenically functional equivalents, or epitopic sequences, may be first designed or predicted and then tested, or may simply be directly tested for cross-reactivity.

As used herein, "pg" means picogram, "ng" means nanogram, "ug" or "μg" mean microgram, "mg" means milligram, "ul" or "μl" mean microliter, "ml" means milliliter, "l" means liter.

A "cell line" is a clone of a primary cell that is capable of stable growth in vitro for many generations.

A "clone" is a population of cells derived from a single cell or common ancestor by mitosis.

A DNA "coding sequence" is a double-stranded DNA sequence which is transcribed and translated into a polypeptide in vivo when placed under the control of appropriate regulatory sequences. The boundaries of the sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxyl) terminus. A coding sequence can include, but is not limited to, prokaryotic sequences, cDNA from eukaryotic mRNA, genetic DNA sequences from eukaryotic (e.g., mammalian) DNA, and even synthetic DNA sequences. A polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence. "DNA molecule" refers to the polymeric form of deoxyribonucleotides (adenine, guanine, thymine, or cytosine) in its either single stranded form, or a double-stranded helix. This term refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, inter alia, in linear DNA molecules (e.g., restriction fragments), viruses, plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the nontranscribed strand of DNA (i.e., the strand having a sequence homologous to the mRNA. Transcriptional and translational control sequences are "DNA regulatory sequences", such as promoters, enhancers, polyadenylation signals, terminators, and the like, that provide for the expression of a coding sequence in a host cell.

An "expression control sequence" is a DNA sequence that controls and regulates the transcription and translation of another DNA sequence. A coding sequence is "under the control" of transcriptional and translational control sequences in a cell when RNA polymerase transcribes the coding sequence into mRNA, which is then translated into the protein encoded by the coding sequence.

As used herein, the term "extracellular matrix proteins," or ECM, refers to four general families of macromolecules, collagens, structural glycoproteins, proteoglycans and elastins, including fibronectin, and fibrinogen, that provide support and modulate cellular behavior.

"Immunologically effective amounts" are those amounts capable of stimulating a B cell and/or T cell response.

As used herein, the term "in vivo vaccine" refers to immunization of animals with proteins so as to elicit a humoral and cellular response that protects against later exposure to the pathogen.

The term "ligand" is used to include molecules, including those within host tissues, to which pathogenic bacteria attach.

The term "MHC II antigens" as used herein refers to cell-surface molecules that are responsible for rapid graft rejections and are required for antigen presentation to T-cells.

The phrase "monoclonal antibody" in its various grammatical forms refers to an antibody having only one species of antibody combining site capable of immunoreacting with a particular antigen.

The term "oligonucleotide," as used herein is defined as a molecule comprised of two or more nucleotides, preferably

more than three. Its exact size will depend upon many factors which, in turn, depend upon the ultimate function and use of the oligonucleotide.

As used herein, the phrase "pharmaceutically acceptable" refers to molecular entities and compositions that are physiologically tolerable and do not typically produce an unacceptable allergic or similar untoward reaction when administered to a human.

The term "primer" as used herein refers to an oligonucleotide, whether occurring naturally as in a purified restriction digest or produced synthetically, which is capable of acting as a point of initiation of synthesis when placed under conditions in which synthesis of a primer extension product, which is complementary to a nucleic acid strand, is induced, i.e., in the presence of nucleotides and an inducing agent such as a DNA polymerase and at a suitable temperature and pH. The primer may be either single-stranded or double-stranded and must be sufficiently long to prime the synthesis of the desired extension product in the presence of the inducing agent. The exact length of the primer will depend upon many factors, including temperature, source of primer and use of the method. For example, for diagnostic applications, depending on the complexity of the target sequence, the oligonucleotide primer typically contains 15-25 or more nucleotides, although it may contain fewer nucleotides.

The primers herein are selected to be substantially complementary to different strands of a particular target DNA sequence. This means that the primers must be sufficiently complementary to hybridize with their respective strands. Therefore, the primer sequence need not reflect the exact sequence of the template. For example, a noncomplementary nucleotide fragment may be attached to the 5' end of the primer, with the remainder of the primer sequence being complementary to the strand. Alternatively, non-complementary bases or longer sequences can be interspersed into the primer, provided that the primer sequence has sufficient complementarity with the sequence of the strand to hybridize therewith and thereby form the template for the synthesis of the extension product.

A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of defining the present invention, the promoter sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site (conveniently defined by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. Eukaryotic promoters will often, but not always, contain "TATA" boxes and "CAT" boxes. Prokaryotic promoters contain Shine-Dalgarno sequences in addition to the -10 and -35 consensus sequences.

A "replicon" is a genetic element (e.g., plasmid, chromosome, virus) that functions as an autonomous unit of DNA replication in vivo; i.e., capable of replication under its own control.

As used herein, the terms "restriction endonucleases" and "restriction enzymes" refer to bacterial enzymes, each of which cut double-stranded DNA at or near a specific palindromic nucleotide sequence.

A "signal sequence" can be included before the coding sequence. This sequence encodes a signal peptide, N-terminal to the polypeptide, that communicates to the host

cell to direct the polypeptide to the cell surface or secrete the polypeptide into the media, and this signal peptide is clipped off by the host cell before the protein leaves the cell. Signal sequences can be found associated with a variety of proteins native to prokaryotes and eukaryotes.

As used herein, the term "site directed mutagen" refers to a compound that can increase the rate at which mutations occur at a certain site within the DNA molecule.

A cell has been "transformed" by exogenous or heterologous DNA when such DNA has been introduced inside the cell. The transforming DNA may or may not be integrated (covalently linked) into chromosomal DNA making up the genome of the cell. In prokaryotes, yeast, and mammalian cells for example, the transforming DNA may be maintained on an episomal element such as a plasmid. With respect to eukaryotic cells, a stably transformed cell is one in which the transforming DNA has become integrated into a chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eukaryotic cell to establish cell lines or clones comprised of a population of daughter cells containing the transforming DNA.

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.

The term "wound" is used herein to mean the epithelial cellular layer, and other surface structures over tissue, damaged by mechanical, chemical or other influence.

By "immunologically effective amount" is meant an amount of a peptide composition that is capable of generating an immune response in the recipient animal. This includes both the generation of an antibody response (B cell response), and/or the stimulation of a cytotoxic immune response (T cell response). The generation of such an immune response will have utility in both the production of useful bioreagents, e.g., CTLs and, more particularly, reactive antibodies, for use in diagnostic embodiments, and will also have utility in various prophylactic or therapeutic embodiments.

The selected combinations of bacterial binding proteins or fragments thereof in the composition used include those binding to fibronectin, fibrinogen, collagen, and elastin. Any such protein, peptide, fragment thereof, or sequence substantially homologous thereto can be used in this invention. Illustrative examples are provided below. In addition, bacterial binding proteins or fragments to MHC II analogous II. Fibronectin-Binding MSCRAMMs

Fibronectin (Fn) is a 440-kDa glycoprotein found in the ECM and body fluids of animals. The primary biological function of fibronectin appears to be related to its ability to serve as a substrate for the adhesion of cells expressing the appropriate integrins. Several bacterial species have been shown to bind fibronectin specifically and to adhere to a fibronectin-containing substratum. Most *S. aureus* isolates bind Fn, but do so in varying extents, which reflects variations in the number of MSCRAMM molecules expressed on the bacterial cell surface. The interaction between Fn and *S. aureus* is highly specific (Kuusela, P., *Nature*, 276:718-20, 1978). Fn binding is mediated by two surface exposed proteins with molecular weights of 110 kDa, named FnBP-A and FnBP-B. The primary Fn binding site consists of a motif of 35-40 amino acids, repeated three to five times. The genes for these have been cloned and sequenced (Jonsson, K., et al., *Eur. J. Biochem.*, 202:1041-1048, 1991).

WO-A-85/05553 discloses bacterial cell surface proteins having fibronectin, fibrinogen, collagen, and or laminin binding ability.

U.S. Pat. Nos. 5,320,951 and 5,571,514 to Hook, et al., discloses the fibronectin binding protein A (fnbA) gene sequence, and products and methods based on this sequence.

U.S. Pat. No. 5,175,096 to Hook et al., discloses the gene sequence of fnbB, a hybrid DNA molecule (fnbB) and biological products and methods based on this sequence.

U.S. Pat. No. 5,652,217 discloses an isolated and purified protein having binding activity that is encoded by a hybrid DNA molecule from *S. aureus* of defined sequence.

U.S. Pat. No. 5,440,014 discloses a fibronectin binding peptide within the D3 homology unit of a fibronectin binding protein of *S. aureus* which can be used for vaccination of ruminants against mastitis caused by staphylococcal infections, for treatment of wounds, for blocking protein receptors, for immunization of other animals, or for use in a diagnostic assay.

U.S. Pat. No. 5,189,015 discloses a method for the prophylactic treatment of the colonization of a *S. aureus* bacterial strain having the ability to bind to fibronectin in a mammal that includes administering to the mammal in need of treatment a prophylactically therapeutically active amount of a protein having fibronectin binding properties, to prevent the generation of infections caused by a *S. aureus* bacterial strain having the ability to bind fibronectin, wherein the protein has a molecular weight of 87 kDa to 165 kDa.

U.S. Pat. No. 5,416,021 discloses a fibronectin binding protein encoding DNA from *Streptococcus dysgalactiae*, along with a plasmid that includes DNA encoding for fibronectin binding protein from *S. dysgalactiae* contained in *E. coli*, DNA encoding a fibronectin binding protein from *S. dysgalactiae* and an *E. coli* microorganism transformed by DNA encoding a fibronectin binding protein from *S. dysgalactiae*.

It has been observed that antibodies to wild type fibronectin binding protein do not substantially inhibit the ability of *S. aureus* to bind to fibronectin, and thus do not exhibit a significant therapeutic effect, in vivo. PCT/US98/01222 discloses antibodies that block the binding of fibronectin to fibronectin binding proteins. The antibodies were raised against a site-directed mutated sequence of fibronectin binding protein that does not bind to fibronectin. It was identified that there is a rapid complexation of fibronectin with fibronectin binding proteins and fragments in vivo. Peptide epitopes that do not bind to fibronectin, even though based on a fibronectin binding domain of a fibronectin binding protein, do not form a complex with fibronectin in vivo. This allows antibodies to be made against the uncomplexed peptide epitope, which inhibit or block the binding of fibronectin to fibronectin binding proteins.

III. Collagen-Binding MSCRAMMs

Collagen is the major constituent of cartilage. Collagen (Cn) binding proteins are commonly expressed by staphylococcal strains. The Cn binding MSCRAMM of *S. aureus* adheres to cartilage in a process that constitutes an important part of the pathogenic mechanism in staphylococcal infections. (Switalski, et al. *Mol. Micro.* 7(1), 99-107, 1993) Cn binding by *S. aureus* is found to play a role in at least, but not only, arthritis and septicemia. CNAs with molecular weights of 133, 110 and 87 kDa (Pati, J., et al., *J. Biol. Chem.*, 267:4766-4772, 1992) have been identified. Strains expressing CNAs with different molecular weights do not differ in their Cn binding ability (Switalski, L. M., et al, *Mol. Microbiol.*, 7:99-107, 1993).

Staphylococcal strains recovered from the joints of patients diagnosed with septic arthritis or osteomyelitis almost invariably express a CBP, whereas significantly

fewer isolates obtained from wound infections express this adhesin (Switalski et al., *Mol. Microbiol.*, 7:99–107, 1993). Similarly, *S. aureus* strains isolated from the bones of patients with osteomyelitis often have an MSCRAMM recognizing the bone-specific protein, bone sialoprotein (BSP) (Ryden et al., *Lancet*, 11:515–518, 1987). *S. aureus* colonization of the articular cartilage within the joint space appears to be an important factor contributing to the development of septic arthritis.

PCT WO 92/07002 discloses a hybrid DNA molecule which includes a nucleotide sequence from *S. aureus* coding for a protein or polypeptide having collagen binding activity and a plasmid or phage comprising the nucleotide sequence. Also disclosed are an *E. coli* strain expressing the collagen binding protein, a microorganism transformed by the recombinant DNA, the method for producing a collagen binding protein or polypeptide, and the protein sequence of the collagen binding protein or polypeptide.

The cloning, sequencing, and expression of a gene *cna*, encoding a *S. aureus* CBP has been reported (Patti, J., et al., *J. Biol. Chem.*, 267:4766–4772, 1992). The *cna* gene encodes an 133-kDa adhesin that contains structural features characteristic of surface proteins isolated from Gram-positive bacteria.

Recently, the ligand-binding site has been localized within the N-terminal half of the CBP (Patti, J. et al, *Biochemistry*, 32:11428–11435, 1993). By analyzing the Col binding activity of recombinant proteins corresponding to different segments of the MSCRAMM, a 168-amino-acid long protein fragment (corresponding to amino acid residues 151–318) that had appreciable Col binding activity was identified. Short truncations of this protein in the N or C terminus resulted in a loss of ligand binding activity but also resulted in conformational changes in the protein as indicated by circular dichroism spectroscopy.

Patti et al. (*J of Biol Chem.*, 270, 12005–12011, 1995) disclose a collagen binding epitope in the *S. aureus* adhesin encoded by the *cna* gene. In their study, the authors synthesized peptides derived from the sequence of the said protein and used them to produce antibodies. Some of these antibodies inhibit the binding of the protein to collagen.

PCT/US97/08210 discloses that certain identified epitopes of the collagen binding protein (M55, M33, and M17) can be used to generate protective antibodies. The application also discloses the crystal structure of the CBP which provides critical information necessary for identifying compositions which interfere with, or block completely, the binding of Col to CBPS. The ligand-binding site in the *S. aureus* CBP and a 25-amino-acid peptide was characterized that directly inhibits the binding of *S. aureus* to 125 I-labeled type II Col.

IV. Fibrinogen-Binding MSCRAMMs

Fibrin is the major component of blood clots, and fibrinogen/fibrin is one of the major plasma proteins deposited on implanted biomaterials. Considerable evidence exists to suggest that bacterial adherence to fibrinogen/fibrin is important in the initiation of device-related infection. For example, as shown by Vaudaux et al, *S. aureus* adheres to in vitro plastic that has been coated with fibrinogen in a dose-dependent manner (*J. Infect. Dis.* 160:865–875 (1989)). In addition, in a model that mimics a blood clot or damage to a heart valve, Herrmann et al demonstrated that *S. aureus* binds avidly via a fibrinogen bridge to platelets adhering to surfaces (*J. Infect. Dis.* 167: 312–322 (1993)). *S. aureus* can adhere directly to fibrinogen in blood clots formed in vitro, and can adhere to cultured endothelial cells via fibrinogen deposited from plasma acting as a bridge

(Moreillon et al., *Infect. Immun.* 63:4738–4743 (1995); Cheung et al., *J. Clin. Invest.* 87:2236–2245 (1991)). As shown by Vaudaux et al. and Moreillon et al., mutants defective in the fibrinogen-binding protein clumping factor (ClfA) exhibit reduced adherence to fibrinogen in vitro, to explanted catheters, to blood clots, and to damaged heart valves in the rat model for endocarditis (Vaudaux et al., *Infect Immun.* 63:585–590 (1995); Moreillon et al., *Infect. Immun.* 63: 4738–4743 (1995)).

An adhesin for fibrinogen, often referred to as “clumping factor,” is located on the surface of *S. aureus* cells. The interaction between bacteria and fibrinogen in solution results in the instantaneous clumping of bacterial cells. The binding site on fibrinogen is located in the C-terminus of the gamma chain of the dimeric fibrinogen glycoprotein. The affinity is very high and clumping occurs in low concentrations of fibrinogen. Scientists have recently shown that clumping factor also promotes adherence to solid phase fibrinogen, to blood clots, and to damaged heart valves (McDevitt et al., *Mol. Microbiol.* 11:237–248 (1994); Vaudaux et al., *Infect. Immun.* 63:585–590 (1995); Moreillon et al., *Infect. Immun.* 63: 4738–4743 (1995)).

Two genes in *S. aureus* have been found that code for two Fg binding proteins, ClfA and ClfB. The gene, *clfA*, was cloned and sequenced and found to code for a polypeptide of 92 kDa. ClfA binds the gamma chain of fibrinogen, and ClfB binds the alpha and beta chains (Eidhin, et al., *Mol Micro*, awaiting publication, 1998). ClfB is a cell wall associated protein with a predicted molecular weight of 88 kDa and an apparent molecular weight of 124 kDa that binds both soluble and immobilized fibrinogen and acts as a clumping factor.

The gene for a clumping factor protein, designated ClfA, was cloned, sequenced and analyzed in detail at the molecular level (McDevitt et al., *Mol. Microbiol.* 11: 237–248 (1994); McDevitt et al., *Mol. Microbiol.* 16:895–907 (1995)). The predicted protein is composed of 933 amino acids. A signal sequence of 39 residues occurs at the N-terminus followed by a 520 residue region (region A), which contains the fibrinogen binding domain. A 308 residue region (region R), composed of 154 repeats of the dipeptide serine-aspartate, follows. The R region sequence is encoded by the 18 basepair repeat GAY TCN GAY TCN GAY AGY (SEQ ID NO: 15) in which Y equals pyrimidines and N equals any base. The C-terminus of ClfA has features present in many surface proteins of gram-positive bacteria such as an LPDTG (SEQ ID NO: 16) motif, which is responsible for anchoring the protein to the cell wall, a membrane anchor, and positive charged residues at the extreme C-terminus.

The platelet integrin alpha IIB β 3 recognizes the C-terminus of the gamma chain of fibrinogen. This is a crucial event in the initiation of blood clotting during coagulation. ClfA and alpha IIB β 3 appear to recognize precisely the same sites on fibrinogen gamma chain because ClfA can block platelet aggregation, and a peptide corresponding to the C-terminus of the gamma chain (198-41 1) can block both the integrin and ClfA interacting with fibrinogen (McDevitt et al., *Eur. J. Biochem.* 247:416–424 (1997)). The fibrinogen binding site of alpha IIB β 3 is close to, or overlaps, a Ca²⁺ binding determinant referred to as an “EF hand”. ClfA region A carries several EF hand-like motifs. A concentration of Ca²⁺ in the range of 3–5 mM blocks these ClfA-fibrinogen interactions and changes the secondary structure of the ClfA protein. Mutations affecting the ClfA EF hand reduce or prevent interactions with fibrinogen. Ca²⁺ and the fibrinogen gamma chain seem to bind to the same, or to overlapping, sites in ClfA region A.

The alpha chain of the leukocyte integrin, alpha MB2, has an insertion of 200 amino acids (A or I domain) which is responsible for ligand binding activities. A novel metal ion-dependent adhesion site (MIDAS) motif in the I domain is required for ligand binding. Among the ligands recognized is fibrinogen. The binding site on fibrinogen is in the gamma chain (residues 190–202). It was recently reported that *Candida albicans* has a surface protein, alpha Intlp, having properties reminiscent of eukaryotic integrins. The surface protein has amino acid sequence homology with the I domain of MB2, including the MIDAS motif. Furthermore, Intlp binds to fibrinogen.

ClfA region A also exhibits some degree of sequence homology with alpha Intlp. Examination of the ClfA region A sequence has revealed a potential MIDAS motif. Mutations in putative cation coordinating residues in the DxSxS portion of the MIDAS motif in ClfA results in a significant reduction in fibrinogen binding. A peptide corresponding to the gamma-chain binding site for alpha M β 2 (190–202) has been shown by O'Connell et al. to inhibit ClfA-fibrinogen interactions (O'Connell, *J. Biol. Chem.*, in press, 1998). Thus it appears that ClfA can bind to the gamma-chain of fibrinogen at two separate sites. The ligand binding sites on ClfA are similar to those employed by eukaryotic integrins and involve divalent cation binding EF-hand and MIDAS motifs.

Also known is the fibrinogen binding protein, ClfB. Used herein are the protein as well as antibodies to the protein and diagnostic kits that include the protein or its antibodies. ClfB has a predicted molecular weight of approximately 88 kDa and an apparent molecular weight of approximately 124 kDa. ClfB is a cell-wall associated protein and binds both soluble and immobilized fibrinogen. In addition, ClfB binds both the alpha and beta chains of fibrinogen and acts as a clumping factor.

Proteins related to the fibrinogen-binding ClfA and ClfB have been found, which bind to the extracellular matrix. The SdrC, SdrD and SdrE proteins are related in primary sequence and structural organization to the ClfA and ClfB proteins, and are also localized on the cell surface. With the A region of these proteins localized on the cell surface, the proteins can interact with the proteins in plasma, the extracellular matrix or with molecules on the surface of host cells. SdrC can bind to the extracellular matrix proteins, for example, vitronectin. SdrE also binds to the extracellular matrix, for example, SdrE binds bone sialoprotein (BSP).

It has been discovered that in the A region of SdrC, SdrD, ClfA and ClfB, there is highly conserved amino acid sequence that can be used to derive a consensus TYTFT-DYVD (SEQ ID NO: 17) motif. The motif can be used in multicomponent vaccines to impart broad spectrum immunity to bacterial infections, and also can be used to produce monoclonal or polyclonal antibodies that impart broad spectrum passive immunity. In an alternative embodiment, any combination of the variable sequence motif derived from the Sdr and Clf protein families, (T/I) (Y/F) (T/V) (F) (T) (D/N) (Y) (V) (D/N), can be used to impart immunity or to induce protective antibodies.

V. Elastin-Binding MSCRAMMs

The primary role of elastin is to confer the property of reversible elasticity to tissues and organs (Rosenbloom, J., et al., *FASEB J.*, 7:1208–1218, 1993). Elastin expression is highest in the lung, skin and blood vessels, but the protein is widely expressed in mammalian hosts for *S. aureus*. *S. aureus* binding to elastin was found to be rapid, reversible, of high affinity and ligand specific. Furthermore, a 25 kDa cell surface elastin binding protein (EbpS) was isolated and

proposed to mediate *S. aureus* binding to elastin-rich host ECM. EbpS binds to a region in the N-terminal 30 kDa fragment of elastin.

PCT/US97/03106 discloses the gene sequences for an elastin binding protein. DNA sequence data disclosed indicates that the ebps open reading frame consists of 606 bp, and encodes a novel polypeptide of 202 amino acids. EbpS protein has a predicted molecular mass of 23,345 daltons and pI of 4.9. EbpS was expressed in *E. coli* as a fusion protein with polyhistidine residues attached to the N-terminus. A polyclonal antibody raised against recombinant EbpS interacted specifically with the 25 kDa cell surface EbpS and inhibited staphylococcal elastin binding. Furthermore, recombinant EbpS bound specifically to immobilized elastin and inhibited binding of *Staphylococcus aureus* to elastin. A degradation product of recombinant EbpS lacking the first 59 amino acids of the molecule and a C-terminal fragment of CNBr-cleaved recombinant EbpS, however, did not interact with elastin. These results strongly suggest that EbpS is the cell surface molecule mediating binding of *Staphylococcus aureus* to elastin. The finding that some constructs of recombinant EbpS do not interact with elastin suggests that the elastin binding site in EbpS is contained in the first 59 amino acids of the molecule.

Several independent criteria indicate that EbpS is the surface protein mediating cellular elastin binding. First, rEbpS binds specifically to immobilized elastin and inhibits binding of *S. aureus* cells to elastin in a dose dependent manner. These results establish that EbpS is an elastin binding protein that is functionally active in a soluble form. Second, an antibody raised against rEbpS recognizes a 25 kDa protein expressed on the cell surface of *S. aureus* cells. In addition to the size similarity and antibody reactivity, further evidence that this 25 kDa protein is cell surface EbpS is provided by the experiment showing that binding of the 25 kDa protein to immobilized anti-rEbpS IgG is inhibited in the presence of excess unlabeled rEbpS. Finally, Fab fragments prepared from the anti-rEbpS antibody, but not from its pre-immune control, inhibit binding of *S. aureus* to elastin. This result suggests that the topology of surface EbpS is such that the elastin binding site is accessible to interact with ligands (i.e. elastin and the anti-rEbpS Fab fragment) and not embedded in the cell wall or membrane domains. The composite data demonstrate that EbpS is the cell surface protein responsible for binding *S. aureus* to elastin.

The present and previous findings suggest the existence of a functionally active 40 kDa intracellular precursor form of EbpS that requires processing at the C terminus prior to surface expression. This notion is based on the following observations: i) there exists an intracellular 40 kDa elastin binding protein that is never detected during cell surface labeling experiments, ii) the 25 kDa EbpS and the 40 kDa elastin binding protein have an identical N-terminal sequence, and iii) a single gene exists for EbpS. Because the size of the ebps open reading frame is not sufficient to encode a 40 kDa protein, at first the inventors disregarded this hypothesis. However, their studies with rEbpS demonstrated that although the actual size of the recombinant protein is 26 kDa, it migrates aberrantly as a 45 kDa protein in SDS-30 PAGE. This finding suggests that full length native EbpS, with a predicted size of 23 kDa, may be migrating in SDS-PAGE as the 40 kDa intracellular precursor, and that the 25 kDa surface form of EbpS is actually a smaller form of the molecule processed at the C-terminus. Although EbpS lacks an N-terminal signal peptide and other known sorting and anchoring signals, this

proposed intracellular processing event may explain some questions regarding how EbpS is targeted to the cell surface. In fact, C-terminal signal peptides have been identified in several bacterial proteins (Fath, M. J. and Kolter, R., *Microbiol. Rev.*, 57:995–1017, 1993) and alternative means of anchoring proteins to the cells surface have been reported in gram positive bacteria (Yother, J. and White, J. M., *J. Bacteriol.*, 176:2976–2985, 1994).

Using overlapping EbpS fragments and recombinant constructs, the elastin binding site in EbpS was mapped to the amino terminal domain of the molecule (PCT/US97/03106). Overlapping synthetic peptides spanning amino acids 14–34 were then used to better define the binding domain. Among these, peptides corresponding to residues 14–23 and 18–34 specifically inhibited elastin binding by more than 95%. Common to all active synthetic peptides and proteolytic and recombinant fragments of EbpS is the hexameric sequence ¹⁸Thr-Asn-Ser-His-Gln-Asp²³ (SEQ ID NO: 18). Further evidence that this sequence is important for elastin binding was the loss of activity when Asp²³ was substituted with Asn in the synthetic peptide corresponding to residues 18–34. However, the synthetic hexamer TNSHQD (SEQ ID NO: 18) by itself did not inhibit staphylococcal binding to elastin. These findings indicate that although the presence of the TNSHQD sequence is essential for EbpS activity, flanking amino acids in the N- or C-terminal direction and the carboxyl side chain of Asp²³ are required for elastin recognition.

VI. MHC II-Analogous Proteins, (MAP)

In addition to fibrinogen, fibronectin, collagen and elastin, *S. aureus* strains associate with other adhesive eukaryotic proteins, many of which belong to the family of adhesive matrix proteins, such as vitronectin. (Chatwal et al., *Infect. Immun.*, 55:1878–1883, 1987). U.S. Pat. No. 5,648,240 discloses a DNA segment comprising a gene encoding a *S. aureus* broad spectrum adhesin that has a molecular weight of about 70 kDa. The adhesin is capable of binding fibronectin or vitronectin and includes a MHC II mimicking unit of about 30 amino acids. Further analyses of the binding specificities of this protein reveal that it functionally resembles an MHC II antigen in that it binds synthetic peptides. Thus, in addition to mediating bacterial adhesion to ECM proteins, it may play a role in staphylococcal infections by suppressing the immune system of the host. The patent further claims a recombinant vector that includes the specified DNA sequence, a recombinant host cell transformed with the vector, and DNA which hybridizes with the DNA of specified sequence. Also disclosed is a composition that includes a protein or polypeptide encoded by the specified DNA sequence and a method of inducing an immune response in an animal that includes administering an immunogenic composition that includes the encoded protein or polypeptide. A method of making a MHC II antigen protein analog comprising the steps of inserting the specified DNA sequence in a suitable expression vector and culturing a host cell transformed with the vector under conditions to produce the MHC II antigen protein analog is additionally claimed in the patent.

VII. SDR Proteins from *Staphylococcus Epidermidis*

Staphylococcus epidermidis, a coagulase-negative bacterium, is a common inhabitant of human skin and a frequent cause of foreign-body infections. Pathogenesis is facilitated by the ability of the organism to first adhere to, and subsequently to form biofilms on, indwelling medical devices such as artificial valves, orthopedic devices, and intravenous and peritoneal dialysis catheters. Device-related infections may jeopardize the success of medical treatment

and significantly increase patient mortality. Accordingly, the ability to develop vaccines that can control or prevent outbreaks of *S. epidermidis* infection is of great importance, as is the development of multicomponent vaccines that can prevent or treat infection from a broad spectrum of bacteria, including both coagulase-positive and coagulase negative bacteria at the same time.

Three Sdr (serine-aspartate (SD) repeat region) proteins that are expressed by *S. epidermidis* have been designated as SdrF, SdrG and SdrH, and the amino acid sequences of these proteins and their nucleic acid sequences are shown in FIGS. 3–5, respectively. In addition, a more complete description of these proteins is provided in a co-pending U.S. patent application of Foster et al. which is based on U.S. provisional application Ser. Nos. 60/098,443 and 60/117,119. These applications are incorporated herein by reference.

In accordance with the present invention, a composition useful as a vaccine is provided that includes the components of any of the above embodiments in combination with an SdrF, SdrG or an SdrH protein. In addition, antibodies to these proteins can be raised using conventional means, and antibodies to the SdrF, SdrG or an SdrH proteins can be employed in any of the above combinations which employ antibodies to the other adhesins discussed herein. The compositions and vaccines which include an SDR protein such as SdrF, SdrG or SdrH can thus be used to treat a broad spectrum of bacterial infections, including those arising both from coagulase-positive and coagulase-negative bacteria.

VIII. Bacterial Components

In an embodiment of the invention, a composition is provided that includes the components of any of the above embodiments in combination with a bacterial component, preferably capsular polysaccharides type 5 or type 8, to increase the rate of opsonization and phagocytosis of *S. aureus*.

Staphylococci contain antigenic polysaccharides, such as capsular polysaccharide types 5 and 8, and proteins as well as other substances important in cell wall structure. Peptidoglycan, a polysaccharide polymer containing linked subunits, provides the rigid exoskeleton of the cell wall. Peptidoglycan is destroyed by strong acids or exposure to lysozyme. It is important in the pathogenesis of infection. It elicits production of interleukin-1 (endogenous pyrogen) and opsonic antibodies by monocytes. It can be a chemoattractant for polymorphonuclear leukocytes, have endotoxin-like activity, produce a localized Shwartzman phenomenon, and activate complement.

Teichoic acids, lipoteichoic acid for example, which are polymers of glycerol or ribitol phosphate, are linked to the peptidoglycan and can be antigenic. Antiteichoic antibodies detectable by gel diffusion may be found in patients with active endocarditis due to *S. aureus*.

Protein A is a cell wall component of many *S. aureus* strains that binds to the Fc portion of IgG molecules except IgG3. The Fab portion of IgG bound to protein A is free to combine with a specific antigen. Protein A has become an important reagent in immunology and diagnostic laboratory technology; for example, protein A with attached IgG molecules directed against a specific bacterial antigen will agglutinate bacteria that have that antigen (“coagglutination”).

Some *S. aureus* strains have capsules, which inhibit phagocytosis by polymorphonuclear leukocytes unless specific antibodies are present. Most strains of *S. aureus* have coagulase, or clumping factor, on the cell wall surface; coagulase binds nonenzymatically to fibrinogen, yielding aggregation of the bacteria.

Staphylococci can produce disease both through their ability to multiply and spread widely in tissues and through their production of many extracellular substances. Some of these substances are enzymes; others are considered to be toxins, though they may function as enzymes. Many of the toxins are under the genetic control of plasmids; some may be under both chromosomal and extrachromosomal control; and for others the mechanism of genetic control is not well defined.

A. Catalase:

Staphylococci produce catalase, which converts hydrogen peroxide into water and oxygen. The catalase test differentiates the staphylococci, which are positive, from the streptococci, which are negative.

B. Coagulase:

S. aureus produces coagulase, an enzyme-like protein that clots oxalated or citrated plasma in the presence of a factor contained in many sera. The serum factor reacts with coagulase to generate both esterase and clotting activities, in a manner similar to the activation of prothrombin to thrombin. The action of coagulase circumvents the normal plasma clotting cascade. Coagulase may deposit fibrin on the surface of staphylococci, perhaps altering their ingestion by phagocytic cells or their destruction within such cells. Coagulase production is considered synonymous with invasive pathogenic potential. However, coagulase-negative bacteria such as *S. epidermidis* also pose a threat for serious infection as well.

C. Other Enzymes:

Other enzymes produced by staphylococci include a hyaluronidase, or spreading factor; a staphylokinase resulting in fibrinolysis but acting much more slowly than streptokinase; proteinases; lipases; and β -lactamase.

D. Exotoxins:

These include several toxins that are lethal for animals on injection, cause necrosis in skin, and contain soluble hemolysins which can be separated by electrophoresis. The alpha toxin (hemolysin) is a heterogeneous protein that can lyse erythrocytes and damage platelets and is probably identical with the lethal and dermonecrotic factors of exotoxin. Alpha toxin also has a powerful action on vascular smooth muscle. Beta toxin degrades sphingomyelin and is toxic for many kinds of cells, including human red blood cells. These toxins and two others, the gamma and delta toxins; are antigenically distinct and bear no relationship to streptococcal lysins. Exotoxin treated with formalin gives a nonpoisonous but antigenic toxoid, but this is not clinically useful.

E. Leukocidin:

This toxin of *S. aureus* can kill exposed white blood cells of many animals. Its role in pathogenic staphylococci may not kill white blood cells and may be phagocytosed as effectively as nonpathogenic varieties. However, they are capable of very active intra-cellular multiplication, whereas the nonpathogenic organisms tend to die inside the cell. Antibodies to leukocidin may plan a role in resistance to recurrent staphylococcal infections.

F. Exfoliative Toxin:

This toxin of *S. aureus* includes at least two proteins that yield the generalized desquamation of the staphylococcal scaled skin syndrome. Specific antibodies protect against the exfoliative action of the toxin.

G. Toxic Shock Syndrome Toxin.

Most *S. aureus* strains isolated from patients with toxic shock syndrome produce a toxin called toxic shock syndrome toxin-1 (TSST-1), which is the same as enterotoxin F and pyrogenic exotoxin C. TSST-1 is the prototypical super-

antigen which promotes the protean manifestations of the toxic shock syndrome. In humans, the toxin is associated with fever, shock, and multisystem involvement, including a desquamative skin rash. In rabbits, TSST-1 produces fever, enhanced susceptibility to the effects of bacterial lipopolysaccharides, and other biologic effects similar to toxic shock syndrome, but the skin rash and desquamation do not occur.

H. Enterotoxins:

There are at least six (A-F) soluble toxins produced by nearly 50% of *S. aureus* strains. Like TSST-1, the enterotoxins are superantigens that bind to MHC class II molecules, yielding T cell stimulation. The enterotoxins are heat-stable (they resist boiling for 30 minutes) and are resistant to the action of gut enzymes. An important cause of food poisoning, enterotoxins are produced when *S. aureus* grows in carbohydrate and protein foods. The gene for enterotoxin production may be on the chromosome, but a plasmid may carry a protein that regulates active toxin production. Ingestion of 25 μ g of enterotoxin B by humans or monkeys results in vomiting and diarrhea. The emetic effect of enterotoxin is probably the result of central nervous system stimulation (vomiting center) after the toxin acts on neural receptors in the gut. Enterotoxins can be assayed by precipitin tests (gel diffusion).

There are also many other antigenic proteins produced by Staphylococcal organisms. These include the MSCRAMMs mentioned above, as well as: bone sialoprotein binding protein, clusterin binding protein, heparin sulfate binding protein, thrombospondin binding protein, transferrin binding protein and vitronectin binding protein. *S. aureus* further expresses virulence factors such as phosphatidyl phospholipase, and toxin expression regulators such as Rap protein.

IX. Proteins and Peptides with Substantial Homology or Equivalent Function to Those Described Herein

The disclosed compositions can include, as desired, full sequence proteins, peptides, protein or peptide fragments, isolated epitopes, fusion proteins, or any alternative which binds to the target ECM, whether in the form of a wild type, a site-directed mutant, or a sequence which is substantially homologous thereto.

Two DNA sequences are "substantially homologous" when at least about 70%, (preferably at least about 80%, and most preferably at least about 90 or 95%) of the nucleotides match over the defined length of the DNA sequences. Sequences that are substantially homologous can be identified by comparing the sequences using standard software available in sequence data banks, or in a Southern hybridization experiment under, for example, stringent conditions as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. See, e.g., Maniatis et al., *Molecular Cloning: A Laboratory Manual*, 1982; *DNA Cloning*, Vols. I & II supra; *Nucleic Acid Hybridization*, [B. D. Hames & S. J. Higgins eds. (1985)].

When used in conjunction with amino acid sequences, the term "substantially similar" means an amino acid sequence which is not identical to published sequences, but which produces a protein having the same functionality and activities, either because one amino acid is replaced with another similar amino acid, or because the change (whether it be substitution, deletion or insertion) does not substantially effect the active site of the protein. Two amino acid sequences are "substantially homologous" when at least about 70%, (preferably at least about 80%, and most preferably at least about 90% or 95%) of the amino acids match over the defined length of the sequences.

It should also be understood that each of the MSCRAMM polypeptides of this invention may be part of a larger protein. For example, a ClfA polypeptide of this invention may be fused at its N-terminus or C-terminus to a ClfB polypeptide, or to a non-fibrinogen binding polypeptide or combinations thereof. Polypeptides which may be useful for this purpose include polypeptides derived any of the MSCRAMM proteins, and serotypic variants of any of the above. Non-MSCRAMM polypeptides which may be useful for this purpose include any of the bacterial components described above.

Modification and changes may be made in the structure of the peptides of the present invention and DNA segments which encode them and still obtain a functional molecule that encodes a protein or peptide with desirable characteristics. The following is a discussion based upon changing the amino acids of a protein to create an equivalent, or even an improved, second generation molecule. The amino acid changes may be achieved by changing the codons of the DNA sequence, according to Table 1. It should be understood by one skilled in the art that the codons specified in Table 1 are for RNA sequences. The corresponding codons for DNA have a T substituted for U. In keeping with standard nomenclature (*J. Biol. Chem.*, 243:3552-3559, 1969), abbreviations for amino acid residues are further shown in Table I.

For example, certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules. Since it is the interactive capacity and nature of a protein that defines that protein's biological functional activity, certain amino acid sequence substitutions can be made in a protein sequence, and, of course, its underlying DNA coding sequence, and nevertheless obtain a protein with like properties. It is thus contemplated by the inventors that various changes may be made in the peptide sequences of the disclosed compositions, or corresponding DNA sequences which encode said peptides without appreciable loss of their biological utility or activity.

TABLE I

Amino Acids		Codons					
Alanine	Ala	A	GCA	GCC	GCG	GCU	
Cysteine	Cys	C	UGC	UGU			
Aspartic acid	Asp	D	GAC	GAU	GAC	GAU	
Glutamic acid	Glu	E	GAA	GAG			
Phenylalanine	Phe	F	UUC	UUU			
Glycine	Gly	G	GGA	GCG	GGG	GGU	
Histidine	His	H	CAC	CAU			
Isoleucine	Ile	I	AUA	AUC	AUU		
Lysine	Lys	K	AAA	AAG			
Leucine	Leu	L	UUA	UUG	CUA	CUC	GUU
Methionine	Met	M	AUG				
Asparagine	Asn	N	AAC	AAU			
Proline	Pro	P	CCA	CCC	CCG	CCU	
Glutamine	Gln	Q	CAA	CAG			
Arginine	Arg	R	AGA	AGG	CGA	CGC	CGG
Serine	Ser	S	AGC	AGU	UCA	UCC	UCG
Threonine	Thr	T	ACA	ACC	ACG	ACU	
Valine	Val	V	GUA	GUC	GUG	GUU	
Tryptophan	Trp	W	UGG				
Tyrosine	Tyr	Y	UAC	UAU			

In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art (Kyte and Doolittle, *J Mol Biol*, 157(1):105-132, 1982, incorporate herein by reference). It is accepted that the relative hydro-

pathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like. Each amino acid has been assigned a hydropathic index on the basis of their hydrophobicity and charge characteristics (Kyte and Doolittle, supra 1982), these are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

It is known in the art that certain amino acids may be substituted by other amino acids having a similar hydropathic index or score and still result in a protein with similar biological activity, i.e., still obtain a biological functionally equivalent protein. In making such changes, the substitution of amino acids whose hydropathic indices are within ± 2 is preferred, those which are within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred. It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U.S. Pat. No. 4,554,101, incorporated herein by reference, states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein.

As detailed in U.S. Pat. No. 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+1.0); aspartate (+3.0 \pm 1); glutamate (+3.0 \pm 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 \pm 1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4). It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent protein. In such changes, the substitution of amino acids whose hydrophilicity values are within ± 2 is preferred, those which are within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred.

As outlined above, amino acid substitutions are generally therefore based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions which take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

The polypeptides of the present invention can be chemically synthesized. The synthetic polypeptides are prepared using the well known techniques of solid phase, liquid phase, or peptide condensation techniques, or any combination thereof, can include natural and unnatural amino acids. Amino acids used for peptide synthesis may be standard Boc (N^{α} -amino protected N^{α} -t-butyloxycarbonyl) amino acid resin with the standard deprotecting, neutralization, coupling and wash protocols of the original solid phase procedure of Merrifield [*J. Am. Chem. Soc.*, 85:2149-2154 (1963)], or the base-labile N^{α} -amino protected 9-fluorenylmethoxycarbonyl (Fmoc) amino acids first described by Carpino and Han [*J. Org. Chem.*,

37:3403–3409 (1972)]. Both Fmoc and Boc N^α-amino protected amino acids can be obtained from Fluka, Bachem, Advanced Chemtech, Sigma, Cambridge Research Biochemical, Bachem, or Peninsula Labs or other chemical companies familiar to those who practice this art. In addition, the method of the invention can be used with other N^α-protecting groups that are familiar to those skilled in this art. Solid phase peptide synthesis may be accomplished by techniques familiar to those in the art and provided, for example, in Stewart and Young, 1984, *Solid Phase Synthesis*, Second Edition, Pierce Chemical Co., Rockford, Ill.; Fields et al., *Int. J. Pept. Protein Res.* 35:161–214 (1990), or using automated synthesizers, such as sold by ABS. Thus, polypeptides 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, Ca-methyl amino acids, and Na-methyl amino acids, etc.) to convey special properties. Synthetic amino acids include ornithine for lysine, fluoro-phenylalanine for phenylalanine, and norleucine for leucine or isoleucine. Additionally, by assigning specific amino acids at specific coupling steps, α-helices, β turns, β sheets, β-turns, and cyclic peptides can be generated.

In a further embodiment, subunits of peptides that confer useful chemical and structural properties will be chosen. For example, peptides comprising D-amino acids will be resistant to L-amino acid-specific proteases in vivo. 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., R₁—CH₂—NH—R₂, where R₁ and R₂ 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, *Life Sciences*, 31:189–199 (1982)); (Hruby et al, *Biochem J.* 268:249–262 (1990)).

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., *J. Am. Chem. Soc.*, 113:2275–2283, 1991); (2S,3S)-methyl-phenylalanine, (2S,3R)-methyl-phenylalanine, (2R,3S)-methyl-phenylalanine and (2R,3R)-methyl-phenylalanine (Kazmierski and Hruby, *Tetrahedron Lett.*, 1991); 2-aminotetrahydro-naphthalene-2-carboxylic acid (Landis, *Ph.D. Thesis, University of Arizona*, 1989); hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (Miyake et al, *J. Takeda Res. Labs.*, 43:53–76, 1989); β-carboline (D and L) (Kazmierski, *Ph.D. Thesis, University of Arizona*, 1988); HIC (histidine isoquinoline carboxylic acid) (Zechel et al, *Int. J. Pep. Protein Res.*, 43, 1991); 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., *J. Org. Chem.*, 50:5834–5838 (1985)); β-sheet inducing analogs [Kemp et al., *Tetrahedron Lett.*, 29:5081–5082 (1988)]; β-turn inducing analogs (Kemp et al., *Tetrahedron Lett.*, 29:5057–5060 (1988)); alpha-helix inducing analogs [Kemp et al., *Tetrahedron Lett.*,

29:4935–4938 (1988)]; β-turn inducing analogs [Kemp et al., *J. Org. Chem.*, 54:109:115 (1989)]; and analogs provided by the following references: Nagai and Sato, *Tetrahedron Lett.*, 26:647–650 (1985); DiMaio et al., *J. Chem. Soc. Perkin Trans.*, p. 1687 (1989); also a Gly-Ala turn analog (Kahn et al., *Tetrahedron Lett.*, 30:2317, 1989); amide bond isostere (Jones et al., *Tetrahedron Lett.*, 29:3853–3856, 1989); tetrazole (Zabrocki et al., *J. Am. Chem. Soc.*, 110:5875–5880, 1988); DTC (Samanen et al., *Int. J. Protein Pep. Res.*, 35:501:509, 1990); and analogs taught in Olson et al., (*J. Am. Chem. Sci.*, 112:323–333, 1990) and Garvey et al., (*J. Org. Chem.*, 56:436, 1990). Conformationally restricted mimetics of beta turns and beta bulges, and peptides containing them, are described in U.S. Pat. No. 5,440,013, issued Aug. 8, 1995 to Kahn.

X. Uses for MSCRAMM and Antibody Compositions

The protein compositions disclosed herein can be used for the treatment of wounds, for blocking protein receptors or for immunization (vaccination). In the latter case, the body creates specific antibodies, which can protect against invasion by bacterial strains comprising such a cell surface protein, and whereby the antibodies block the adherence of the bacterial strains to a damaged tissue.

The protein composition can be dispersed in a sterile, isotonic saline solution, optionally with the addition of a pharmaceutically acceptable dispersing agent. Different types of adjuvants can further be used to sustain the release in the tissue, and thus expose the peptide for a longer time to the immune defense system of a body.

The proteins, nucleic acid molecules or antibodies are useful for interfering with the initial physical interaction between a pathogen and mammalian host responsible for infection, such as the adhesion of bacteria, particularly gram positive bacteria, to mammalian extracellular matrix proteins on in-dwelling devices or to extracellular matrix proteins in wounds; to block protein-mediated mammalian cell invasion; to block bacterial adhesion between mammalian extracellular matrix proteins and bacterial proteins that mediate tissue damage; and, to block the normal progression of pathogenesis in infections initiated other than by the implantation of in-dwelling devices or surgical techniques. Medical devices or polymeric biomaterials to be coated with the antibodies, proteins and active fragments described herein include, but are not limited to, staples, sutures, replacement heart valves, cardiac assist devices, hard and soft contact lenses, intraocular lens implants (anterior chamber, posterior chamber or phasic), other implants such as corneal inlays, kerato-prostheses, vascular stents, epikeratophalia devices, glaucoma shunts, retinal staples, scleral buckles, dental prostheses, thyroplastic devices, laryngoplastic devices, vascular grafts, soft and hard tissue prostheses including, but not limited to, pumps, electrical devices including stimulators and recorders, auditory prostheses, pacemakers, artificial larynx, dental implants, mammary implants, penile implants, cranio/facial tendons, artificial joints, tendons, ligaments, menisci, and disks, artificial bones, artificial organs including artificial pancreas, artificial hearts, artificial limbs, and heart valves; stents, wires, guide wires, intravenous and central venous catheters, laser and balloon angioplasty devices, vascular and heart devices (tubes, catheters, balloons), ventricular assists, blood dialysis components, blood oxygenators, urethra/ureteral/urinary devices (Foley catheters, stents, tubes and balloons), airway catheters (endotracheal and tracheostomy tubes and cuffs), enteral feeding tubes (including nasogastric, intragastric and jejunal tubes), wound drainage tubes, tubes used to drain the body cavities such as the

pleural, peritoneal, cranial, and pericardial cavities, blood bags, test tubes, blood collection tubes, vacutainers, syringes, needles, pipettes, pipette tips, and blood tubing.

The term "coated" or "coating", as used herein, means to apply the protein, antibody, or active fragment to a surface of the device, preferably an outer surface that would be exposed to *S. aureus* infection. The surface of the device need not be entirely covered by the protein, antibody or active fragment.

XI. Preparation of Proteins DNA, and Antibodies

The skilled reader can employ conventional molecular biology, microbiology, and recombinant DNA techniques to prepare the proteins, peptides, and antibody compositions described herein. Such techniques are explained fully in the literature. See, e.g., Sambrook et al, *Molecular Cloning: A Laboratory Manual* (1989); *Current Protocols in Molecular Biology* Volumes I-III (Ausubel, R. @-I ed., 1994); *Cell Biology: A Laboratory Handbook* Volumes I-III (J. E. Celis, ed., 1994); *Current Protocols in Immunology* Volumes I-III (Coligan, J. E., ed., 1994); *Oligonucleotide Synthesis* (M. J. Gait ed. 1984); *Nucleic Acid Hybridization* (B. D. Hames & S. J. Higgins eds., 1985); *Transcription And Translation* (B. D. Hames & S. J. Higgins, eds., 1984); *Animal Cell Culture* [R. I. Freshney, ed. 1, (1986); *Immobilized Cells And Enzymes* [IRL Press, (1986)]; B. Perbal, *A Practical Guide To Molecular Cloning* (1984).

Reference to antibodies throughout the specification includes whole polyclonal and monoclonal antibodies, and parts thereof, either alone or conjugated with other moieties. Antibody parts include Fab and F(ab)2 fragments and single chain antibodies. The antibodies may be made in vivo in suitable laboratory animals or in vitro using recombinant DNA techniques. An antibody can be a polygonal or a monoclonal antibody. In a preferred embodiment, an antibody is a polyclonal antibody. Means for preparing and characterizing antibodies are well known in the art (See, e.g., Harlow and Lane, *Antibodies: a Laboratory Manual*, Cold Spring Harbor, N.Y., 1988).

Briefly, a polyclonal antibody is prepared by immunizing an animal with an immunogen comprising a polypeptide of the present invention and collecting antisera from that immunized animal. A wide range of animal species can be used for the production of antisera. Typically an animal used for production of anti-antisera is a rabbit, a mouse, a rat, a hamster or a guinea pig. Because of the relatively large blood volume of rabbits, a rabbit is a preferred choice for production of polyclonal antibodies.

Antibodies, both polyclonal and monoclonal, specific for MSCRAMM epitopes may be prepared using conventional immunization techniques, as will be generally known to those of skill in the art. A composition containing antigenic epitopes of particular binding MSCRAMMs (either synthetic peptides, site-specifically mutated, or truncated peptides) can be used to immunize one or more experimental animals, such as a rabbit or mouse, which will then proceed to produce specific antibodies against epitope-containing MSCRAMM peptides.

Polyclonal antisera may be obtained, after allowing time for antibody generation, simply by bleeding the animal and preparing serum samples from the whole blood.

The amount of immunogen composition used in the production of polyclonal antibodies varies upon the nature of the immunogen, as well as the animal used for immunization. A variety of routes can be used to administer the immunogen (subcutaneous, intramuscular, intradermal, intravenous and intraperitoneal). The production of polyclonal antibodies may be monitored by sampling blood of

the immunized animal at various points following immunization. A second, booster injection, also may be given. The process of boosting and titering is repeated until a suitable titer is achieved. When a desired level of immunogenicity is obtained, the immunized animal can be bled and the serum isolated and stored.

One of the important features provided by the present invention is a polygonal sera that is relatively homogenous with respect to the specificity of the antibodies therein. Typically, polygonal antisera is derived from a variety of different "clones," i.e., B-cells of different lineage. Monoclonal antibodies, by contrast, are defined as coming from antibody-producing cells with a common B-cell ancestor, hence their "mono" clonality.

When peptides are used as antigens to raise polyclonal sera, one expects considerably less variation in the clonal nature of the sera than if a whole antigen were employed. Unfortunately, if incomplete fragments of an epitope are presented, the peptide may very well assume multiple (and probably non-native) conformations. As a result, even short peptides can produce polyclonal antisera with relatively plural specificities and, unfortunately, an antisera that does not react or reacts poorly with the native molecule.

Polyclonal antisera according to the present invention is produced against peptides that are predicted to comprise whole, intact epitopes. It is believed that these epitopes are, therefore, more stable in an immunologic sense and thus express a more consistent immunologic target for the immune system. Under this model, the number of potential B-cell clones that will respond to this peptide is considerably smaller and, hence, the homogeneity of the resulting sera will be higher. In various embodiments, the present invention provides for polyclonal antisera where the clonality, i.e., the percentage of clone reacting with the same molecular determinant, is at least 80%. Even higher clonality—90%, 95% or greater—is contemplated.

To obtain monoclonal antibodies, one also initially immunizes an experimental animal, often preferably a mouse, with an MSCRAMM-derived epitope-containing composition. One then, after a period of time sufficient to allow antibody generation, obtains a population of spleen or lymph cells from the animal. The spleen or lymph cells are then fused with cell lines, such as human or mouse myeloma strains, to produce antibody-secreting hybridomas. These hybridomas may be isolated to obtain individual clones which can then be screened for production of antibody to the desired peptide. Following immunization, spleen cells are removed and fused, using a standard fusion protocol with plasmacytoma cells to produce hybridomas secreting monoclonal antibodies against MSCRAMM-derived epitopes. Hybridomas which produce monoclonal antibodies to the selected antigens are identified using standard techniques, such as ELISA and Western blot methods. Hybridoma clones can then be cultured in liquid media and the culture supernatants unified to provide the MSCRAMM-derived epitope-specific monoclonal antibodies.

Immortal antibody-producing cell lines can also be created by techniques other than fusion, such as direct transformation of B lymphocytes with oncogenic DNA, or transfection with Epstein-Barr virus. See, e.g., M. Schreier et al., *Hybridoma Techniques* (1980); Hammerling et al., *Monoclonal Antibodies And T-cell Hybridomas* (1981); Kennett et al., *Monoclonal Antibodies* (1980); see also U.S. Pat. Nos. 4,341,761; 4,399,121; 4,427,783; 4,444,887; 4,451,570; 4,466,917; 4,472,500; 4,491,632; 4,493,890.

It is proposed that the monoclonal antibodies of the present invention will find useful application in standard

immunochemical procedures, such as ELISA and Western blot methods, as well as other procedures which may utilize antibody specific to the MSCRAMM epitopes.

Additionally, it is proposed that monoclonal antibodies specific to the particular MSCRAMM-derived peptides may be utilized in other useful applications. For example, their use in immunoabsorbent protocols may be useful in purifying native or recombinant peptide species or synthetic or natural variants thereof.

In general, both poly- and monoclonal antibodies against these peptides may be used in a variety of embodiments. For example, they may be employed in antibody cloning protocols to obtain cDNAs or genes encoding the peptides discussed herein or related proteins. They may also be used in inhibition studies to analyze the effects of MSCRAMM-derived peptides in cells or animals. Anti-MSCRAMM epitope antibodies will also be useful in immunolocalization studies to analyze the distribution of MSCRAMMs during various cellular events, for example, to determine the cellular or tissue-specific distribution of the MSCRAMM peptides under different physiological conditions. A particularly useful application of such antibodies is in purifying native or recombinant MSCRAMMs, for example, using an antibody affinity column. The operation of all such immunological techniques will be known to those of skill in the art in light of the present disclosure.

Techniques for the production of single chain antibodies are known to those skilled in the art and described in U.S. Pat. No. 4,946,778 and can be used to produce single chain antibodies to the proteins described herein. Phage display technology may be used to select antibody genes having binding, activities for MSCRAMMs, or antigenic portions thereof, from PCR-amplified ν genes of lymphocytes from humans screened for having antibodies to MSCRAMMs or naive libraries. Bispecific antibodies have two antigen binding domains wherein each domain is directed against a different epitope.

The antibody may be labeled directly with a detectable label for identification and quantification of a staphylococcal bacterium such as *S. aureus*. Labels for use in immunoassays are generally known to those skilled in the art and include enzymes, radioisotopes, and fluorescent, luminescent and chromogenic substances including colored particles such as colloidal gold and latex beads. Suitable immunoassays include enzyme-linked immunosorbent assays (ELISA).

Alternatively, the antibody may be labeled indirectly by reaction with labeled substances that have an affinity for immunoglobulin, such as protein A or G or second antibodies. The antibody may be conjugated with a second substance and detected with a labeled third substance having an affinity for the second substance conjugated to the antibody. For example, the antibody may be conjugated to biotin and the antibody-biotin conjugate detected using labeled avidin or streptavidin. Similarly, the antibody may be conjugated to a hapten and the antibody-hapten conjugate detected using labeled anti-hapten antibody. These and other methods of labeling antibodies and assay conjugates are well known to those skilled in the art. Antibodies to the binding proteins may also be used in production facilities or laboratories to isolate additional quantities of the protein, such as by affinity chromatography.

In general, the preparation of bispecific antibodies is also well known in the art, as exemplified by Glennie et al. (*J Immunol*, 139:2367-2375, 1987). Bispecific antibodies have been employed clinically, for example, to treat cancer patients (Bauer et al, *Vox Sang*, 61:156-157, 1991). One method for the preparation of bispecific antibodies involves

the separate preparation of antibodies having specificity for different epitopes of one or more fibronectin binding domains from one or more fibronectin binding protein(s).

While numerous methods are known in the art for the preparation of bispecific antibodies, the Glennie et al., (1987 supra) method involves the preparation of peptic F(ab'Y)₂ fragments from the two chosen antibodies, followed by reduction of each to provide separate Fab'YSH fragments. The SH groups on one of the two partners to be coupled are then alkylated with a cross-linking reagent such as o-phenylenedimaleimide to provide free maleimide groups on one partner. This partner may then be conjugated to the other by means of a thioether linkage, to give the desired F(ab'Y)₂ heterodconjugate.

Due to ease of preparation, high yield and reproducibility, the Glennie et al., (1987 supra) method is often preferred for the preparation of bispecific antibodies, however, there are numerous other approaches that can be employed and that are envisioned by the inventors. For example, other techniques are known wherein cross-linking with SPDP or protein A is carried out, or a specific construct is prepared (Titus et al, *J. Immunol.*, 138:4018-4022, 1987; Tutt et al, *Eur J Immunol*, 21:1351-1358, 1991).

Another method for producing bispecific antibodies is by the fusion of two hybridomas to form a quadroma (Flavell et al, *Br. J. Cancer*, 64(2):274-280, 1991; Pimm et al, *J. Cancer Res Clin Oncol*, 118:367-370, 1992; French et al, *Cancer Res*, 51:2358-2361, 1991; Embleton et al., *Br. J. Cancer*, 63(5):670-674, 1991). As used herein, the term "quadroma" is used to describe the productive fusion of two B cell hybridomas. Using now standard techniques, two antibody producing hybridomas are fused to give daughter cells, and those cells that have maintained the expression of both sets of clonotype immunoglobulin genes are then selected.

A preferred method of generating a quadroma involves the selection of an enzyme deficient mutant of at least one of the parental hybridomas. This first mutant hybridoma cell line is then fused to cells of a second hybridoma that had been lethally exposed, e.g., to iodoacetamide, precluding its continued survival. Cell fusion allows for the rescue of the first hybridoma by acquiring the gene for its enzyme deficiency from the lethally treated hybridoma, and the rescue of the second hybridoma through fusion to the first hybridoma. Preferred, but not required, is the fusion of immunoglobulins of the same isotype, but of a different subclass. A mixed subclass antibody permits the use if an alternative assay for the isolation of a preferred quadroma.

In more detail, one method of quadroma development and screening involves obtaining a hybridoma line that secretes the first chosen mAb and making this deficient for the essential metabolic enzyme, hypoxanthine-guanine phosphoribosyltransferase (HGPRT). To obtain deficient mutants of the hybridoma, cells are grown in the presence of increasing concentrations of 8-azaguanine (1×10^7 M to 1×10^{-5} M). The mutants are subcloned by limiting dilution and tested for their hypoxanthine/aminopterin/thymidine (HAT) sensitivity. The culture medium may consist of, for example, DMEM supplemented with 10% FCS, 2 mM L-Glutamine and 1 mM penicillin-streptomycin.

A complementary hybridoma cell line that produces the second desired MAb is used to generate the quadromas by standard cell fusion techniques (Galfre et al, *Methods Enzymol*, 73:1-46, 1981), or by using the protocol described by Clark et al (*Int J Cancer*, 2:15-17, 1988). Briefly, 4.5×10^7 HAT-sensitive first cells are mixed with 2.8x phosphate buffered saline) for 30 in minutes on ice before fusion. Cell

fusion is induced using polyethylene glycol (PEG) and the cells are plated out in 96 well microculture plates. Quadromas are selected using Hat-containing medium. Bispecific antibody-containing cultures are identified using, for example, a solid phase isotype-specific ELISA and isotype-specific immunofluorescence staining.

In one identification embodiment to identify the bispecific antibody, the wells of microliter plates (Falcon, Becton Dickinson Labware) are coated with a reagent that specifically interacts with one of the parent hybridoma antibodies and that lacks cross-reactivity with both antibodies. The plates are washed, blocked, and the supernatants (SNs) to be tested are added to each well. Plates are incubated at room temperature for 2 hours, the supernatants discarded, the plates washed, and diluted alkaline phosphatase-antibody conjugate added for 2 hours at room temperature. The plates are washed and a phosphatase substrate, e.g., p-Nitrophenyl phosphate (Sigma, St. Louis) is added to each well. Plates are incubated, 3N NaOH is added to each well to stop the reaction, and the OD410 values determined using an ELISA reader.

In another identification embodiment, microliter plates pre-treated with poly-L-lysine are used to bind one of the target cells to each well, the cells are then fixed, e.g. using 1% glutaraldehyde, and the bispecific antibodies are tested for their ability to bind to the intact cell. In addition, FACS, immunofluorescence staining, idiotype specific antibodies, antigen binding competition assays, and other methods common in the art of antibody characterization may be used in conjunction with the present invention to identify preferred quadromas.

Following the isolation of the quadroma, the bispecific antibodies are purified away from other cell products. This may be accomplished by a variety of protein isolation Procedures, known to those skilled in the art of immunoglobulin purification. Means for preparing and characterizing antibodies are well known in the art (See, e.g., *Antibodies: A Laboratory Manual*, 1988).

For example, supernatants from selected quadromas are passed over protein A or protein G sepharose columns to bind IgG (depending on the isotype). The bound antibodies are then eluted with, e.g. a pH 5.0 citrate buffer. The elute fractions containing the BsAbs, are dialyzed against an isotonic buffer. Alternatively, the eluate is also passed over an anti-immunoglobulin-sepharose column. The BsAb is then eluted with 3.5 M magnesium chloride. BsAbs purified in this way are then tested for binding activity by, e.g., an isotype-specific ELISA and immunofluorescence staining assay of the target cells, as described above.

Purified BsAbs and parental antibodies may also be characterized and isolated by SDS PAGE electrophoresis, followed by staining with silver or Coomassie. This is possible when one of the parental antibodies has a higher molecular weight than the other, wherein the band of the BsAbs migrates midway between that of the two parental antibodies. Reduction of the samples verifies the presence of heavy chains with two different apparent molecular weights.

Furthermore, recombinant technology is now available for the preparation of antibodies in general, allowing the preparation of recombinant antibody genes encoding an antibody having the desired dual specificity (Van Duk et al., *Int J. Cancer*, 43:344-349, 1989). Thus, after selecting the monoclonal antibodies having the most preferred binding characteristics, the respective genes for these antibodies can be isolated, e.g., by immunological screening of a phage expression library (Oi and Morrison, 1986; Winter and Milstein, 1991). Then, through rearrangement of Fab coding domains, the appropriate chimeric construct can be readily obtained.

Humanized monoclonal antibodies are antibodies of animal origin that have been modified using genetic engineering techniques to replace constant region and/or variable region framework sequences with human sequences, while retaining the original antigen specificity.

Such antibodies are commonly derived from rodent antibodies with specificity against human antigens. Such antibodies are generally useful for in vivo therapeutic applications. This strategy reduces the host response to the foreign antibody and allows selection of the human effector functions.

The techniques for producing humanized immunoglobulins are well known to those of skill in the art. For example U.S. Pat. No. 5,693,762 discloses methods for producing, and compositions of, humanized immunoglobulins having one or more complementarily determining regions (CDR's). When combined into an intact antibody, the humanized immunoglobulins are substantially non-immunogenic in humans and retain substantially the same affinity as the donor immunoglobulin to the antigen, such as a protein or other compound containing an epitope.

Other U.S. patents, each incorporated herein by reference, that teach the production of antibodies useful in the present invention include U.S. Pat. No. 5,565,332, which describes the production of chimeric antibodies using a combinatorial approach; U.S. Pat. No. 4,816,567 which describes recombinant immunoglobulin preparations and U.S. Pat. No. 4,867,973 which describes antibody-therapeutic agent conjugates.

U.S. Pat. No. 5,565,332 describes methods for the production of antibodies, or antibody fragments, which have the same binding specificity as a parent antibody but which have increased human characteristics. Humanized antibodies may be obtained by chain shuffling, perhaps using phage display technology, in as much as such methods will be useful in the present invention the entire text of U.S. Pat. No. 5,565,332 is incorporated herein by reference.

Using the peptide antigens described herein, the present invention also provides methods of generating an immune response, which methods generally comprise administering to an animal, a pharmaceutically-acceptable composition comprising an immunologically effective amount of an MSCRAMM-derived peptide composition. Preferred animals include mammals, and particularly humans. Other preferred animals include murines, bovines, equines, porcines, canines, and felines. The composition may include partially or significantly purified MSCRAMM-derived peptide epitopes, obtained from natural or recombinant sources, which proteins or peptides may be obtainable naturally or either chemically synthesized, or alternatively produced in vitro from recombinant host cells expressing DNA segments encoding such epitopes. Smaller peptides that include reactive epitopes, such as those between about 30 and about 100 amino acids in length will often be preferred. The antigenic proteins or peptides may also be combined with other agents, such as other staphylococcal or streptococcal peptide or nucleic acid compositions, if desired. The composition may also include staphylococcal produced bacterial components such as those discussed above, obtained from natural or recombinant sources, which proteins may be obtainable naturally or either chemically synthesized, or alternatively produced in vitro from recombinant host cells expressing DNA segments encoding such peptides.

Immunoformulations of this invention, whether intended for vaccination, treatment, or for the generation of antibodies useful in the detection of staphylococci and streptococci, or prevention of bacterial adhesion to ECM components such as fibronectin, collagen, elastin, fibrinogen or vitrone-

tin may comprise site-specifically mutated, truncated, or synthetically-derived antigenic peptide fragments from these proteins. As such, antigenic functional equivalents of the proteins and peptides described herein also fall within the scope of the present invention.

Further means contemplated by the inventors for generating an immune response in an animal includes administering to the animal, or human subject, a pharmaceutically-acceptable composition comprising an immunologically effective amount of a nucleic acid composition encoding a peptide epitope, or an immunologically effective amount of an attenuated live organism that includes and expresses such a nucleic acid composition.

The amount of expressible DNA or transcribed RNA to be introduced into a vaccine recipient will have a very broad dosage range and may depend on the strength of the transcriptional and translational promoters used. In addition, the magnitude of the immune response may depend on the level of protein expression and on the immunogenicity of the expressed gene product. In general, effective dose ranges of about 1 ng to 5 mg, 100 ng to 2.5 mg, 1 μ g to 750 μ g, and preferably about 10 μ g to 300 μ g of DNA is administered directly into muscle tissue. Subcutaneous injection, intradermal introduction, impression through the skin, and other modes of administration such as intraperitoneal, intravenous, or inhalation delivery are also suitable. It is also contemplated that booster vaccinations may be provided. Following vaccination with an MSCRAMM polynucleotide immunogen, boosting with MSCRAMM protein immunogens such as the M55 gene product is also contemplated.

The polynucleotide may be "naked", that is, unassociated with any proteins, adjuvants or other agents which affect the recipients' immune system. In this case, it is desirable for the polynucleotide to be in a physiologically acceptable solution, such as, but not limited to, sterile saline or sterile buffered saline. Alternatively, the DNA may be associated with liposomes, such as lecithin liposomes or other liposomes known in the art, as a DNA-liposome mixture, or the DNA may be associated with an adjuvant known in the art to boost immune responses, such as a protein or other carrier. Agents which assist in the cellular uptake of DNA, such as, but not limited to, calcium ions, may also be used. These agents are generally referred to herein as transfection facilitating reagents and pharmaceutically acceptable carriers. Techniques for coating microprojectiles coated with polynucleotide are known in the art and are also useful in connection with this invention. For DNA intended for human use it may be useful to have the final DNA product in a pharmaceutically acceptable carrier or buffer solution. Pharmaceutically acceptable carriers or buffer solutions are known in the art and include those described in a variety of texts such as Remington's Pharmaceutical Sciences.

In another embodiment, the invention is a polynucleotide which comprises contiguous nucleic acid sequences capable of being expressed to produce a gene product upon introduction of said polynucleotide into eukaryotic tissues in vivo. The encoded gene product preferably either acts as an immunostimulant or as an antigen capable of generating an immune response. Thus, the nucleic acid sequences in this embodiment encode an MSCRAMM immunogenic epitope, and optionally a cytokine or a T-cell costimulatory element, such as a member of the B7 family of proteins.

There are several advantages of immunization with a gene rather than its gene product. The first is the relative simplicity with which native or nearly native antigen can be presented to the immune system. Mammalian proteins expressed recombinantly in bacteria, yeast, or even mam-

malian cells often require extensive treatment to insure appropriate antigenicity. A second advantage of DNA immunization is the potential for the immunogen to enter the MHC class I pathway and evoke a cytotoxic T cell response.

5 Immunization of mice with DNA encoding the influenza A nucleoprotein (NP) elicited a CD8⁺ response to NP that protected mice against challenge with heterologous strains of flu. (Montgomery, D. L. et al., *Cell Mol Biol*, 43(3): 285-292, 1997; Ulmer, J. et al., *Vaccine*, 15(8):792-794, 10 1997)

Cell-mediated immunity is important in controlling infection. Since DNA immunization can evoke both humoral and cell-mediated immune responses, its greatest advantage may be that it provides a relatively simple method to survey a large number of *S. aureus* genes for their vaccine potential.

15 Immunization by DNA injection also allows the ready assembly of multicomponent subunit vaccines. Simultaneous immunization with multiple influenza genes has recently been reported. (Donnelly, J. et al., *Vaccines*, 55-59, 1994). The inclusion in a *S. aureus* vaccine of genes whose products activate varied and multiple responses from the immune system may also provide thorough protection from subsequent challenge.

Further provided is a composition comprising an isolated nucleic acid segment that encodes a peptide of a binding domain of a binding protein, wherein the peptide does or does not specifically bind to its ligand. It is also contemplated that attenuated organisms may be engineered to express recombinant MSCRAMM gene products and themselves be delivery vehicles for the invention. Particularly preferred are attenuated bacterial species such as Mycobacterium, and in particular *M. bovis*, *M. smegmatis*, or BCG. Alternatively, pox-, polio-, adeno-, or other viruses, and bacteria such as Salmonella, Shigella, Listeria, Streptococcus species may also be used in conjunction with the methods and compositions disclosed herein.

The naked DNA technology, often referred to as genetic immunization, has been shown to be suitable for protection against infectious organisms. Such DNA segments could be used in a variety of forms including naked DNA and plasmid DNA, and may administered to the subject in a variety of ways including parenteral, mucosal, and so-called microprojectile-based "gene-gun" inoculations. The use of nucleic acid compositions of the present invention in such immunization techniques is thus proposed to be useful as a vaccination strategy against at least streptococcal and staphylococcal infection.

It is recognized by those skilled in the art that an optimal dosing schedule of a DNA vaccination regimen may include as many as five to six, but preferably three to five, or even more preferably one to three administrations of the immunizing entity given at intervals of as few as two to four weeks, to as long as five to ten years, or occasionally at even longer intervals.

Particular aspects of the invention concern the use of plasmid vectors for the cloning and expression of recombinant peptides, and particular peptide epitopes comprising either native, or site-specifically mutated binding site epitopes. The generation of recombinant vectors, transformation of host cells, and expression of recombinant proteins is well-known to those of skill in the art. Prokaryotic hosts are preferred for expression of the peptide compositions of the present invention. An example of a preferred prokaryotic host is *E. coli*, and in particular, *E. coli*, strains ATCC 69791, BL21(DE3), JM101, XL1-BlueTM, RRI, LE392, B, λ ⁷⁷⁶ (ATCC 31537), and W3110 (F, λ , prototrophic, ATCC 273325). Alternatively, other Enterobacteriaceae species

such as *Salmonella typhimurium* and *Serratia marcescens*, or even other Gram-negative hosts including various *Pseudomonas* species may be used in the recombinant expression of the genetic constructs discussed herein. Additional hosts may include well known eukaryotic and prokaryotic hosts, such as strains of *Bacillus*, *Streptomyces*, fungi such as yeasts, and animal cells, such as CHO, R1.1, B-W and L-M cells, African Green Monkey kidney cells (e.g., COS 1, COS 7, BSC1, BSC40, and BMT10), insect cells (e.g., Sf9), and human cells and plant cells in tissue culture.

A wide variety of host/expression vector combinations may be employed in expressing the DNA. 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\mu* 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.

As is well known in the art, DNA sequences may be expressed by operatively linking them to an expression control sequence in an appropriate expression vector and employing that expression vector to transform an appropriate unicellular host. Such operative linking of a DNA sequence of this invention to an expression control sequence, of course, includes, if not already part of the DNA sequence, the provision of an initiation codon, ATG, in the correct reading frame upstream of the DNA sequence.

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-phospho-glycerate 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.

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

In selecting an expression control sequence, a variety of factors will normally be considered. These include, for

example, the relative strength of the system, its controllability, and its compatibility with the particular DNA sequence or gene to be expressed, particularly with regard to potential secondary structures. Suitable unicellular hosts will be selected by consideration of, e.g., their compatibility with the chosen vector, their secretion characteristics, their ability to fold proteins correctly, and their fermentation requirements, as well as the toxicity to the host of the product encoded by the DNA sequences to be expressed, and the ease of purification of the expression products. Considering these and other factors a person skilled in the art will be able to construct a variety of vector/expression control sequence/host combinations that will express the DNA sequences encoding the components of this invention on fermentation or in large scale animal culture.

In certain embodiments, it is also contemplated that the nucleic acid segments discussed herein will be used to transect appropriate host cells. Technology for introduction of DNA into cells is well-known to those of skill in the art. Four general methods for delivering a nucleic segment into cells have been described: (1) chemical methods (Graham and VanDerEb, *Virology*, 54 (2):536–539, 1973); physical methods such as microinjection (Capecchi, *Cell*, 22(2):479–488, 1980), electroporation (Wong and Neuman, *Biochim Biophys Res Commun*, 107(2):584–587, 1982; Fromm et al., *Proc Natl Acad Sci USA*, 82(17):5824–5828, 1985) and the gene gun (Yang et al., *Proc Natl Acad Sci USA*, 87:4144–4148, 1990); (3) viral vectors (Eglitis and Anderson, *Bio/techniques*, 6(7):608–614, 1988); and (4) receptor-mediated mechanisms (Wagner, et al., *Proc Natl Acad Sci USA*, 89(13):6099–6103, 1992).

DNA sequences encoding MSCRAMM can be prepared synthetically or cloned. The DNA sequence can be designed with the appropriate codons for the MSCRAMM amino acid sequence. In general, one will select preferred codons for the intended host if the sequence will be used for expression. The complete sequence is assembled from overlapping oligonucleotides prepared by standard methods and assembled into a complete coding sequence. See, e.g., Edge, *Nature*, 292:756 (1981); Nambair et al., *Science*, 223:1299 (1984); Jay et al., *J. Biol. Chem.*, 259:6311 (1984).

Synthetic DNA sequences allow convenient construction of genes which will express MSCRAMM analogs. Alternatively, DNA encoding analogs can be made by site-directed mutagenesis of native MSCRAMM genes or cDNAs, and analogs can be made directly using conventional polypeptide synthesis. 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.

XII. Antisense Oligonucleotides and Ribozymes

The present invention extends to the preparation of antisense oligonucleotides and ribozymes that may be used to interfere with the expression of the MSCRAMM at the translational level. This approach utilizes antisense nucleic acid and ribozymes to block translation of a specific mRNA, either by masking the mRNA with an antisense nucleic acid or cleaving it with a ribozyme.

Antisense nucleic acids are DNA or RNA molecules that are complementary to at least a portion of a specific mRNA molecule. In the cell, they hybridize to that specific mRNA, forming a double stranded molecule. The cell does not translate an mRNA in this double-stranded form. Therefore, antisense nucleic acids interfere with the expression of mRNA into protein. Oligomers of about fifteen nucleotides and molecules that hybridize to the AUG initiation codon

will be particularly efficient, since they are easy to synthesize and are likely to pose fewer problems than larger molecules when introducing them into MSCRAMM-producing cells. Antisense methods have been used to inhibit the expression of many genes in vitro (Marcus-Sekura, 1988; Hambor et al., 1988).

Ribozymes are RNA molecules possessing the ability to specifically cleave other single stranded RNA molecules in a manner somewhat analogous to DNA restriction endonucleases. Ribozymes were discovered from the observation that certain mRNAs have the ability to excise their own introns. By modifying the nucleotide sequence of these RNAs, researchers have been able to engineer molecules that recognize specific nucleotide sequences in an RNA molecule and cleave it (Cech, 1988.). Because they are sequence-specific, only mRNAs with particular sequences are inactivated.

Investigators have identified two types of ribozymes, Tetrahymena-type and "hammerhead"-type. (Hasselhoff and Gerlach, *Nature*, 334(6183):585-591, 1988) Tetrahymena-type ribozymes recognize four-base sequences, while "hammerhead"-type recognizes eleven- to eighteen-base sequences. The longer the recognition sequence, the more likely it is to occur exclusively in the target mRNA species. Therefore, hammerhead-type ribozymes are preferable to Tetrahymena-type ribozymes for inactivating a specific mRNA species, and eighteen base recognition sequences are preferable to shorter recognition sequences.

The DNA sequences described herein may thus be used to prepare antisense molecules against, and ribozymes that cleave mRNAs for MSCRAMM and their ligands.

XIII. Pharmaceutical Compositions

A pharmaceutical composition is provided that comprises the binding proteins, the peptides, the antibodies, or the nucleic acids as described above optionally in combination with bacterial components, in a pharmaceutically acceptable excipient, in an effective amount to treat *S. aureus* infection. The compositions are typically used in the preparation of an immunization formulation that optionally includes an adjuvant and other customary additives. The compositions can also comprise diagnostic kits as described herein.

Methods for preparing pharmaceutical compositions which contain polypeptides, analogs or active fragments as active ingredients are well understood in the art. Typically, such compositions are prepared 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.

A polypeptide, analog or active fragment 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.

The therapeutic polypeptide-, analog- or active fragment-containing compositions are conventionally administered intravenously, as by injection of a unit dose, for example. 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.

The compositions are administered in a manner compatible with the dosage formulation, and in a therapeutically effective amount. The quantity to be administered depends on the subject to be treated, capacity of the subject's immune system to utilize the active ingredient, and degree of inhibition or neutralization of MSCRAMM binding capacity desired. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are peculiar to each individual. However, suitable dosages may range from about 0.1 to 20, preferably about 0.5 to about 10, and more preferably one to several, milligrams of active ingredient per kilogram body weight of individual per day and depend on the route of administration. Suitable regimes for initial administration and booster shots are also variable, but are typified by an initial administration followed by repeated doses at one or more hour intervals by a subsequent injection or other administration. Alternatively, continuous intravenous infusion sufficient to maintain concentrations often nanomolar to ten micromolar in the blood are contemplated.

The therapeutic compositions may further include an effective amount of the MSCRAMM/MSCRAMM antagonist or analog thereof, and one or more of the following active ingredients: an antibiotic, a steroid.

The preparation of vaccines that contain peptide sequences as active ingredients is generally well understood in the art, as exemplified by U.S. Pat. Nos. 4,608,251; 4,601,903; 4,599,231; 4,599,230; 4,596,792; and 4,578,770, all incorporated herein by reference. Typically, such vaccines are prepared as injectables, either as liquid solutions or suspensions, solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The preparation may also be emulsified. The active immunogenic ingredient is often mixed with excipients that 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 vaccine may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, or adjuvants that enhance the effectiveness of the vaccines.

The preparation of such compositions that are essentially free from endotoxin can be achieved by following the published methodology, for example, U.S. Pat. No. 4,271,147 (incorporated herein by reference) discloses methods for the preparation of *Neisseria meningitidis* membrane proteins for use in vaccines.

The immunological compositions, such as vaccines, and other pharmaceutical compositions can be used alone or in combination with other blocking agents to protect against human and animal infections caused by staphylococcal bacteria such as *S. aureus*. In particular, the compositions can be used to protect humans against endocarditis or to protect humans or ruminants against mastitis caused by staphylococcal infections. The vaccine can also be used to

protect canine and equine animals against similar staphylococcal infections.

To enhance immunogenicity, the proteins may be conjugated to a carrier molecule. Suitable immunogenic carriers include proteins, polypeptides or peptides such as albumin, hemocyanin, thyroglobulin and derivatives thereof, particularly bovine serum albumin (BSA) and keyhole limpet hemocyanin (KLH), polysaccharides, carbohydrates, polymers, and solid phases. Other protein derived or non-protein derived substances are known to those skilled in the art. An immunogenic carrier typically has a molecular weight of at least 1,000 daltons, preferably greater than 10,000 daltons. Carrier molecules often contain a reactive group to facilitate covalent conjugation to the hapten. The carboxylic acid group or amine group of amino acids or the sugar groups of glycoproteins are often used in this manner. Carriers lacking such groups can often be reacted with an appropriate chemical to produce them. Preferably, an immune response is produced when the immunogen is injected into animals such as mice, rabbits, rats, goats, sheep, guinea pigs, chickens, and other animals, most preferably mice and rabbits. Alternatively, a multiple antigenic peptide comprising multiple copies of the protein or polypeptide, or an antigenically or immunologically equivalent polypeptide may be sufficiently antigenic to improve immunogenicity without the use of a carrier.

The MSCRAMM protein or proteins may be administered with an adjuvant in an amount effective to enhance the immunogenic response against the conjugate. At this time, the only adjuvant widely used in humans has been alum (aluminum phosphate or aluminum hydroxide). Saponin and its purified component Quil A, Freund's complete adjuvant and other adjuvants used in research and veterinary applications have toxicities which limit their potential use in human vaccines. However, chemically defined preparations such as muramyl dipeptide, monophosphoryl lipid A, phospholipid conjugates such as those described by Goodman-Snitkoff et al. (*J. Immunol.* 147:410-415, 1991) and incorporated by reference herein, encapsulation of the conjugate within a proteoliposome as described by Miller et al., (*J. Exp. Med.* 176:1739-1744, 1992) and incorporated by reference herein, and encapsulation of the protein in lipid vesicles such as Novasome™ lipid vesicles (Micro Vesicular Systems, Inc., Nashua, N.H.) may also be useful.

In certain embodiments, the inventors contemplate the use of liposomes and/or nanocapsules for the introduction of particular peptides or nucleic acid segments into host cells. In particular, the malonyltyrosyl and phosphotyrosyl peptides of the present invention may be formulated for delivery in solution with DMSO or encapsulated in liposomes.

Such formulations may be preferred for the introduction of pharmaceutically acceptable formulations of the nucleic acids, peptides, and/or antibodies disclosed herein. The formation and use of liposomes is generally known to those of skill in the art (see for example, Couvreur et al, *FEBS Lett*, 84:323-326, 1977; and *Crit Rev Ther Drug Carrier Syst*, 5:1-20, 1988 which describes the use of liposomes and nanocapsules in the targeted antibiotic therapy of intracellular bacterial infections and diseases). Recently, liposomes were developed with improved serum stability and circulation half-times (Gabizon and Papahadjopoulos, *Proc Natl Acad Sci USA*, 85:6949-6953, 1988; Allen and Choun, *FEBS Lett*, 223:42-46, 1987).

Liposomes have been used successfully with a number of cell types that are normally resistant to transfection by other procedures including T cell suspensions, primary hepatocyte cultures and PC 12 cells (Muller et al., *DNA Cell Biol*,

9(3):221-229, 1990). In addition, liposomes are free of the DNA length constraints that are typical of viral-based delivery systems. Liposomes have been used effectively to introduce genes, drugs, radiotherapeutic agents, enzymes, viruses, transcription factors and allosteric effectors into a variety of cultured cell lines and animals. In addition, several successful clinical trials examining the effectiveness of liposome-mediated drug delivery have been completed (Lopez-Berestein et al, *Cancer Drug Review*, 2(3):183-189, 1985; Sculier et al, *Eur J Cancer Clin Oncol*, 24(3):527-538, 1988). Furthermore, several studies suggest that the use of liposomes is not associated with autoimmune responses, toxicity or gonadal localization after systemic delivery (Mori and Fukatsu, *Epilepsia*, 33(6):994-1000, 1992).

Liposomes are formed from phospholipids that are dispersed in an aqueous medium and spontaneously form multilamellar concentric bilayer vesicles (also termed multilamellar vesicles (MLVs)). MLVs generally have diameters of from 25 nm to 4 micron. Sonication of MLVs results in the formation of small unilamellar vesicles (SUVS) with diameters in the range of 200 to 500 Å, containing an aqueous solution in the core.

Liposomes bear many resemblances to cellular membranes and are contemplated for use in connection with the present invention as carriers for the peptide compositions. They are widely suitable, as both water- and lipid-soluble substances can be entrapped, i.e., in the aqueous spaces and within the bilayer itself, respectively. It is possible that the drug-bearing liposomes may even be employed for site-specific delivery of active agents by selectively modifying the liposomal formulation.

In addition to the teachings of Couvreur et al. (*FEBS Lett*, 84:323-326, 1977; and *Crit Rev Ther Drug Carrier Syst*, 5:1-20, 1988), the following information may be utilized in generating liposomal formulations. Phospholipids can form a variety of structures other than liposomes when dispersed in water, depending on the molar ratio of lipid to water. At low ratios the liposome is the preferred structure. The physical characteristics of liposomes depend on pH, ionic strength and the presence of divalent cations. Liposomes can show low permeability to ionic and polar substances, but at elevated temperatures undergo a phase transition which markedly alters their permeability. The phase transition involves a change from a closely packed, ordered structure, known as the gel state, to a loosely packed, less-ordered structure, known as the fluid state. This occurs at a characteristic phase-transition temperature and results in an increase in permeability to ions, sugars and drugs.

In addition to temperature, exposure to proteins can alter the permeability of liposomes. Certain soluble proteins such as cytochrome C bind, deform and penetrate the bilayer, thereby causing changes in permeability. Cholesterol inhibits this penetration of proteins, apparently by packing the phospholipids more tightly. It is contemplated that the most useful liposome formations for antibiotic and inhibitor delivery will contain cholesterol.

The ability to trap solutes varies between different types of liposomes. For example, MLVs are moderately efficient at trapping solutes, but SUVs are extremely inefficient. SUVs offer the advantage of homogeneity and reproducibility in size distribution, however, and a compromise between size and trapping efficiency is offered by large unilamellar vesicles (LUVs). These are prepared by ether evaporation and are three to four times more efficient at solute entrapment than MLVS.

In addition to liposome characteristics, an important determinant in entrapping compounds is the physicochemi-

cal properties of the compound itself. Polar compounds are trapped in the aqueous spaces and nonpolar compounds bind to the lipid bilayer of the vesicle. Polar compounds are released through permeation or when the bilayer is broken, but nonpolar compounds remain affiliated with the bilayer unless it is disrupted by temperature or exposure to lipoproteins. Both types show maximum efflux rates at the phase transition temperature.

Liposomes interact with cells via four different mechanisms: Endocytosis by phagocytic cells of the reticuloendothelial system such as macrophages and neutrophils; adsorption to the cell surface, either by nonspecific weak hydrophobic or electrostatic forces, or by specific interactions with cell-surface components; fusion with the plasma cell membrane by insertion of the lipid bilayer of the liposome into the plasma membrane, with simultaneous release of liposomal contents into the cytoplasm; and by transfer of liposomal lipids to cellular or subcellular membranes, or vice versa, without any association of the liposome contents. It often is difficult to determine which mechanism is operative and more than one may operate at the same time.

The fate and disposition of intravenously injected liposomes depends on their physical properties, such as size, fluidity and surface charge. They may persist in tissues for hours or days, depending on their composition, and half lives in the blood range from minutes to several hours. Larger liposomes, such as MLVs and LUVS, are taken up rapidly by phagocytic cells of the reticuloendothelial system, but physiology of the circulatory system restrains the exit of such large species at most sites. They can exit only in places where large openings or pores exist in the capillary endothelium, such as the sinusoids of the liver or spleen. Thus, these organs are the predominate site of uptake. On the other hand, SUVs show a broader tissue distribution but still are sequestered highly in the liver and spleen. In general, this in vivo behavior limits the potential targeting of liposomes to only those organs and tissues accessible to their large size. These include the blood, liver, spleen, bone marrow and lymphoid organs.

Targeting is generally not a limitation in terms of the present invention. However, should specific targeting be desired, methods are available for this to be accomplished. Antibodies may be used to bind to the liposome surface and to direct the antibody and its drug contents to specific antigenic receptors located on a particular cell-type surface. Carbohydrate determinants (glycoprotein or glycolipid cell-surface components that play a role in cell-cell recognition, interaction and adhesion) may also be used as recognition sites as they have potential in directing liposomes to particular cell types. Mostly, it is contemplated that intravenous injection of liposomal preparations would be used, but other routes of administration are also conceivable.

Alternatively, the invention provides for pharmaceutically-acceptable nanocapsule formulations of the peptides of the present invention. Nanocapsules can generally entrap compounds in a stable and reproducible way (Henry-Michelland et al, *Int J. Pharm.*, 35:121-127, 1987). To avoid side effects due to intracellular polymeric overloading, such ultrafine particles (sized around 0.1 micron) should be designed using polymers able to be degraded in vivo. Biodegradable polyalkyl-cyanoacrylate nanoparticles that meet these requirements are contemplated for use in the present invention, and such particles may be easily made, as described by Couvreur et al, (supra, 1977 and 1988).

Suitable methods of administration include, but are not limited to, topical, oral, anal, vaginal, intravenous,

intraperitoneal, intramuscular, subcutaneous, intranasal and intradermal administration.

For topical administration, the composition is formulated in the form of an ointment, cream, gel, lotion, drops (such as eye drops and ear drops), or solution (such as mouthwash). Wound or surgical dressings, sutures and aerosols may be impregnated with the composition. The composition may contain conventional additives, such as preservatives, solvents to promote penetration, and emollients. Topical formulations may also contain conventional carriers such as cream or ointment bases, ethanol, or oleyl alcohol.

In a preferred embodiment, a vaccine is packaged in a single dosage for immunization by parenteral (i.e., intramuscular, intradermal or subcutaneous) administration or nasopharyngeal (i.e., intranasal) administration. The vaccine is most preferably injected intramuscularly into the deltoid muscle. The vaccine is preferably combined with a pharmaceutically acceptable carrier to facilitate administration. The carrier is usually water or a buffered saline, with or without a preservative. The vaccine may be lyophilized for resuspension at the time of administration or in solution.

The carrier to which the protein may be conjugated may also be a polymeric delayed release system. Synthetic polymers are particularly useful in the formulation of a vaccine to effect the controlled release of antigens. For example, the polymerization of methyl methacrylate into spheres having diameters less than one micron has been reported by Kreuter, J., *Microcapsules And Nanoparticles In Medicine And Pharmacology*, M. Donbrow (Ed). CRC Press, p. 125-148.

Microencapsulation of the protein will also give a controlled release. A number of factors contribute to the selection of a particular polymer for microencapsulation. The reproducibility of polymer synthesis and the microencapsulation process, the cost of the microencapsulation materials and process, the toxicological profile, the requirements for variable release kinetics and the physicochemical compatibility of the polymer and the antigens are all factors that must be considered. Examples of useful polymers are polycarbonates, polyesters, polyurethanes, polyorthoesters polyamides, poly (d,l-lactide-co-glycolide) (PLGA) and other biodegradable polymers. The use of PLGA for the controlled release of antigen is reviewed by Eldridge, J. H., et al. *Current Topics In Microbiology And Immunology*, 146:59-66 (1989).

The preferred dose for human administration is from 0.01 mg/kg to 10 mg/kg, preferably approximately 1 mg/kg. Based on this range, equivalent dosages for heavier body weights can be determined. The dose should be adjusted to suit the individual to whom the composition is administered and will vary with age, weight and metabolism of the individual. The vaccine may additionally contain stabilizers such as thimerosal (ethyl(2-mercaptobenzoate-S) mercury sodium salt) (Sigma Chemical Company, St. Louis, Mo.) or physiologically acceptable preservatives.

It will be readily apparent to one skilled in the art that various substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

XIV. Kits

This invention also includes a kit comprising anti-MSCRAMM antibody or an MSCRAMM antigen for the detection and diagnosis of infections caused or exacerbated by Staphylococcus bacteria such as *S. aureus* or *S. epidermidis*. The preferred kit contains sufficient antibody to bind substantially all of the antigen in the sample in about ten minutes or less, or sufficient antigen to bind antibodies for MSCRAMMs. The antibody or antigen can be immobilized

on a solid support, and can be labeled with a detectable agent, as described above. The kit optionally contains a means for detecting the detectable agent. If the antibody or antigen is labeled with a fluorochrome or radioactive label, no means for detecting the agent will typically be provided, as the user will be expected to have the appropriate spectrophotometer, scintillation counter, or microscope. If the detectable agent is an enzyme, a means for detecting the detectable agent can be supplied with the kit, and would typically include a substrate for the enzyme in sufficient quantity to detect all of the antigen-antibody complex. One preferred means for detecting a detectable agent is a substrate that is converted by an enzyme into a colored product. A common example is the use of the enzyme horseradish peroxidase with 2,2'-azino-di-[3-ethyl-benzothiazoline sulfonate] (ABTS).

The kit can optionally contain a lysing agent that lyses cells present in the sample of body fluid. Suitable lysing agents include surfactants such as Tween-80, Nonidet P40, and Triton X-100. Preferably, the lysing agent is immobilized onto the solid support along with the antibody.

The kit can also contain a buffer solution for washing the substrate between steps. The buffer solution is typically a physiological solution such as a phosphate buffer, physiological saline, citrate buffer, or Tris buffer.

The kit can optionally include different concentrations of a preformed antigen to calibrate the assay. The kit can additionally contain a visual or numeric representation of amounts of antigen in a calibrated standard assay for reference purposes. For example, if an assay is used that produces a colored product, a sheet can be included that provides a depiction of increasing intensities associated with differing amounts of antigen.

The kit can optionally include two antibodies in the detection system. The first antibody which is present in small amounts is specific for the antigen being assayed for. The second antibody provided in higher amounts is used to detect the first antibody. For example, a rabbit antibody can be used to detect the LOOH/amine antigen, and then an anti-rabbit IgG antibody can be used to detect the bound rabbit antibody. Goat antibodies and anti-antibodies are also commonly used.

As one nonlimiting example, a kit for the detection of the lipid peroxidation state of a patient is provided that includes a rabbit antibody specific for desired antigen, anti-rabbit IgG antibody in sufficient amounts to detect the bound first antibody, an enzyme conjugated to the second antibody and a substrate for the enzyme which changes color on exposure to the enzyme. In addition, a kit may be prepared using one or more MSCRAMM antigens such as the M55 domain of the collagen binding protein and the ClfA fibrinogen binding protein, and this kit will enable the detection of samples with antibodies to collagen binding and fibrinogen binding MSCRAMMs.

EXAMPLES

The following examples are included to demonstrate preferred embodiments of the present invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventors to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1

Preparation of Prototype Four Component MSCRAMM Vaccine

A series of recombinant proteins, representing domains from the collagen, Fn, and Fbg-binding MSCRAMMs (FIG. 1), were overexpressed in *E. coli* and affinity purified by metal chelating chromatography as previously described (see, e.g., Joh et al., *Biochemistry*. 33 (20):6086-6092, 1994; Patti et al., *J. Biol. Chem.* 270, 12005-12011, 1995; McDevitt et al., *Mol. Micro.* 11 (2):237-248, 1994; Ni Eidhin et al., *Infect. Immun.* Submitted, 1998). Used were the following: amino acids contained in the recombinant collagen-binding MSCRAMM expressed from *cna* (M55, such as disclosed in co-pending U.S. patent application Ser. No. 08/856,253, incorporated herein by reference); amino acids contained in the recombinant fibrinogen-binding MSCRAMM expressed from *clfA* (pCF40, such as disclosed in U.S. patent application Ser. No. 08/293,728, incorporated herein by reference); amino acids contained in the recombinant fibrinogen-binding MSCRAMM expressed from *clfB* (Region A, such as disclosed in U.S. application Ser. No. 09/200,650, incorporated herein by reference); and amino acids contained in the recombinant fibronectin-binding MSCRAMM (DUD4, such as those disclosed in co-pending U.S. application Ser. No. 09/010,317, incorporated herein by reference). The recombinant FN-binding MSCRAMM protein DUD4 was treated with formalin (5% formalin overnight, 4° C.) prior to combining it with the M55, Region A from ClfA, and Region A from ClfB.

Example 2

Example of growing *E. coli* Strains for Production of Recombinant Proteins

Overnight cultures of *E. coli* JM101 or TOP 3 cells (Stratagene) harboring the recombinant plasmids were diluted 1:50 in 1 L of Luria Broth (Gibco BRL) containing 50 mg/mL ampicillin. *E. coli* cells were grown until the culture reached an OD₆₀₀ of 0.5-0.8. Expression of the recombinant proteins was induced by adding IPTG to a final concentration of 0.2 mM. After a three hour induction period, cells were collected by centrifugation, resuspended in 15 mL of Buffer A (5 mM imidazole, 0.5 M NaCl, 20 mM Tris-HCl, pH 7.9) and lysed by passage through a French press twice at 20,000 lb./in². Cell debris was removed by centrifugation at 50,000xg for 10 min and the supernatant was passed through a 0.45 μm filter.

Example 3

Purification of HIS₆ Containing Recombinant Proteins Expressed from pQE-30 (Qiagen®; Qiagen Inc. Chatsworth, Calif.) or PV-4 Based Recombinant Plasmids

The recombinant proteins were purified by immobilized metal chelate chromatography, using a column of iminodiacetic acid/Sepharoses® 6B Fast Flow (Sigma, St. Louis, Mo.) charged with Ni²⁺; (Porath et al. 1975; Hochuli et al. 1988). The HIS₆ tagged proteins were purified by immobilized metal chelate affinity chromatography. More specifically, a column containing iminodiacetic acid Sepharose® 6B FF, connected to a FPLC® system (Pharmacia), was charged with 150 mM Ni⁺⁺ and equilibrated with buffer A (5 mM imidazole, 0.5 M NaCl, 20 mM Tris, pH 7.9). After equilibration, the bacterial supernatant

was applied to the column and the column was washed with 10 bed volumes of buffer A. Subsequently, the column was eluted with buffer B (200 mM imidazole, 0.5 M NaCl, 20 mM Tris, pH 7.9). The eluate was monitored for protein by the absorbance at 280 nm and peak fractions were analyzed by SDS-PAGE. Endotoxin was removed from the purified recombinant proteins by detergent extraction with 1% Triton X-114 followed by metal chelate affinity chromatography and passage through a polymyxin B-sepharose column. The level of endotoxin was quantitated using a chromogenic Limulus Amebocyte Lysate (BioWhittaker, Walkersville, Md.) assay.

Example 4

Immunization of Animals with Four Component MSCRAMM Vaccine—MSCRAMM IV Rhesus Monkeys

100 μ g of M55 (1 EU/mg), ClfA (2.5 EU/mg), ClfB (<1.0 EU/mg), and DUD4 (<10 EU/mg) were mixed together to form the MSCRAMM IV vaccine. The cocktail was mixed with TiterMax™ Gold (CytRX, Norcross, Ga.) in a 1:1 ratio. Two female rhesus monkeys, ID#495Z & 664U (~9.4 kg), were vaccinated intramuscularly (IM) in the hind quadriceps with 200 μ l of the vaccine. Twenty-eight days later the two monkeys were boosted IM with 200 μ l of the same vaccine formulation. Two additional female monkeys, ID#215W & 203U (~8.0 kg), were immunized with the MSCRAMM IV that was compounded in a 1:1 ratio with aluminum hydroxide (2% Alhydrogel; Superfos, Denmark). Twenty-eight days later the two monkeys were boosted IM with 200 μ l of the same vaccine formulation. The clinical regimen followed is described below.

Day 0	15 ml pre-immunization plasma sample, complete blood chemistry
Day 1	Vaccinate IM hind quadriceps with 0.2 ml MSCRAMM IV (100 μ g), injection site exam, temperature recorded
Day 7	Liver panel, temperature recorded, injection site exam
Day 14	15 ml plasma sample
Day 21	15 ml plasma sample
Day 28	Complete blood chemistry, temperature recorded 15 ml plasma sample, boost with IM injection of 0.2 ml MSCRAMM IV (100 μ g)
Day 30	Liver panel, temperature recorded, injection site exam
Day 35	Liver panel, temperature recorded, injection site, 15 ml plasma sample
Day 42	15 ml plasma sample
Day 49	15 ml plasma sample
Day 106	15 ml plasma sample

All 4 animals seroconverted following the initial immunization. Antibody levels >3 times above background could be detected by ELISA 106 days after the primary vaccination. The four animals received another booster immunization in the 21st week of the study. Each animal was given a booster of four subcutaneous injections of 125 μ l of the vaccine for a total booster of 600 μ l of the vaccine. Antibody levels at least 3 times above background, and as much as 15 times above background, could be detected by ELISA 189 days after the primary vaccination. See FIG. 2. No adverse injection site reactions were detected by direct observation by veterinarians. In addition, liver enzyme profiles, CBC, and hematology profiles were within the normal range for rhesus monkeys.

Example 5

Analysis of Plasma Samples from the Vaccinated Monkeys were Analyzed by ELISA

Immulon-2 microtiter plates (Dynex Technologies, Chantilly, Va.) were coated overnight at 4° C. with 10 μ g/ml

(50 μ l) of the collagen binding MSCRAMM (M55), fibrinogen binding MSCRAMM (clfA; pCF44), fibrinogen binding MSCRAMM (ClfB; Region A), and the fibronectin binding MSCRAMM (DUD4). Fifty microliters of the diluted plasma samples were added to the MSCRAMM coated wells and incubated for 1 hr at room temperature. Wash buffer consisting of PBS containing 0.05% vol/vol Tween-20, a blocking solution of 1% wt/vol BSA, 0.05% Tween-20 in PBS, and antibody dilution buffer consisting of PBS containing 0.1% BSA, 0.05% Tween-20. Incubation with primary and secondary antibodies was for 60 min at 25° C. The secondary antibody was alkaline phosphatase-conjugated goat anti-monkey immunoglobulin G, (Rockland, Gilbertsville, Pa.), diluted 3500-fold in antibody dilution buffer. ELISA plates were developed for 30 min at 37° C. with 1 mg/ml p-nitrophenyl phosphate (Sigma) in 1 M diethanolamine, 0.5 mM MgCl₂, pH 9.8, and quantified at 405 nm on a Perkin Elmer HTS 7000 Bio-Assay reader. Each plasma sample was diluted 100-fold in phosphate buffered saline, containing 0.05% Tween 20, 0.1% BSA, pH 7.4. ELISA data are shown in FIG. 2.

Example 6

Inhibition Assays

Methicillin resistant *S. aureus* strain 601 (Smeltzer, M. S., *Gene*. 196:249–159, 1997) was cultured under constant rotation for 15 h at 37° C. in BHI broth. A 1:100 dilution of the overnight culture was made into BHI and the bacteria were grown at 37° C. until mid exponential phase. The bacteria were harvested by centrifugation, washed three times in sterile PBS, pH 7.4, and then resuspended in a carbonate buffer (50 mM NaHCO₃, pH 8.5). The bacteria were mixed with 1 mg/ml FITC (Sigma; F-7250) in 50 mM NaHCO₃, pH 8.5 and incubated end-over-end in the dark for 1 hr at 25° C. The FITC labeling reaction was stopped by centrifugation of the bacterial cells and removing the supernatant containing the unreacted FITC. The labeled bacteria were washed three times in PBS to remove unincorporated FITC, resuspended in PBS, adjusted to ~1×10⁸ cfu/ml and stored at -20° C. in PBS, pH 7.4.

Example 7

Purification of IgG from Immunized Monkeys

IgG was purified from the monkey plasma by affinity chromatography on PROSEP®-A high capacity resin (Bioprocessing Inc., Princeton, N.J.). Briefly, the plasma was thawed and passed through 0.45 μ filter. The plasma was applied to a benchtop column containing PROSEP®-A high capacity resin. The unbound material was removed by washing the column extensively with PBS. The IgG was eluted from the column with 0.1 M sodium citrate, pH 3.0. The pH of eluted IgG was immediately neutralized to pH 6.8–7.4 by the addition of 1M Tris, pH 9.0. The IgG was then dialyzed into PBS, pH 7.4, concentrated and filter sterilized. The concentration of the purified IgG was determined by absorbance at 280 nm.

Example 8

Competitive Inhibition ELISA

Costar 96 well black plates were coated overnight at 4° C. or at room temperature for 2 hr with a 10 μ g/ml solution of matrix components consisting of bovine collagen, human fibrinogen, and bovine fibronectin in PBS, pH 7.4. The

matrix protein coated plates were washed three times with PBS, 0.05% Tween 20 and then blocked with PBS, 1% BSA. The blocked plates were washed three times with PBS, 0.05% Tween 20. A 500 μ l aliquot of FITC-labeled *S. aureus* cells were mixed with an increasing amount of purified monkey IgG in PBS, 0.05% Tween 20, 0.1% BSA. The labeled cells and IgG were mixed on an end-over-end shaker for 1 hr at 25° C. Fifty μ l of the labeled cells/IgG mixture was added to each well on the microtiter plate and incubated at 25° C. on a rocker platform. The wells were washed three times with PBS, 0.05% Tween 20. The amount of bacteria bound to the immobilized matrix proteins was determined on a Perkin Elmer HTS 7000 Bio-Assay reader with the excitation filter set at 485 nm and the emission filter set at 535 nm.

Example 2

Animal Model of Sepsis

Using a mouse model of sepsis (Bremell, T. A., et al., *Infect. Immun.* 62 (7):2976–2985, 1992) we have demonstrated that passive immunization with IgG purified from rhesus monkeys immunized with the MSCRAMM IV can protect mice against sepsis induced death. Naive male NMRI mice 5–8 weeks old were passively immunized i.p. on day-1 with 20 mg of either purified IgG from rhesus monkeys immunized with MSCRAMM IV (n=12), or IgG from non-immunized rhesus monkeys (n=13). On day 0, the mice were challenged i.v. with 2.4×10^7 CFU/mouse *S. aureus* strain LS-1. Mortality and weight change was monitored over the next 3 days. Three days after the inoculation 3/13 mice (13%) were dead in the control group, compared to 0/12 mice (0%) in the control group. Mortality in control group at day 13 was 53.8% (7/13) compared to only 16.2% (2/12) for the MSCRAMM IV passively immunized group. The control mice exhibited a significant decrease in their body weight compared to MSCRAMM IV IgG passively immunized mice ($28.0 \pm 2.5\%$ vs $21.3 \pm 3.1\%$; $p < 0.01$).

Example 10

Multicomponent Vaccines Containing M55 (Collagen-Binding MSCRA and ClfA (Fibrinogen-binding MSCRAMM))

Sixty female Swiss Webster mice received a total of 50 μ g of either ovalbumin, M55 (collagen-binding MSCRAMM) or a combination of M55 and ClfA (fibrinogen-binding MSCRAMM) proteins via a subcutaneous injection. The primary injection was prepared by emulsifying the antigens in Freund's Complete Adjuvant. The mice received a second injection of 25 μ g total protein in Freund's Incomplete Adjuvant 14 days after the primary injection. A final injection of 25 μ g total protein in PBS was given 28 days after the primary injection. Post bleed samples from all mice were obtained two weeks after the final injection to determine antibody titers against the different MSCRAMM proteins. The mice were then challenged (42 days after primary injection) via a single intravenous injection with 1.2×10^8 CFU of *S. aureus* 601. At day 5 post-challenge, the mice were sacrificed and their kidneys harvested. The kidneys were then homogenized and plated on blood agar plates. The plates were incubated at 37° C. overnight and the bacterial load in the kidneys was determined by colony counts. The results of the experiment showed a two log difference in bacterial load between the ovalbumin group (7.03 ± 0.93 log CFU/g) and the M55/ClfA group (4.83 ± 3.04 log CFU/g, $p = 0.006$). A difference in bacterial load was also observed in the M55 group (5.86 ± 3.42 log CFU/g, $p = 0.003$) when compared to the ovalbumin group.

As shown in the above specification and examples, immunological compositions, including vaccines, and other pharmaceutical compositions containing the MSCRAMM proteins are included within the scope of the present invention. One or more of the binding proteins, or active or antigenic fragments thereof, or fusion proteins thereof can be formulated and packaged, alone or in combination with other antigens, using methods and materials known to those skilled in the art for vaccines. The immunological response may be used therapeutically or prophylactically and may provide antibody immunity or cellular immunity such as that produced by T lymphocytes such as cytotoxic T lymphocytes or CD4+ T lymphocytes.

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Ser	Asp	Ala	Asp	Ser	Asp	Ser	Asp	Ala	Asp	Ser	Asp	Ser	Asp	Ser	Asp	
				1285					1290					1295		
agt	gat	tcg	gac	gca	gac	agt	gac	tcg	gac	tca	gat	agt	gat	tca	gat	3936
Ser	Asp	Ser	Asp	Ala	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	
		1300						1305					1310			
gca	gac	agc	gat	tca	gac	tca	gat	agc	gac	tcg	gat	tca	gac	agc	gac	3984
Ala	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	
		1315					1320						1325			

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tcc gac gca gac agc gac tcg gat tca gat agt gat tct gac tca gac	4032
Ser Asp Ala Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp	
1330 1335 1340	
agt gac tca gat tcc gat agt gat tcg gat tca gat agt gat tcc gac	4080
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp	
1345 1350 1355 1360	
gca gac agc gat tcg gat tcc gat agc gat tca gac tca gac agc gat	4128
Ala Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp	
1365 1370 1375	
tca gat tca gac agc gac tca gat tca gat agt gat tcc gac gca gac	4176
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Ala Asp	
1380 1385 1390	
agc gat gca gac agc gac tca gac gca gac agt gat tca gat gca gac	4224
Ser Asp Ala Asp Ser Asp Ser Asp Ala Asp Ser Asp Ser Asp Ala Asp	
1395 1400 1405	
agc gat tct gac tca gat agt gac tca gac gca gat agt gat tcc gat	4272
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ala Asp Ser Asp	
1410 1415 1420	
tcc gat agc gat tca gat tct gat agt gac tca gac tca gac agt gac	4320
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp	
1425 1430 1435 1440	
tca gat tcc gat agc gac tcg gat tca gat agt gat tcc gac gca gac	4368
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Ala Asp	
1445 1450 1455	
agt gac tca gac tca gat agt gac tcg gat tcc gat agt gat tcc gac	4416
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp	
1460 1465 1470	
gca gac agc gat tct gac tca gat agt gac tca gac gca gat agt gat	4464
Ala Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ala Asp Ser Asp	
1475 1480 1485	
tcc gat tcc gat agc gat tcg gat gca gac agc gac tcg gat tca gat	4512
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ala Asp Ser Asp Ser Asp	
1490 1495 1500	
agt gat tcc gac gca gac agt gac tca gac tca gat agt gac tcg gat	4560
Ser Asp Ser Asp Ala Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp	
1505 1510 1515 1520	
tcc gat agt gat tcc gac gca gac agc gat tcg gat tcc gat agc gat	4608
Ser Asp Ser Asp Ser Asp Ala Asp Ser Asp Ser Asp Ser Asp Ser Asp	
1525 1530 1535	
tca gac tcc gac agc gat tca gat tca gac agc gac tca gat tcc gat	4656
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp	
1540 1545 1550	
agt gat tcc gat tca gac agt gac tcg gat tcc gat agt gac tca gac	4704
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp	
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Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp	
1570 1575 1580	
tcg gac tca gat agt gac tcc gat tca gac agt gat tcg gat tcc gat	4800
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp	
1585 1590 1595 1600	
agc gat tcg gat tcc gat agt gac tcg gat tca gac agt gat tcg gac	4848
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp	
1605 1610 1615	
tca gac agc gac tcc gat tca gat agt gat tcc gac tca gac agc gat	4896
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp	
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tcg gat tcc gat agt gac tcg gat tca gac agt gat tcg gac tca gac	4944
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp	
1635 1640 1645	

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agc gac tcc gat tca gat agt gat tcc gac gca gac agc gac tcc gat 4992
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ala Asp Ser Asp Ser Asp
1650 1655 1660

tca gat agt gat tcg gac gca gac agc gat tcc gat agt gac tcg gat 5040
Ser Asp Ser Asp Ser Asp Ala Asp Ser Asp Ser Asp Ser Asp Ser Asp
1665 1670 1675 1680

tca gac agt gat tcg gac tca gac agc gat tcc gat tca gac agt gac 5088
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
1685 1690 1695

tcg gac tca gat agc gac tcg gat tca gac agt gac tcg gac tca gat 5136
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
1700 1705 1710

agt gac tcc gat tca gac agc gac tcg gat tct gat aaa aat gca aaa 5184
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Lys Asn Ala Lys
1715 1720 1725

gat aaa tta cct gat aca gga gca aat gaa gat cat gat tct aaa ggc 5232
Asp Lys Leu Pro Asp Thr Gly Ala Asn Glu Asp His Asp Ser Lys Gly
1730 1735 1740

aca tta ctt gga act tta ttt gca ggt tta gga gca tta tta tta gga 5280
Thr Leu Leu Gly Thr Leu Phe Ala Gly Leu Gly Ala Leu Leu Leu Gly
1745 1750 1755 1760

aga cgt cgt aaa aaa gat aat aaa gaa aaa tag cac tat tga ttc att 5328
Arg Arg Arg Lys Lys Asp Asn Lys Glu Lys His Tyr Phe Ile
1765 1770 1775

cat aag tta ttt caa gcc agg tct ata tgg cct ggt ttg aaa tca tat 5376
His Lys Leu Phe Gln Ala Arg Ser Ile Trp Pro Gly Leu Lys Ser Tyr
1780 1785 1790

taa att gaa agg aga aaa aga tga gta tgg 5406
Ile Glu Arg Arg Lys Arg Val Trp
1795 1800

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<210> SEQ ID NO 2
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus epidermidis

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<400> SEQUENCE: 2

Tyr Trp Ile Asn Tyr Ala Tyr Lys Val Phe Thr
1 5 10

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<210> SEQ ID NO 3
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus epidermidis

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<400> SEQUENCE: 3

Lys Cys Lys Cys Asn Leu Gln Val Asn Ile Gln Ile Ile Ser Leu
1 5 10 15

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<210> SEQ ID NO 4
<211> LENGTH: 1742
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus epidermidis

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<400> SEQUENCE: 4

Asn Ile Tyr Phe Asn Trp Arg Tyr Ser Met Lys Lys Arg Arg Gln Gly
1 5 10 15

Pro Ile Asn Lys Arg Val Asp Phe Leu Ser Asn Lys Val Asn Lys Tyr
20 25 30

Ser Ile Arg Lys Phe Thr Val Gly Thr Ala Ser Ile Leu Val Gly Ala
35 40 45

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

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Thr	Asn	Thr	Thr	Thr	Tyr	Thr	Phe	Thr	Asn	Tyr	Val	Asp	Gln	Tyr	Gln
465					470					475					480
Asn	Ile	Thr	Gly	Ser	Phe	Asp	Leu	Ile	Ala	Thr	Pro	Lys	Arg	Glu	Thr
				485					490					495	
Ala	Ile	Lys	Asp	Asn	Gln	Asn	Tyr	Pro	Met	Glu	Val	Thr	Ile	Ala	Asn
			500					505					510		
Glu	Val	Val	Lys	Lys	Asp	Phe	Ile	Val	Asp	Tyr	Gly	Asn	Lys	Lys	Asp
		515					520					525			
Asn	Thr	Thr	Thr	Ala	Ala	Val	Ala	Asn	Val	Asp	Asn	Val	Asn	Asn	Lys
530						535				540					
His	Asn	Glu	Val	Val	Tyr	Leu	Asn	Gln	Asn	Asn	Gln	Asn	Pro	Lys	Tyr
545					550					555					560
Ala	Lys	Tyr	Phe	Ser	Thr	Val	Lys	Asn	Gly	Glu	Phe	Ile	Pro	Gly	Glu
				565					570					575	
Val	Lys	Val	Tyr	Glu	Val	Thr	Asp	Thr	Asn	Ala	Met	Val	Asp	Ser	Phe
			580					585					590		
Asn	Pro	Asp	Leu	Asn	Ser	Ser	Asn	Val	Lys	Asp	Val	Thr	Ser	Gln	Phe
		595					600					605			
Ala	Pro	Lys	Val	Ser	Ala	Asp	Gly	Thr	Arg	Val	Asp	Ile	Asn	Phe	Ala
610						615					620				
Arg	Ser	Met	Ala	Asn	Gly	Lys	Lys	Tyr	Ile	Val	Thr	Gln	Ala	Val	Arg
625					630					635					640
Pro	Thr	Gly	Thr	Gly	Asn	Val	Tyr	Thr	Glu	Tyr	Trp	Leu	Thr	Arg	Asp
				645					650					655	
Gly	Thr	Thr	Asn	Thr	Asn	Asp	Phe	Tyr	Arg	Gly	Thr	Lys	Ser	Thr	Thr
			660					665					670		
Val	Thr	Tyr	Leu	Asn	Gly	Ser	Ser	Thr	Ala	Gln	Gly	Asp	Asn	Pro	Thr
		675					680					685			
Tyr	Ser	Leu	Gly	Asp	Tyr	Val	Trp	Leu	Asp	Lys	Asn	Lys	Asn	Gly	Val
690						695					700				
Gln	Asp	Asp	Asp	Glu	Lys	Gly	Leu	Ala	Gly	Val	Tyr	Val	Thr	Leu	Lys
705					710					715					720
Asp	Ser	Asn	Asn	Arg	Glu	Leu	Gln	Arg	Val	Thr	Thr	Asp	Gln	Ser	Gly
				725					730					735	
His	Tyr	Gln	Phe	Asp	Asn	Leu	Gln	Asn	Gly	Thr	Tyr	Thr	Val	Glu	Phe
			740					745					750		
Ala	Ile	Pro	Asp	Asn	Tyr	Thr	Pro	Ser	Pro	Ala	Asn	Asn	Ser	Thr	Asn
		755					760					765			
Asp	Ala	Ile	Asp	Ser	Asp	Gly	Glu	Arg	Asp	Gly	Thr	Arg	Lys	Val	Val
770						775					780				
Val	Ala	Lys	Gly	Thr	Ile	Asn	Asn	Ala	Asp	Asn	Met	Thr	Val	Asp	Thr
785					790					795					800
Gly	Phe	Tyr	Leu	Thr	Pro	Lys	Tyr	Asn	Val	Gly	Asp	Tyr	Val	Trp	Glu
			805						810					815	
Asp	Thr	Asn	Lys	Asp	Gly	Ile	Gln	Asp	Asp	Asn	Glu	Lys	Gly	Ile	Ser
			820					825					830		
Gly	Val	Lys	Val	Thr	Leu	Lys	Asn	Lys	Asn	Gly	Asp	Thr	Ile	Gly	Thr
		835					840					845			
Thr	Thr	Thr	Asp	Ser	Asn	Gly	Lys	Tyr	Glu	Phe	Thr	Gly	Leu	Glu	Asn
850						855					860				
Gly	Asp	Tyr	Thr	Ile	Glu	Phe	Glu	Thr	Pro	Glu	Gly	Tyr	Thr	Pro	Thr
865					870					875					880
Lys	Gln	Asn	Ser	Gly	Ser	Asp	Glu	Gly	Lys	Asp	Ser	Asn	Gly	Thr	Lys

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885					890					895					
Thr	Thr	Val	Thr	Val	Lys	Asp	Ala	Asp	Asn	Lys	Thr	Ile	Asp	Ser	Gly
			900					905					910		
Phe	Tyr	Lys	Pro	Thr	Tyr	Asn	Leu	Gly	Asp	Tyr	Val	Trp	Glu	Asp	Thr
		915					920					925			
Asn	Lys	Asp	Gly	Ile	Gln	Asp	Asp	Ser	Glu	Lys	Gly	Ile	Ser	Gly	Val
	930					935					940				
Lys	Val	Thr	Leu	Lys	Asp	Lys	Asn	Gly	Asn	Ala	Ile	Gly	Thr	Thr	Thr
945						950					955				960
Thr	Asp	Ala	Ser	Gly	His	Tyr	Gln	Phe	Lys	Gly	Leu	Glu	Asn	Gly	Ser
				965					970					975	
Tyr	Thr	Val	Glu	Phe	Glu	Thr	Pro	Ser	Gly	Tyr	Thr	Pro	Thr	Lys	Ala
			980						985					990	
Asn	Ser	Gly	Gln	Asp	Ile	Thr	Val	Asp	Ser	Asn	Gly	Ile	Thr	Thr	Thr
		995					1000					1005			
Gly	Ile	Ile	Asn	Gly	Ala	Asp	Asn	Leu	Thr	Ile	Asp	Ser	Gly	Phe	Tyr
	1010						1015					1020			
Lys	Thr	Pro	Lys	Tyr	Ser	Val	Gly	Asp	Tyr	Val	Trp	Glu	Asp	Thr	Asn
025						1030					1035				1040
Lys	Asp	Gly	Ile	Gln	Asp	Asp	Asn	Glu	Lys	Gly	Ile	Ser	Gly	Val	Lys
				1045					1050					1055	
Val	Thr	Leu	Lys	Asp	Glu	Lys	Gly	Asn	Ile	Ile	Ser	Thr	Thr	Thr	Thr
			1060					1065						1070	
Asp	Glu	Asn	Gly	Lys	Tyr	Gln	Phe	Asp	Asn	Leu	Asp	Ser	Gly	Asn	Tyr
		1075					1080						1085		
Ile	Ile	His	Phe	Glu	Lys	Pro	Glu	Gly	Met	Thr	Gln	Thr	Thr	Ala	Asn
		1090					1095					1100			
Ser	Gly	Asn	Asp	Asp	Glu	Lys	Asp	Ala	Asp	Gly	Glu	Asp	Val	Arg	Val
105						1110					1115				1120
Thr	Ile	Thr	Asp	His	Asp	Asp	Phe	Ser	Ile	Asp	Asn	Gly	Tyr	Phe	Asp
				1125					1130					1135	
Asp	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ala	Asp	Ser	Asp	Ser	Asp	Ser	Asp
			1140					1145					1150		
Ser	Asp	Ser	Asp	Ala	Asp	Ser	Asp	Ser	Asp	Ala	Asp	Ser	Asp	Ser	Asp
		1155					1160						1165		
Ala	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ala	Asp	Ser	Asp
	1170						1175					1180			
Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ala	Asp
185						1190					1195				1200
Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ala	Asp	Ser	Asp	Ser	Asp
				1205				1210					1215		
Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp
			1220					1225					1230		
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		1235					1240						1245		
Ser	Asp	Ser	Asp	Ser	Asp	Ala	Asp	Ser	Asp	Ser	Asp	Ala	Asp	Ser	Asp
	1250						1255					1260			
Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ala	Asp	Ser	Asp	Ser	Asp	Ser	Asp
265						1270					1275				1280
Ser	Asp	Ser	Asp	Ala	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp
				1285				1290						1295	
Ser	Asp	Ser	Asp	Ser	Asp	Ala	Asp	Ser	Asp	Ser	Asp	Ser	Asp	Ser	Asp
				1300				1305						1310	

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Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
 1315 1320 1325
 Ser Asp Ser Asp Ala Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
 1330 1335 1340
 Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
 345 1350 1355 1360
 Ser Asp Ala Asp Ser Asp Ala Asp Ser Asp Ser Asp Ala Asp Ser Asp
 1365 1370 1375
 Ser Asp Ala Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ala Asp
 1380 1385 1390
 Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
 1395 1400 1405
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 505 1510 1515 1520
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 1650 1655 1660
 Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
 665 1670 1675 1680
 Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
 1685 1690 1695
 Lys Asn Ala Lys Asp Lys Leu Pro Asp Thr Gly Ala Asn Glu Asp His
 1700 1705 1710
 Asp Ser Lys Gly Thr Leu Leu Gly Thr Leu Phe Ala Gly Leu Gly Ala
 1715 1720 1725

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Leu Leu Leu Gly Arg Arg Arg Lys Lys Asp Asn Lys Glu Lys
 1730 1735 1740

<210> SEQ ID NO 5
 <211> LENGTH: 18
 <212> TYPE: PRT
 <213> ORGANISM: Staphylococcus epidermidis

<400> SEQUENCE: 5

Phe Ile His Lys Leu Phe Gln Ala Arg Ser Ile Trp Pro Gly Leu Lys
 1 5 10 15

Ser Tyr

<210> SEQ ID NO 6
 <211> LENGTH: 6
 <212> TYPE: PRT
 <213> ORGANISM: Staphylococcus epidermidis

<400> SEQUENCE: 6

Ile Glu Arg Arg Lys Arg
 1 5

<210> SEQ ID NO 7
 <211> LENGTH: 2976
 <212> TYPE: DNA
 <213> ORGANISM: Staphylococcus epidermidis
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (3)..(2975)

<400> SEQUENCE: 7

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Ile Ala Lys Lys Thr Tyr Ile Leu Tyr Cys Ile Leu Leu Lys	
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cga ttt tta ctt gaa aat tac att gaa ata gtc aaa gat aag gag ttt	95
Arg Phe Leu Leu Glu Asn Tyr Ile Glu Ile Val Lys Asp Lys Glu Phe	
20 25 30	
tta tga tta aaa aat aat tta cta act aaa aag aaa cct ata gca	143
Leu Leu Lys Lys Asn Asn Leu Leu Thr Lys Lys Lys Pro Ile Ala	
35 40 45	
aat aaa tcc aat aaa tat gca att aga aaa ttc aca gta ggt aca gcg	191
Asn Lys Ser Asn Lys Tyr Ala Ile Arg Lys Phe Thr Val Gly Thr Ala	
50 55 60	
tct att gta ata ggt gca gca tta ttg ttt ggt tta ggt cat aat gag	239
Ser Ile Val Ile Gly Ala Ala Leu Leu Phe Gly Leu Gly His Asn Glu	
65 70 75	
gcc aaa gct gag gag aat aca gta caa gac gtt aaa gat tcg aat atg	287
Ala Lys Ala Glu Glu Asn Thr Val Gln Asp Val Lys Asp Ser Asn Met	
80 85 90 95	
gat gat gaa tta tca gat agc aat gat cag tcc agt aat gaa gaa aag	335
Asp Asp Glu Leu Ser Asp Ser Asn Asp Gln Ser Ser Asn Glu Glu Lys	
100 105 110	
aat gat gta atc aat aat agt cag tca ata aac acc gat gat gat aac	383
Asn Asp Val Ile Asn Asn Ser Gln Ser Ile Asn Thr Asp Asp Asp Asn	
115 120 125	
caa ata aaa aaa gaa gaa acg aat agc aac gat gcc ata gaa aat cgc	431
Gln Ile Lys Lys Glu Glu Thr Asn Ser Asn Asp Ala Ile Glu Asn Arg	
130 135 140	
tct aaa gat ata aca cag tca aca aca aat gta gat gaa aac gaa gca	479
Ser Lys Asp Ile Thr Gln Ser Thr Thr Asn Val Asp Glu Asn Glu Ala	
145 150 155	
aca ttt tta caa aag acc cct caa gat aat act cag ctt aaa gaa gaa	527

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Thr Phe Leu Gln Lys Thr Pro Gln Asp Asn Thr Gln Leu Lys Glu Glu 160 165 170 175	
gtg gta aaa gaa ccc tca tca gtc gaa tcc tca aat tca tca atg gat Val Val Lys Glu Pro Ser Ser Val Glu Ser Ser Asn Ser Ser Met Asp 180 185 190	575
act gcc caa caa cca tct cat aca aca ata aat agt gaa gca tct att Thr Ala Gln Gln Pro Ser His Thr Thr Ile Asn Ser Glu Ala Ser Ile 195 200 205	623
caa aca agt gat aat gaa gaa aat tcc cgc gta tca gat ttt gct aac Gln Thr Ser Asp Asn Glu Glu Asn Ser Arg Val Ser Asp Phe Ala Asn 210 215 220	671
tct aaa ata ata gag agt aac act gaa tcc aat aaa gaa gag aat act Ser Lys Ile Ile Glu Ser Asn Thr Glu Ser Asn Lys Glu Glu Asn Thr 225 230 235	719
ata gag caa cct aac aaa gta aga gaa gat tca ata aca agt caa ccg Ile Glu Gln Pro Asn Lys Val Arg Glu Asp Ser Ile Thr Ser Gln Pro 240 245 250 255	767
tct agc tat aaa aat ata gat gaa aaa att tca aat caa gat gag tta Ser Ser Tyr Lys Asn Ile Asp Glu Lys Ile Ser Asn Gln Asp Glu Leu 260 265 270	815
tta aat tta cca ata aat gaa tat gaa aat aag gtt aga ccg tta tct Leu Asn Leu Pro Ile Asn Glu Tyr Glu Asn Lys Val Arg Pro Leu Ser 275 280 285	863
aca aca tct gcc caa cca tcg agt aag cgt gta acc gta aat caa tta Thr Thr Ser Ala Gln Pro Ser Ser Lys Arg Val Thr Val Asn Gln Leu 290 295 300	911
gcg gca gaa caa ggt tcg aat gtt aat cat tta att aaa gtt act gat Ala Ala Glu Gln Gly Ser Asn Val Asn His Leu Ile Lys Val Thr Asp 305 310 315	959
caa agt att act gaa gga tat gat gat agt gat ggt att att aaa gca Gln Ser Ile Thr Glu Gly Tyr Asp Asp Ser Asp Gly Ile Ile Lys Ala 320 325 330 335	1007
cat gat gct gaa aac tta atc tat gat gta act ttt gaa gta gat gat His Asp Ala Glu Asn Leu Ile Tyr Asp Val Thr Phe Glu Val Asp Asp 340 345 350	1055
aag gtg aaa tct ggt gat acg atg aca gtg aat ata gat aag aat aca Lys Val Lys Ser Gly Asp Thr Met Thr Val Asn Ile Asp Lys Asn Thr 355 360 365	1103
gtt cca tca gat tta acc gat agt ttt gca ata cca aaa ata aaa gat Val Pro Ser Asp Leu Thr Asp Ser Phe Ala Ile Pro Lys Ile Lys Asp 370 375 380	1151
aat tct gga gaa atc atc gct aca ggt act tat gac aac aca aat aaa Asn Ser Gly Glu Ile Ile Ala Thr Gly Thr Tyr Asp Asn Thr Asn Lys 385 390 395	1199
caa att acc tac act ttt aca gat tat gta gat aaa tat gaa aat att Gln Ile Thr Tyr Thr Phe Thr Asp Tyr Val Asp Lys Tyr Glu Asn Ile 400 405 410 415	1247
aaa gcg cac ctt aaa tta aca tca tac att gat aaa tca aag gtt cca Lys Ala His Leu Lys Leu Thr Ser Tyr Ile Asp Lys Ser Lys Val Pro 420 425 430	1295
aat aat aac act aag tta gat gta gaa tat aag acg gcc ctt tca tca Asn Asn Asn Thr Lys Leu Asp Val Glu Tyr Lys Thr Ala Leu Ser Ser 435 440 445	1343
gta aat aaa aca att acg gtt gaa tat caa aaa cct aac gaa aat cgg Val Asn Lys Thr Ile Thr Val Glu Tyr Gln Lys Pro Asn Glu Asn Arg 450 455 460	1391
act gct aac ctt caa agt atg ttc aca aac ata gat acg aaa aac cat Thr Ala Asn Leu Gln Ser Met Phe Thr Asn Ile Asp Thr Lys Asn His 465 470 475	1439

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aca gtt gag caa acg att tat att aac cct ctt cgt tat tca gcc aaa	1487
Thr Val Glu Gln Thr Ile Tyr Ile Asn Pro Leu Arg Tyr Ser Ala Lys	
480 485 490 495	
gaa aca aat gta aat att tca ggg aat ggc gat gaa ggt tca aca att	1535
Glu Thr Asn Val Asn Ile Ser Gly Asn Gly Asp Glu Gly Ser Thr Ile	
500 505 510	
atc gac gat agt aca atc att aaa gtt tat aag gtt gga gat aat caa	1583
Ile Asp Asp Ser Thr Ile Ile Lys Val Tyr Lys Val Gly Asp Asn Gln	
515 520 525	
aat tta cca gat agt aac aga att tat gat tac agt gaa tat gaa gat	1631
Asn Leu Pro Asp Ser Asn Arg Ile Tyr Asp Tyr Ser Glu Tyr Glu Asp	
530 535 540	
gtc aca aat gat gat tat gcc caa tta gga aat aat aat gac gtg aat	1679
Val Thr Asn Asp Asp Tyr Ala Gln Leu Gly Asn Asn Asn Asp Val Asn	
545 550 555	
att aat ttt ggt aat ata gat tca cca tat att att aaa gtt att agt	1727
Ile Asn Phe Gly Asn Ile Asp Ser Pro Tyr Ile Ile Lys Val Ile Ser	
560 565 570 575	
aaa tat gac cct aat aag gac gat tac acg acg ata cag caa act gtg	1775
Lys Tyr Asp Pro Asn Lys Asp Asp Tyr Thr Thr Ile Gln Gln Thr Val	
580 585 590	
aca atg caa acg act ata aat gag tat act ggt gag ttt aga aca gca	1823
Thr Met Gln Thr Thr Ile Asn Glu Tyr Thr Gly Glu Phe Arg Thr Ala	
595 600 605	
tcc tat gat aat aca att gct ttc tct aca agt tca ggt caa gga caa	1871
Ser Tyr Asp Asn Thr Ile Ala Phe Ser Thr Ser Ser Gly Gln Gly Gln	
610 615 620	
ggg gac ttg cct cct gaa aaa act tat aaa atc gga gat tac gta tgg	1919
Gly Asp Leu Pro Pro Glu Lys Thr Tyr Lys Ile Gly Asp Tyr Val Trp	
625 630 635	
gaa gat gta gat aaa gat ggt att caa aat aca aat gat aat gaa aaa	1967
Glu Asp Val Asp Lys Asp Gly Ile Gln Asn Thr Asn Asp Asn Glu Lys	
640 645 650 655	
ccg ctt agt aat gta ttg gta act ttg acg tat cct gat gga act tca	2015
Pro Leu Ser Asn Val Leu Val Thr Leu Thr Tyr Pro Asp Gly Thr Ser	
660 665 670	
aaa tca gtc aga aca gat gaa gag ggg aaa tat caa ttt gat ggg tta	2063
Lys Ser Val Arg Thr Asp Glu Glu Gly Lys Tyr Gln Phe Asp Gly Leu	
675 680 685	
aaa aac gga ttg act tat aaa att aca ttc gaa aca ccg gaa gga tat	2111
Lys Asn Gly Leu Thr Tyr Lys Ile Thr Phe Glu Thr Pro Glu Gly Tyr	
690 695 700	
acg ccg acg ctt aaa cat tca gga aca aat cct gca cta gac tca gaa	2159
Thr Pro Thr Leu Lys His Ser Gly Thr Asn Pro Ala Leu Asp Ser Glu	
705 710 715	
ggc aat tct gta tgg gta act att aac gga caa gac gat atg act att	2207
Gly Asn Ser Val Trp Val Thr Ile Asn Gly Gln Asp Asp Met Thr Ile	
720 725 730 735	
gat agc gga ttt tat caa aca cct aaa tat agc tta ggg aac tat gta	2255
Asp Ser Gly Phe Tyr Gln Thr Pro Lys Tyr Ser Leu Gly Asn Tyr Val	
740 745 750	
tgg tat gac act aat aaa gat ggt att caa ggt gat gat gaa aaa gga	2303
Trp Tyr Asp Thr Asn Lys Asp Gly Ile Gln Gly Asp Asp Glu Lys Gly	
755 760 765	
atc tct gga gta aaa gtg acg tta aaa gat gaa aac gga aat atc att	2351
Ile Ser Gly Val Lys Val Thr Leu Lys Asp Glu Asn Gly Asn Ile Ile	
770 775 780	
agt aca aca aca act gat gaa aat gga aag tat caa ttt gat aat tta	2399
Ser Thr Thr Thr Asp Glu Asn Gly Lys Tyr Gln Phe Asp Asn Leu	
785 790 795	

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aat agt ggt aat tat att gtt cat ttt gat aaa cct tca ggt atg act 2447
Asn Ser Gly Asn Tyr Ile Val His Phe Asp Lys Pro Ser Gly Met Thr
800                               805                               810                               815

caa aca aca aca gat tct ggt gat gat gac gaa cag gat gct gat ggg 2495
Gln Thr Thr Thr Asp Ser Gly Asp Asp Asp Glu Gln Asp Ala Asp Gly
                               820                               825                               830

gaa gaa gtc cat gta aca att act gat cat gat gac ttt agt ata gat 2543
Glu Glu Val His Val Thr Ile Thr Asp His Asp Asp Phe Ser Ile Asp
                               835                               840                               845

aac gga tac tat gat gac gac tca gat tca gat agt gat tca gac tca 2591
Asn Gly Tyr Tyr Asp Asp Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
                               850                               855                               860

gat agc gac gac tca gac tcc gat agc gat tcc gac tca gac agc gac 2639
Asp Ser Asp Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
865                               870                               875

tca gat tcc gat agt gat tca gat tca gac agt gac tca gac tca gat 2687
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
880                               885                               890                               895

agt gat tca gat tca gac agc gat tcc gac tca gac agt gac tca gga 2735
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Gly
                               900                               905                               910

tta gac aat agc tca gat aag aat aca aaa gat aaa tta ccg gat aca 2783
Leu Asp Asn Ser Ser Asp Lys Asn Thr Lys Asp Lys Leu Pro Asp Thr
                               915                               920                               925

gga gct aat gaa gat cat gat tct aaa ggc aca tta ctt gga gct tta 2831
Gly Ala Asn Glu Asp His Asp Ser Lys Gly Thr Leu Leu Gly Ala Leu
930                               935                               940

ttt gca ggt tta gga gcg tta tta tta ggg aag cgt cgc aaa aat aga 2879
Phe Ala Gly Leu Gly Ala Leu Leu Leu Gly Lys Arg Arg Lys Asn Arg
945                               950                               955

aaa aat aaa aat taa att att caa atg aaa tta gtg aaa gaa gca gat 2927
Lys Asn Lys Asn Ile Ile Gln Met Lys Leu Val Lys Glu Ala Asp
960                               965                               970                               975

acg aca ttt gaa tag aaa gta tat tta gtc caa caa ata taa ggt gtt g 2976
Thr Thr Phe Glu Lys Val Tyr Leu Val Gln Gln Ile Gly Val
                               980                               985                               990

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<210> SEQ ID NO 8
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus epidermidis

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<400> SEQUENCE: 8
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```
Ile Ala Lys Lys Thr Tyr Ile Leu Tyr Cys Ile Leu Leu
  1                               5                               10
```

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<210> SEQ ID NO 9
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus epidermidis

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<400> SEQUENCE: 9
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```
Lys Arg Phe Leu Leu Glu Asn Tyr Ile Glu Ile Val Lys Asp Lys Glu
  1                               5                               10                               15
```

```
Phe Leu
```

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<210> SEQ ID NO 10
<211> LENGTH: 930
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus epidermidis

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<400> SEQUENCE: 10

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

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Lys Thr Ile Thr Val Glu Tyr Gln Lys Pro Asn Glu Asn Arg Thr Ala
 420 425 430
 Asn Leu Gln Ser Met Phe Thr Asn Ile Asp Thr Lys Asn His Thr Val
 435 440 445
 Glu Gln Thr Ile Tyr Ile Asn Pro Leu Arg Tyr Ser Ala Lys Glu Thr
 450 455 460
 Asn Val Asn Ile Ser Gly Asn Gly Asp Glu Gly Ser Thr Ile Ile Asp
 465 470 475 480
 Asp Ser Thr Ile Ile Lys Val Tyr Lys Val Gly Asp Asn Gln Asn Leu
 485 490 495
 Pro Asp Ser Asn Arg Ile Tyr Asp Tyr Ser Glu Tyr Glu Asp Val Thr
 500 505 510
 Asn Asp Asp Tyr Ala Gln Leu Gly Asn Asn Asn Asp Val Asn Ile Asn
 515 520 525
 Phe Gly Asn Ile Asp Ser Pro Tyr Ile Ile Lys Val Ile Ser Lys Tyr
 530 535 540
 Asp Pro Asn Lys Asp Asp Tyr Thr Thr Ile Gln Gln Thr Val Thr Met
 545 550 555 560
 Gln Thr Thr Ile Asn Glu Tyr Thr Gly Glu Phe Arg Thr Ala Ser Tyr
 565 570 575
 Asp Asn Thr Ile Ala Phe Ser Thr Ser Ser Gly Gln Gly Gln Gly Asp
 580 585 590
 Leu Pro Pro Glu Lys Thr Tyr Lys Ile Gly Asp Tyr Val Trp Glu Asp
 595 600 605
 Val Asp Lys Asp Gly Ile Gln Asn Thr Asn Asp Asn Glu Lys Pro Leu
 610 615 620
 Ser Asn Val Leu Val Thr Leu Thr Tyr Pro Asp Gly Thr Ser Lys Ser
 625 630 635 640
 Val Arg Thr Asp Glu Glu Gly Lys Tyr Gln Phe Asp Gly Leu Lys Asn
 645 650 655
 Gly Leu Thr Tyr Lys Ile Thr Phe Glu Thr Pro Glu Gly Tyr Thr Pro
 660 665 670
 Thr Leu Lys His Ser Gly Thr Asn Pro Ala Leu Asp Ser Glu Gly Asn
 675 680 685
 Ser Val Trp Val Thr Ile Asn Gly Gln Asp Asp Met Thr Ile Asp Ser
 690 695 700
 Gly Phe Tyr Gln Thr Pro Lys Tyr Ser Leu Gly Asn Tyr Val Trp Tyr
 705 710 715 720
 Asp Thr Asn Lys Asp Gly Ile Gln Gly Asp Asp Glu Lys Gly Ile Ser
 725 730 735
 Gly Val Lys Val Thr Leu Lys Asp Glu Asn Gly Asn Ile Ile Ser Thr
 740 745 750
 Thr Thr Thr Asp Glu Asn Gly Lys Tyr Gln Phe Asp Asn Leu Asn Ser
 755 760 765
 Gly Asn Tyr Ile Val His Phe Asp Lys Pro Ser Gly Met Thr Gln Thr
 770 775 780
 Thr Thr Asp Ser Gly Asp Asp Glu Gln Asp Ala Asp Gly Glu Glu
 785 790 795 800
 Val His Val Thr Ile Thr Asp His Asp Asp Phe Ser Ile Asp Asn Gly
 805 810 815
 Tyr Tyr Asp Asp Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
 820 825 830

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Asp Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
 835 840 845
 Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
 850 855 860
 Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Gly Leu Asp
 865 870 875 880
 Asn Ser Ser Asp Lys Asn Thr Lys Asp Lys Leu Pro Asp Thr Gly Ala
 885 890 895
 Asn Glu Asp His Asp Ser Lys Gly Thr Leu Leu Gly Ala Leu Phe Ala
 900 905 910
 Gly Leu Gly Ala Leu Leu Leu Gly Lys Arg Arg Lys Asn Arg Lys Asn
 915 920 925
 Lys Asn
 930

<210> SEQ ID NO 11
 <211> LENGTH: 15
 <212> TYPE: PRT
 <213> ORGANISM: Staphylococcus epidermidis
 <400> SEQUENCE: 11

Ile Ile Gln Met Lys Leu Val Lys Glu Ala Asp Thr Thr Phe Glu
 1 5 10 15

<210> SEQ ID NO 12
 <211> LENGTH: 8
 <212> TYPE: PRT
 <213> ORGANISM: Staphylococcus epidermidis
 <400> SEQUENCE: 12

Lys Val Tyr Leu Val Gln Ile
 1 5

<210> SEQ ID NO 13
 <211> LENGTH: 1464
 <212> TYPE: DNA
 <213> ORGANISM: Staphylococcus epidermidis
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)..(1464)

<400> SEQUENCE: 13

atg aaa aag ttt aac att aaa cat tca ttt atg ctt acg ggc ttt gct	48
Met Lys Lys Phe Asn Ile Lys His Ser Phe Met Leu Thr Gly Phe Ala	
1 5 10 15	
ttc atg gta act aca tca tta ttc agt cac caa gca cat gct gaa ggt	96
Phe Met Val Thr Thr Ser Leu Phe Ser His Gln Ala His Ala Glu Gly	
20 25 30	
aat cat cct att gac att aat ttt tct aaa gat caa att gat aga aat	144
Asn His Pro Ile Asp Ile Asn Phe Ser Lys Asp Gln Ile Asp Arg Asn	
35 40 45	
aca gct aag agc aat att atc aat cga gtg aat gac act agt cgc aca	192
Thr Ala Lys Ser Asn Ile Ile Asn Arg Val Asn Asp Thr Ser Arg Thr	
50 55 60	
gga att agt atg aat tcg gat aat gat tta gat aca gat atc gtt tca	240
Gly Ile Ser Met Asn Ser Asp Asn Asp Leu Asp Thr Asp Ile Val Ser	
65 70 75 80	
aat agt gac tca gaa aat gac aca tat tta gat agt gat tca gat tca	288
Asn Ser Asp Ser Glu Asn Asp Thr Tyr Leu Asp Ser Asp Ser Asp Ser	
85 90 95	
gac agt gac tca gat tca gat agt gac tca gat tca gat agt gac tca	336

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Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser	100	105	110	
gat tca gat agt gac tca gat tca gac agt gat tca gac tca gat agt				384
Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser	115	120	125	
gac tca gat tca gac agt gat tca gac tca gat agt gat tca gat tca				432
Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser	130	135	140	
gac agt gat tca gat tca gac agt gac tca gac tca gac agt gat tca				480
Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser	145	150	155	160
gat tca gat agt gat tca gat tca gat agt gat tca gat tca gat agt				528
Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser	165	170	175	
gat tca gat tca gac agt gac tca gac tca gac agt gat tca gat tca				576
Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser	180	185	190	
gat agt gat tca gac tca gat agt gac tca gat tca gat agt gat tca				624
Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser	195	200	205	
gac tct ggt aca agt tca ggt aag ggt tca cat acc gga aaa aaa cct				672
Asp Ser Gly Thr Ser Ser Gly Lys Gly Ser His Thr Gly Lys Lys Pro	210	215	220	
ggt aac cct aaa gga aat aca aat aga cct tct caa aga cat acg aat				720
Gly Asn Pro Lys Gly Asn Thr Asn Arg Pro Ser Gln Arg His Thr Asn	225	230	235	240
caa ccc caa agg cct aaa tac aat caa aca aat caa aac aat ata aac				768
Gln Pro Gln Arg Pro Lys Tyr Asn Gln Thr Asn Gln Asn Asn Ile Asn	245	250	255	
aat ata aac cat aat att aat cat aca cgt act agt gga gat ggt gcg				816
Asn Ile Asn His Asn Ile Asn His Thr Arg Thr Ser Gly Asp Gly Ala	260	265	270	
cct ttt aaa cgt caa caa aat att att aat tct aat tca ggt cat aga				864
Pro Phe Lys Arg Gln Gln Asn Ile Ile Asn Ser Asn Ser Gly His Arg	275	280	285	
aat caa aat aat ata aat caa ttt ata tgg aac aaa aat ggc ttt ttt				912
Asn Gln Asn Asn Ile Asn Gln Phe Ile Trp Asn Lys Asn Gly Phe Phe	290	295	300	
aaa tct caa aat aat acc gaa cat aga atg aat agt agc gat aat acc				960
Lys Ser Gln Asn Asn Thr Glu His Arg Met Asn Ser Ser Asp Asn Thr	305	310	315	320
aat tca tta att agc aga ttc aga caa tta gcc acg ggt gct tat aag				1008
Asn Ser Leu Ile Ser Arg Phe Arg Gln Leu Ala Thr Gly Ala Tyr Lys	325	330	335	
tac aat ccg ttt ttg att aat caa gta aaa aat ttg aat caa tta gat				1056
Tyr Asn Pro Phe Leu Ile Asn Gln Val Lys Asn Leu Asn Gln Leu Asp	340	345	350	
gga aag gtg aca gat agt gac att tat agc ttg ttt aga aag caa tca				1104
Gly Lys Val Thr Asp Ser Asp Ile Tyr Ser Leu Phe Arg Lys Gln Ser	355	360	365	
ttt aga gga aat gaa tat tta aat tca tta caa aaa ggg aca agc tat				1152
Phe Arg Gly Asn Glu Tyr Leu Asn Ser Leu Gln Lys Gly Thr Ser Tyr	370	375	380	
ttc aga ttt caa tat ttt aat cca ctt aat tct agt aaa tac tat gaa				1200
Phe Arg Phe Gln Tyr Phe Asn Pro Leu Asn Ser Ser Lys Tyr Tyr Glu	385	390	395	400
aat tta gat gat cag gtt tta gct tta att aca gga gaa atc ggc tca				1248
Asn Leu Asp Asp Gln Val Leu Ala Leu Ile Thr Gly Glu Ile Gly Ser	405	410	415	

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atg cca gaa ctt aaa aaa cct acg gat aaa gaa gat aaa aat cat agc 1296
 Met Pro Glu Leu Lys Lys Pro Thr Asp Lys Glu Asp Lys Asn His Ser
 420 425 430

gcc ttc aaa aac cat agt gca gat gag ata aca aca aat aat gat gga 1344
 Ala Phe Lys Asn His Ser Ala Asp Glu Ile Thr Thr Asn Asn Asp Gly
 435 440 445

cac tcc aaa gat tat gat aag aaa aag aaa ata cat cga agt ctt tta 1392
 His Ser Lys Asp Tyr Asp Lys Lys Lys Lys Ile His Arg Ser Leu Leu
 450 455 460

tcg tta agt att gca ata att gga att ttt cta gga gtc act gga cta 1440
 Ser Leu Ser Ile Ala Ile Ile Gly Ile Phe Leu Gly Val Thr Gly Leu
 465 470 475 480

tat atc ttt aga aga aaa aag taa 1464
 Tyr Ile Phe Arg Arg Lys Lys
 485

<210> SEQ ID NO 14
 <211> LENGTH: 487
 <212> TYPE: PRT
 <213> ORGANISM: Staphylococcus epidermidis

<400> SEQUENCE: 14

Met Lys Lys Phe Asn Ile Lys His Ser Phe Met Leu Thr Gly Phe Ala
 1 5 10 15

Phe Met Val Thr Thr Ser Leu Phe Ser His Gln Ala His Ala Glu Gly
 20 25 30

Asn His Pro Ile Asp Ile Asn Phe Ser Lys Asp Gln Ile Asp Arg Asn
 35 40 45

Thr Ala Lys Ser Asn Ile Ile Asn Arg Val Asn Asp Thr Ser Arg Thr
 50 55 60

Gly Ile Ser Met Asn Ser Asp Asn Asp Leu Asp Thr Asp Ile Val Ser
 65 70 75 80

Asn Ser Asp Ser Glu Asn Asp Thr Tyr Leu Asp Ser Asp Ser Asp Ser
 85 90 95

Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
 100 105 110

Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
 115 120 125

Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
 130 135 140

Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
 145 150 155 160

Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
 165 170 175

Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
 180 185 190

Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser
 195 200 205

Asp Ser Gly Thr Ser Ser Gly Lys Gly Ser His Thr Gly Lys Lys Pro
 210 215 220

Gly Asn Pro Lys Gly Asn Thr Asn Arg Pro Ser Gln Arg His Thr Asn
 225 230 235 240

Gln Pro Gln Arg Pro Lys Tyr Asn Gln Thr Asn Gln Asn Asn Ile Asn
 245 250 255

Asn Ile Asn His Asn Ile Asn His Thr Arg Thr Ser Gly Asp Gly Ala
 260 265 270

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Pro Phe Lys Arg Gln Gln Asn Ile Ile Asn Ser Asn Ser Gly His Arg
 275 280 285

Asn Gln Asn Asn Ile Asn Gln Phe Ile Trp Asn Lys Asn Gly Phe Phe
 290 295 300

Lys Ser Gln Asn Asn Thr Glu His Arg Met Asn Ser Ser Asp Asn Thr
 305 310 315 320

Asn Ser Leu Ile Ser Arg Phe Arg Gln Leu Ala Thr Gly Ala Tyr Lys
 325 330 335

Tyr Asn Pro Phe Leu Ile Asn Gln Val Lys Asn Leu Asn Gln Leu Asp
 340 345 350

Gly Lys Val Thr Asp Ser Asp Ile Tyr Ser Leu Phe Arg Lys Gln Ser
 355 360 365

Phe Arg Gly Asn Glu Tyr Leu Asn Ser Leu Gln Lys Gly Thr Ser Tyr
 370 375 380

Phe Arg Phe Gln Tyr Phe Asn Pro Leu Asn Ser Ser Lys Tyr Tyr Glu
 385 390 395 400

Asn Leu Asp Asp Gln Val Leu Ala Leu Ile Thr Gly Glu Ile Gly Ser
 405 410 415

Met Pro Glu Leu Lys Lys Pro Thr Asp Lys Glu Asp Lys Asn His Ser
 420 425 430

Ala Phe Lys Asn His Ser Ala Asp Glu Ile Thr Thr Asn Asn Asp Gly
 435 440 445

His Ser Lys Asp Tyr Asp Lys Lys Lys Lys Ile His Arg Ser Leu Leu
 450 455 460

Ser Leu Ser Ile Ala Ile Ile Gly Ile Phe Leu Gly Val Thr Gly Leu
 465 470 475 480

Tyr Ile Phe Arg Arg Lys Lys
 485

<210> SEQ ID NO 15
 <211> LENGTH: 18
 <212> TYPE: DNA
 <213> ORGANISM: Staphylococcus aureus
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <223> OTHER INFORMATION: n=(a or c or g or t)

<400> SEQUENCE: 15
 gaytcngayt cngayagy

18

<210> SEQ ID NO 16
 <211> LENGTH: 5
 <212> TYPE: PRT
 <213> ORGANISM: Staphylococcus aureus

<400> SEQUENCE: 16
 Leu Pro Asp Thr Gly
 1 5

<210> SEQ ID NO 17
 <211> LENGTH: 9
 <212> TYPE: PRT
 <213> ORGANISM: Staphylococcus aureus

<400> SEQUENCE: 17
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 1 5

<210> SEQ ID NO 18

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<211> LENGTH: 6
 <212> TYPE: PRT
 <213> ORGANISM: *Staphylococcus aureus*

<400> SEQUENCE: 18

Thr Asn Ser His Gln Asp
 1 5

What is claimed is:

1. A multicomponent vaccine consisting essentially of immunologically effective amounts of the collagen binding domain of a Staphylococcal collagen binding protein, and the fibrinogen binding domain of a Staphylococcal fibrinogen binding protein, and a pharmaceutically acceptable carrier or excipient.
2. A vaccine according to claim 1 further comprising the fibronectin binding domain of a Staphylococcal fibronectin binding protein.
3. A vaccine according to claim 1 wherein the staphylococcal organisms are *Staphylococcus aureus*.
4. A vaccine according to claim 3, wherein the *Staphylococcus aureus* binding proteins are selected from the group consisting of the collagen binding adhesin CNA, clumping factor A (ClfA) and clumping factor B (ClfB).

5. A vaccine according to claim 1 further comprising an SdrH protein from *Staphylococcus epidermidis*.

6. A vaccine according to claim 1 wherein the collagen binding protein is selected from the group consisting of the collagen binding adhesin CNA and the collagen binding adhesin subdomain M55.

7. A vaccine according to claim 1 wherein the fibrinogen binding protein is selected from the group consisting of clumping factor A (ClfA) and clumping factor B (ClfB).

8. A vaccine according to claim 2 wherein the fibronectin binding protein is selected from the group consisting of fibronectin binding protein A (FnBP-A) and fibronectin binding protein B (FnBP-B).

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