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(54) Title: COMPOSITIONS AND METHODS FOR TREATING FUNGAL AND BACTERIAL PATHOGENS

(57) Abstract: The invention features fragments of the Candida cell surface proteins Als3 and Hyr1 and combinations thereof useful in immunizing a subject against fungal or bacterial infections or both.

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COMPOSITIONS AND METHODS FOR TREATING FUNGAL AND BACTERIAL PATHOGENS

FIELD OF THE INVENTION

The present invention relates generally to compositions and methods for detecting, treating and preventing infectious diseases in a subject.

BACKGROUND OF THE INVENTION

The fungus *Candida*, the third most common cause of healthcare-associated bloodstream infections, causes approximately 60,000 cases of hematogenously disseminated candidiasis per year in the United States, resulting in billions of dollars of healthcare expenditures. Despite current antifungal therapy, mortality remains unacceptably high. Because of the rising incidence of life-threatening candidiasis and high treatment failure rates, more effective prophylactic and therapeutic strategies are needed.

Lethal infections of antibiotic resistant pathogenic bacteria, like infections resulting from Candida, are becoming increasingly frequent. Moreover, the risk of contracting these lethal infections is extremely high for many at-risk patients in intensive care units (ICUs) every year as well as for soldiers deployed to front line combat zones. *Acinetobacter* species are a frequent source of infection in hospitalized patients and soldiers, in particular the species *Acinetobacter baumannii*. *Acinetobacter* is a genus of gram negative bacteria belonging to the Gammaproteobacteria. *Acinetobacter* species contribute to the mineralization of aromatic compounds in the soil. Unfortunately, no technology presently exists that prevents *Acinetobacter* infections, aside from standard hand washing and other infection control practices in hospital settings.

Another bacterium, *Staphylococcus aureus* is the leading cause of skin and skin structure infections including cellulitis and

furunculosis, and is among the most common causes of bacteremia. Strains of *S. aureus* that exhibit the methicillin-resistant (MRSA) phenotype are predominant causes of healthcare-and community-acquired infections, including invasive disease in immune competent hosts, in immune suppression (e.g. neutropenia, solid-organ or bone marrow transplants), and in inherited immune dysfunctions manifesting recurring cutaneous infection (e.g. Job's Syndrome, Chronic Granulomatous Disease). The significant impact of MRSA on public health is of special concern in light of high rates of mortality associated with invasive *S. aureus* disease even with appropriate antimicrobial therapy (e.g. 15-40% in bacteremia and endocarditis). Increasing rates of life-threatening infections and decreasing susceptibility to antibiotics call for development of an effective vaccine targeting *S. aureus*.

There accordingly exists a need for compounds and methods that reduce the risk of infectious diseases related to fungal and bacterial infections and provide effective therapies. The present invention satisfies this need and provides related advantages as well.

SUMMARY OF THE INVENTION

Fragments of the *Candida* cell surface proteins Als3 and Hyr1 and combinations thereof useful in immunizing a subject against fungal or bacterial infections or both are described below.

The amino acid sequence of native C. albicans SC5314 Als3 polypeptide is as follows:

1	MLQQYTLLLIYLSVATAKTI	TGVFNSFNSLTWSNAATYNY	KGPGTPTWNAVLGWSLDGTS
61	ASPGDTFTLNMPCVFKFTTS	QTSVDLTAHGVKYATCQFQA	GEEFMTFSTLTCTVSNTLTP
121	SIKALGTVTLPLAFNVGGTG	SSVDLEDSKCFTAGTNTVTF	NDGGKKISINVDFERSNVDP
181	KGYLTDSRVIPSLNKVSTLF	VAPQCANGYTSGTMGFANTY	GDVQIDCSNIHVGITKGLND
241	WNYPVSSESFSYTKTCSSNG	IFITYKNVPAGYRPFVDAYI	SATDVNSYTLSYANEYTCAG
301	GYWQRAPFTLRWTGYRNSDA	GSNGIVIVATTRTVTDSTTA	VTTLPFDPNRDKTKTIEILK
361	PIPTTTITTSYVGVTTSYST	KTAPIGETATVIVDIPYHTT	TTVTSKWTGTITSTTTHTNP
421	TDSIDTVIVQVPSPNPTVTT	TEYWSQSFATTTTITGPPGN	TDTVLIREPPNHTVTTTEYW
481	SESYTTTSTFTAPPGGTDSV	IIKEPPNPTVTTTEYWSESY	TTTSTFTAPPGGTDSVIIKE
541	PPNHTVTTTEYWSQSYTTTT	TVTAPPGGTDTVLVREPPNH	TVTTTEYWSQSYTTTTTVIA
601	PPGGTDSVIIREPPNPTVTT	TEYWSQSYATTTTITAPPGE	TDTVLIREPPNHTVTTTEYW
661	SQSYATTTTITAPPGETDTV	LIREPPNHTVTTTEYWSQSF	ATTTTVTAPPGGTDTVIIRE

721 PPNHTVTTTEYWSQSYATTT TITAPPGETDTVLIREPPNH TVTTTEYWSQSYATTTTIIA
781 PPGETDTVLIREPPNPTVTT TEYWSQSYTTATTVTAPPGG TDTVIIYDTMSSSEISSFSR
841 PHYTNHTTLWSTTWVIETKT ITETSCEGDKGCSWVSVSTR IVTIPNNIETPMVTNTVDST
901 TTESTSQSPSGIFSESGVSV ETESSTVTTAQTNPSVPTTE SEVVFTTKGNNENGPYESPS
961 TNVKSSMDENSEFTTSTAAS TSTDIENETIATTGSVEASS PIISSSADETTTVTTTAEST
1021 SVIEQPTNNNGGGKAPSATS SPSTTTTANNDSVITGTTST NQSQSQSQYNSDTQQTTLSQ
1081 QMTSSLVSLHMLTTFDGSGS VIQHSTWLCGLITLLSLFI

Select Als fragments are as follows.

Als3 (18-324)

In one aspect, the invention features and Als3 (18-324 amino acid fragment). In particular, the invention features an isolated polypeptide optionally fused to a heterologous fusion partner, wherein the amino acid sequence of the polypeptide consists of an amino acid sequence having at least 95% identity to

KTI TGVFNSFNSLTWSNAATYNY KGPGTPTWNAVLGWSLDGTS
ASPGDTFTLNMPCVFKFTTS QTSVDLTAHGVKYATCQFQA GEEFMTFSTLTCTVSNTLTP
SIKALGTVTLPLAFNVGGTG SSVDLEDSKCFTAGTNTVTF NDGGKKISINVDFERSNVDP
KGYLTDSRVIPSLNKVSTLF VAPQCANGYTSGTMGFANTY GDVQIDCSNIHVGITKGLND
WNYPVSSESFSYTKTCSSNG IFITYKNVPAGYRPFVDAYI SATDVNSYTLSYANEYTCAG
GYWQRAPFTLRWTGYRNSDA GSNG (SEQ ID NO: 2).

Als3 (Ser/Thr-rich sequence)

In another aspect, the invention features an isolated polypeptide optionally fused to a heterologous fusion partner, wherein the amino acid sequence of the polypeptide consists of an amino acid sequence having at least 95% identity to

IVIVATTRTVTDSTTA VTTLPFDPNRDKTKTIEILK
PIPTTTITTSYVGVTTSYST KTAPIGETATVIVDIPYHTT TTVTSKWTGTITSTTTHTNP
TDSIDTVIVQVP (SEQ ID NO: 3).

Hyr1

In other aspect, the invention features fragments of Hyr1. The amino acid sequence of native C. albicans SC5314 Hyr1 polypeptide is as follows:

1 MKVVSNFIFTILLTLNLSAA LEVVTSRIDRGGIQGFHGDV KVHSGATWAILGTTLCSFFG

61	GLEVEKGASLFIKSDNGPVL	ALNVALSTLVRPVINNGVIS	LNSKSSTSFSNFDIGGSSFT
121	NNGEIYLDSSGLVKSTAYLY	AREWTNNGLIVAYQNQKAAG	NIAFGTAYQTITNNGQICLR
181	HQDFVPATKIKGTGCVTADE	DTWIKLGNTILSVEPTHNFY	LKDSKSSLIVHAVSSNQTFT
241	VHGFGNGNKLGLTLPLTGNR	DHFRFEYYPDTGILQLRADA	LPQYFKIGKGYDSKLFRIVN
301	SRGLKNAVTYDGPVPNNEIP	AVCLIPCTNGPSAPESESDL	NTPTTSSIETSSYSSAATES
361	SVVSESSSAVDSLTSSSLSS	KSESSDVVSSTTNIESSSTA	IETTMNSESSTDAGSSSISQ
421	SESSSTAITSSSETSSSESM	SASSTTASNTSIETDSGIVS	QSESSSNALSSTEQSITSSP
481	GQSTIYVNSTVTSTITSCDE	NKCTEDVVTIFTTVPCSTDC	VPTTGDIPMSTSYTQRTVTS
541	TITNCDEVSCSQDVVTYTTN	VPHTTVDATTTTTTSTGGDN	STGGNESGSNHGPGNGSTEG
601	SGNGSGAGSNEGSQSGPNNG	SGSGSEGGSNNGSGSDSGSN	NGSGSGSNNGSGSGSTEGSE
661	GGSGSNEGSQSGSGSQPGPN	EGSEGGSGSNEGSNHGSNEG	SGSGSGSGSNNGSGSGSQSG
721	SGSGSQSGSESGSNSGSNEG	SNPGAGNGSNEGSGQGSGNG	SEAGSGQGSGPNNGSGSGHN
781	DGSGSGSNQGSNPGAGSGSG	SESGSKAGSHSGSNEGAKTD	SIEGFHTESKPGFNTGAHTD
841	ATVTGNSVANPVTTSTESDT	TISVTVSITSYMTGFDGKPK	PFTTVDVIPVPHSMPSNTTD
901	SSSSVPTIDTNENGSSIVTGG	KSILFGLIVSMVVLFM	

Select fragments of Hyr1 are as follows.

Hyr1 (hydrophobic sequence)

In particular, the invention features an isolated polypeptide optionally fused to a heterologous fusion partner, wherein the amino acid sequence of the polypeptide consists of an amino acid sequence having at least 95% identity to

TSRIDRGGIQGFHGDVKVHS GATWAILGTTLCSFFGGLEV EKGASLFIKSDNGPVLALNV
ALSTLVRPVINNGVISLNSK SSTSFSNFDIGGSSFTNNGE IYLDSSGLVKSTAYLYAREW
TNNGLIVAY (SEQ ID NO: 5).

Hyr1 (154-350)

In another aspect, the invention features an isolated polypeptide optionally fused to a heterologous fusion partner, wherein the amino acid sequence of the polypeptide consists of an amino acid sequence having at least 95% identity to

	QNQKAAG	NIAFGTAYQTITNNGQICLR
HQDFVPATKIKGTGCVTADE	DTWIKLGNTILSVEPTHNFY	LKDSKSSLIVHAVSSNQTFT
VHGFGNGNKLGLTLPLTGNR	DHFRFEYYPDTGILQLRADA	LPQYFKIGKGYDSKLFRIVN
SRGLKNAVTYDGPVPNNEIP	AVCLIPCTNGPSAPESESDL	NTPTTSSIET (SEQ ID NO: 6).

Hyr1 (201-350)

In another aspect, the invention features an isolated polypeptide optionally fused to a heterologous fusion partner, wherein the amino acid sequence of the polypeptide consists of an amino acid sequence having at least 95% identity to

DTWIKLGNTILSVEPTHNFY LKDSKSSLIVHAVSSNQTFT
VHGFGNGNKLGLTLPLTGNR DHFRFEYYPDTGILQLRADA LPQYFKIGKGYDSKLFRIVN
SRGLKNAVTYDGPVPNNEIP AVCLIPCTNGPSAPESESDL NTPTTSSIET (SEQ ID NO: 7).

Hyr1 (25-469)

In another aspect, the invention features an isolated polypeptide optionally fused to a heterologous fusion partner, wherein the amino acid sequence of the polypeptide consists of an amino acid sequence having at least 95% identity to

TSRIDRGGIQGFHGDV KVHSGATWAILGTTLCSFFG
GLEVEKGASLFIKSDNGPVL ALNVALSTLVRPVINNGVIS LNSKSSTSFSNFDIGGSSFT
NNGEIYLDSSGLVKSTAYLY AREWTNNGLIVAYQNQKAAG NIAFGTAYQTITNNGQICLR
HQDFVPATKIKGTGCVTADE DTWIKLGNTILSVEPTHNFY LKDSKSSLIVHAVSSNQTFT
VHGFGNGNKLGLTLPLTGNR DHFRFEYYPDTGILQLRADA LPQYFKIGKGYDSKLFRIVN
SRGLKNAVTYDGPVPNNEIP AVCLIPCTNGPSAPESESDL NTPTTSSIETSSYSSAATES
SVVSESSSAVDSLTSSSLSS KSESSDVVSSTTNIESSSTA IETTMNSESSTDAGSSSISQ
SESSSTAITSSSETSSSESM SASSTTASNTSIETDSGIVS QSESSSNAL (SEQ ID NO: 8).

Hyr1 (201-469)

In another aspect, the invention features an isolated polypeptide optionally fused to a heterologous fusion partner, wherein the amino acid sequence of the polypeptide consists of an amino acid sequence having at least 95% identity to

DTWIKLGNTILSVEPTHNFY LKDSKSSLIVHAVSSNQTFT
VHGFGNGNKLGLTLPLTGNR DHFRFEYYPDTGILQLRADA LPQYFKIGKGYDSKLFRIVN
SRGLKNAVTYDGPVPNNEIP AVCLIPCTNGPSAPESESDL NTPTTSSIETSSYSSAATES
SVVSESSSAVDSLTSSSLSS KSESSDVVSSTTNIESSSTA IETTMNSESSTDAGSSSISQ
SESSSTAITSSSETSSSESM SASSTTASNTSIETDSGIVS QSESSSNAL (SEQ ID NO: 9).

Hyr1 (Ser/Thr-rich sequence)

In another aspect, the invention features an isolated polypeptide optionally fused to a heterologous fusion partner, wherein the amino acid sequence of the polypeptide consists of an amino acid sequence having at least 95% identity to

SSYSSAATESSVVS ESSSAVDSLTSSSLSSKSES
SDVVSSTTNIESSSTAIETT MNSESSTDAGSSSISQSESS STAITSSSETSSSESMSASS
TTASNTSIETDSGIVSQSES SSNAL (SEQ ID NO: 10).

Hyr1 (154-469)

In another aspect, the invention features an isolated polypeptide optionally fused to a heterologous fusion partner, wherein the amino acid sequence of the polypeptide consists of an amino acid sequence having at least 95% identity to

QNQKAAG NIAFGTAYQTITNNGQICLR
HQDFVPATKIKGTGCVTADE DTWIKLGNTILSVEPTHNFY LKDSKSSLIVHAVSSNQTFT
VHGFGNGNKLGLTLPLTGNR DHFRFEYYPDTGILQLRADA LPQYFKIGKGYDSKLFRIVN
SRGLKNAVTYDGPVPNNEIP AVCLIPCTNGPSAPESESDL NTPTTSSIETSSYSSAATES
SVVSESSSAVDSLTSSSLSS KSESSDVVSSTTNIESSSTA IETTMNSESSTDAGSSSISQ
SESSSTAITSSSETSSSESM SASSTTASNTSIETDSGIVS QSESSSNAL (SEQ ID NO: 33).

Any of the above-described polypeptide fragments may be produced recombinantly in *E. coli* or *S. cerevesiae*. Additionally, the invention features Als3/Hyr1 fusion polypeptides and recombinant expression systems producing the same.

E. coli expressed Als3/Hyr1 fusion polypeptides

In another aspect, the invention relates to fragments of combinations of Als3 and Hyr1 polypeptides expressed in *E. coli*. In particular, these fragments and linkers joining such fragments are as follows:

Als3

A= KTITGVFNSFNSLTWSNAAT YNYKGPGTPTWNAVLGWSLD GTSASPGDTFTLNMPCVFKF
TTSQTSVDLTAHGVKYATCQ FQAGEEFMTFSTLTCTVSNT LTPSIKALGTVTLPLAFNVG
GTGSSVDLEDSKCFTAGTNT VTFNDGGKKISINVDFERSN VDPKGYLTDSRVIPSLNKVS
TLFVAPQCANGYTSGTMGFA NTYGDVQIDCSNIHVGITKG LNDWNYPVSSESFSYTKTCS

SNGIFITYKNVPAGYRPFVD AYISATDVNSYTLSYANEYT CAGGYWQRAPFTLRWTGYRN SDAGSNG (SEQ ID NO: 2).

B= IVIVATTRTVTDS TTAVTTLPFDPNRDKTKTIE ILKPIPTTTIITTSYVGVTTS YSTKTAPIGETATVIVDIPY HTTTTVTSKWTGTITSTTTH TNPTDSIDTVIVQVP (SEQ ID NO: 3)

Hyr1

C= TSRIDRGGIQGFHGDVKVHS GATWAILGTTLCSFFGGLEV EKGASLFIKSDNGPVLALNV ALSTLVRPVINNGVISLNSK SSTSFSNFDIGGSSFTNNGE IYLDSSGLVKSTAYLYAREW TNNGLIVAY (SEQ ID NO: 5)

D= QNQKAAGNIAF GTAYQTITNNGQICLRHQDF VPATKIKGTGCVTADEDTWI KLGNTILSVEPTHNFYLKDS KSSLIVHAVSSNQTFTVHGF GNGNKLGLTLPLTGNRDHFR FEYYPDTGILQLRADALPQY FKIGKGYDSKLFRIVNSRGL KNAVTYDGPVPNNEIPAVCL IPCTNGPSAPESESDLNTPT TSSIET (SEQ ID NO: 6)

X= is present or absent (designated as –X), wherein X is a linker peptide.

Exemplary fusion polypeptides are as follows:

E1=	A–B–X–C–D (SEQ ID NO: 11)	E1(–X)=	A–B–C–D (SEQ ID NO: 12)
E2=	A–X–C–D (SEQ ID NO: 13)	E2(–X)=	A–C–D (SEQ ID NO: 14)
E3=	A–X–D (SEQ ID NO: 15)	E3(–X)=	A–D (SEQ ID NO: 16)
E4=	C–D–X–A–B (SEQ ID NO: 17)	E4(–X)=	C–D–A–B (SEQ ID NO: 18)
E5=	C–D–X–A (SEQ ID NO: 19)	E5(–X)=	C–D–A (SEQ ID NO: 20)
E6=	D–X–A–B (SEQ ID NO: 21)	E6(–X)=	D–A–B (SEQ ID NO: 22)
E7=	D–X–A (SEQ ID NO: 23)	E7(–X)=	D–A (SEQ ID NO: 24)

E1= A–B–X–C–D

In another aspect, the invention features an isolated polypeptide including a sequence having substantial identity to the amino acid sequence

A–B–X–C–D (SEQ ID NO: 11),

wherein A is SEQ ID NO: 2;

wherein B is SEQ ID NO: 3;

wherein X is absent or is a linker peptide;
wherein C is SEQ ID NO: 5; and
wherein D is SEQ ID NO: 6.

In some embodiments, the isolated polypeptide is substantially identical to A-B-C-D (SEQ ID NO: 12. In other embodiments, the polypeptide is A-B-C-D (SEQ ID NO: 12).

E2= A-X-C-D

In another aspect, the invention features an isolated polypeptide including a sequence having substantial identity to the amino acid sequence

A-X-C-D (SEQ ID NO: 13),

wherein A is SEQ ID NO: 2;
wherein X is absent or is a linker peptide;
wherein C is SEQ ID NO: 5; and
wherein D is SEQ ID NO: 6.

In some embodiments, the polypeptide is substantially identical to A-C-D (SEQ ID NO: 14). In other embodiments, the polypeptide is A-C-D (SEQ ID NO: 14).

E3= A-X-D

In another aspect, the invention features an isolated polypeptide including a sequence having substantial identity to the amino acid sequence

A-X-D (SEQ ID NO: 15),

wherein A is SEQ ID NO: 2;
wherein X is absent or is a linker peptide; and
wherein D is SEQ ID NO: 6.

In some embodiments, the polypeptide is substantially identical to A-D SEQ ID NO: 16). In other embodiments, the polypeptide is A-D (SEQ ID NO: 16).

E4= C-D-X-A-B

In another aspect, the invention features an isolated polypeptide including a sequence having substantial identity to the amino acid sequence

C-D-X-A-B (SEQ ID NO: 17),

wherein C is SEQ ID NO: 5;
wherein D is SEQ ID NO: 6;
wherein X is absent or is a linker peptide;
wherein A is SEQ ID NO: 2; and
wherein B is SEQ ID NO: 3.

In some embodiments, the polypeptide is substantially identical to C-D-A-B (SEQ ID NO: 18). In other embodiments, the polypeptide is C-D-A-B (SEQ ID NO: 18).

E5= C-D-X-A

In another aspect, the invention features an isolated polypeptide including a sequence having substantial identity to the amino acid sequence

C-D-X-A (SEQ ID NO: 19),

wherein C is SEQ ID NO: 5;
wherein D is SEQ ID NO: 6;
wherein X is absent or is a linker peptide; and
wherein A is SEQ ID NO: 2.

In some embodiments, the polypeptide is substantially identical to C-D-A (SEQ ID NO: 20). In other embodiments, the polypeptide is C-D-A (SEQ ID NO: 20).

E6= D-X-A-B

In another aspect, the invention features an isolated polypeptide including a sequence having substantial identity to the amino acid sequence

D-X-A-B (SEQ ID NO: 21),

wherein D is SEQ ID NO: 6;
wherein X is absent or is a linker peptide;
wherein A is SEQ ID NO: 2; and

wherein B is SEQ ID NO: 3.

In some embodiments, the polypeptide is substantially identical to D-A-B (SEQ ID NO: 22). In other embodiments, the polypeptide is D-A-B (SEQ ID NO: 22).

E7 D-X-A

In another aspect, the invention features an isolated polypeptide including a sequence having substantial identity to the amino acid sequence

D-X-A (SEQ ID NO: 23),

wherein D is SEQ ID NO: 6;
wherein X is absent or is a linker peptide; and
wherein A is SEQ ID NO: 2.

In some embodiments, the polypeptide is substantially identical to D-A (SEQ ID NO: 24). In other embodiments, the polypeptide is D-A (SEQ ID NO: 24).

S. cerevisiae expressed Als3/Hyr1 fusion polypeptides

In another aspect, the invention relates to fragments of combinations of Als3 and Hyr1 polypeptides expressed in S. cerevisiae. In particular, these fragments and linkers joining such fragments are as follows:

Als3

A= KTITGVFNSFNSLTWSNAAT YNYKGPGTPTWNAVLGWSLD GTSASPGDTFTLNMPCVFKF
TTSQTSVDLTAHGVKYATCQ FQAGEEFMTFSTLTCTVSNT LTPSIKALGTVTLPLAFNVG
GTGSSVDLEDSKCFTAGTNT VTFNDGGKKISINVDFERSN VDPKGYLTDSRVIPSLNKVS
TLFVAPQCANGYTSGTMGFA NTYGDVQIDCSNIHVGITKG LNDWNYPVSSESFSYTKTCS
SNGIFITYKNVPAGYRPFVD AYISATDVNSYTLSYANEYT CAGGYWQRAPFTLRWTGYRN
SDAGSNG (SEQ ID NO: 2)

B= IVIVATTRTVTDS TTAVTTLPFDPNRDKTKTIE ILKPIPTTTITTSYVGVTTS
YSTKTAPIGETATVIVDIPY HTTTTVTSKWTGTITSTTTH TNPTDSIDTVIVQVP (SEQ
ID NO: 3)

Hyr1

C= TSRIDRGGIQGFHGDVKVHS GATWAILGTTLCSFFGGLEV EKGASLFIKSDNGPVLALNV
ALSTLVRPVINNGVISLNSK SSTSFSNFDIGGSSFTNNGE IYLDSSGLVKSTAYLYAREW
TNNGLIVAY (SEQ ID NO: 5)

D= QNQKAAGNIAF GTAYQTITNNGQICLRHQDF VPATKIKGTGCVTADEDTWI
KLGNTILSVEPTHNFYLKDS KSSLIVHAVSSNQTFTVHGF GNGNKLGLTLPLTGNRDHFR
FEYYPDTGILQLRADALPQY FKIGKGYDSKLFRIVNSRGL KNAVTYDGPVPNNEIPAVCL
IPCTNGPSAPESESDLNTPT TSSIET (SEQ ID NO: 6)

E= SSYSSAATESSVVS ESSSAVDSLTSSSLSSKSES
SDVVSSTTNIESSSTAIETT MNSESSTDAGSSSISQSESS STAITSSSETSSSESMSASS
TTASNTSIETDSGIVSQSES SSNAL (SEQ ID NO: 10)

X= is present or absent, wherein X is a linker peptide.

S1=	A-B-X-C-D (SEQ ID NO: 11)	S1(-X)=	A-B-C-D (SEQ ID NO: 12)
S2=	A-X-C-D-E (SEQ ID NO: 25)	S2(-X)=	A-C-D-E ((SEQ ID NO: 26)
S3=	A-X-D-E (SEQ ID NO: 27)	S3(-X)=	A-D-E (SEQ ID NO: 28)
S4=	C-D-E-X-A-B (SEQ ID NO: 29)	S4(-X)=	C-D-E-A-B (SEQ ID NO: 30)
S5=	C-D-X-A-B (SEQ ID NO: 17)	S5(-X)=	C-D-A-B (SEQ ID NO: 18)
S6=	D-X-A-B (SEQ ID NO: 21)	S6(-X)=	D-A-B (SEQ ID NO: 22)
S7=	D-X-A (SEQ ID NO: 23)	S7(-X)=	D-A (SEQ ID NO: 24)

S1= A-B-X-C-D

In another aspect, the invention features an isolated polypeptide including a sequence having substantial identity to the amino acid sequence

A-B-X-C-D (SEQ ID NO: 11),

wherein A is SEQ ID NO: 2;
wherein B is SEQ ID NO: 3;
wherein X is absent or is a linker peptide;
wherein C is SEQ ID NO: 5; and
wherein D is SEQ ID NO: 6.

In some embodiments, the polypeptide is substantially identical to A-B-C-D (SEQ ID NO: 12). In other embodiments, the polypeptide is A-B-C-D (SEQ ID NO: 12).

S2= A-X-C-D-E

In another aspect, the invention features an isolated polypeptide isolated polypeptide including a sequence having substantial identity to the amino acid sequence

A-X-C-D-E (SEQ ID NO: 25),

wherein A is SEQ ID NO: 2;
wherein X is absent or is a linker peptide;
wherein C is SEQ ID NO: 5;
wherein D is SEQ ID NO: 6; and
wherein E is SEQ ID NO: 10.

In some embodiments, the polypeptide is substantially identical to A-C-D-E (SEQ ID NO: 26). In other embodiments, the polypeptide is A-C-D-E (SEQ ID NO: 26).

S3= A-X-D-E

In another aspect, the invention features an isolated polypeptide isolated polypeptide including a sequence having substantial identity to the amino acid sequence

A-X-D-E (SEQ ID NO: 27),

wherein A is SEQ ID NO: 2;
wherein X is absent or is a linker peptide;
wherein D is SEQ ID NO: 6; and
wherein E is SEQ ID NO: 10.

In some embodiments, the polypeptide is substantially identical to A-D-E (SEQ ID NO: 28). In other embodiments, the polypeptide is A-D-E (SEQ ID NO: 28).

S4= C-D-E-X-A-B

In another aspect, the invention features an isolated polypeptide isolated polypeptide including a sequence having substantial identity to the amino acid sequence

C-D-E-X-A-B (SEQ ID NO: 29),

wherein C is SEQ ID NO: 5;

wherein D is SEQ ID NO: 6;

wherein E is SEQ ID NO: 10;

wherein X is absent or is a linker peptide;

wherein A is SEQ ID NO: 2; and

wherein B is SEQ ID NO: 3.

In some embodiments, the polypeptide is substantially identical to C-D-E-A-B (SEQ ID NO: 30). In other embodiments, the polypeptide is C-D-E-A-B (SEQ ID NO: 30).

S5= C-D-X-A-B

In another aspect, the invention features an isolated polypeptide isolated polypeptide including a sequence having substantial identity to the amino acid sequence

C-D-X-A-B (SEQ ID NO: 17),

wherein C is SEQ ID NO: 5;

wherein D is SEQ ID NO: 6;

wherein X is absent or is a linker peptide;

wherein A is SEQ ID NO: 2; and

wherein B is SEQ ID NO: 3.

In some embodiments, the polypeptide is substantially identical to C-D-A-B (SEQ ID NO: 18). In other embodiments, the polypeptide is C-D-A-B (SEQ ID NO: 18).

S6= D-X-A-B

In another aspect, the invention features an isolated polypeptide isolated polypeptide including a sequence having substantial identity to the amino acid sequence

D-X-A-B (SEQ ID NO: 21),

wherein D is SEQ ID NO: 6;

wherein X is absent or is a linker peptide;

wherein A is SEQ ID NO: 2; and

wherein B is SEQ ID NO: 3.

In some embodiments, the polypeptide is substantially identical to D-A-B (SEQ ID NO: 22). In other embodiments, the polypeptide is D-A-B (SEQ ID NO: 22).

S7= D-X-A

In another aspect, the invention features an isolated polypeptide isolated polypeptide including a sequence having substantial identity to the amino acid sequence

D-X-A (SEQ ID NO: 23),

wherein D is SEQ ID NO: 6;
wherein X is absent or is a linker peptide; and
wherein A is SEQ ID NO: 2.

In some embodiments, the polypeptide is substantially identical to D-A (SEQ ID NO: 24). In other embodiments, the polypeptide is D-A (SEQ ID NO: 24).

In other aspects, the invention features an isolated nucleic acid molecule which encodes any of the polypeptides or fusion polypeptides described herein.

In another aspect, the invention features an isolated nucleic acid molecule including a nucleic acid sequence which is substantially identical to any of the isolated nucleic acid molecule which encodes any of the polypeptides or fusion polypeptides described herein.

The invention further features a vector including any of the nucleic acid molecules encoding the polypeptides or fusion polypeptides described herein. The invention accordingly also provides vectors containing the nucleic acids of the invention. Suitable expression vectors are well-known in the art and include vectors capable of expressing a nucleic acid operatively linked to a regulatory sequence or element such as a promoter region or enhancer region that is capable of regulating expression of the nucleic acid. Appropriate expression vectors include vectors that are replicable in eukaryotic cells and/or prokaryotic cells and vectors that remain episomal or integrate into the host cell genome.

The invention also provides a method for expression of a polypeptide as disclosed herein by culturing cells containing a

nucleic acid that encodes the polypeptide under conditions suitable for expression of polypeptide. Thus, there is provided a method for the recombinant production of a polypeptide of the invention by expressing the nucleic acid sequences encoding the polypeptide in suitable host cells. Recombinant DNA expression systems that are suitable for production of polypeptides are described herein and are well-known in the art. For example, the above-described nucleotide sequences can be incorporated into vectors for further manipulation. Vectors can include a recombinant DNA or RNA plasmid or virus containing discrete elements that are used to introduce heterologous DNA into cells for either expression or replication thereof.

Similarly, the invention features a cell including any of the nucleic acid molecules encoding polypeptides or fusion polypeptides described herein.

In another aspect, the invention features a method of producing a recombinant polypeptide, the method including the steps of: (a) providing a cell transformed with the nucleic acid molecule of encoding an polypeptide or fusion polypeptide described herein positioned for expression in the cell; (b) culturing the transformed cell under conditions for expressing the nucleic acid molecule, wherein the culturing results in expression of the recombinant polypeptide; and (c) isolating the recombinant polypeptide. In some embodiments, cell is a bacterium (e.g., E. coli). In other embodiments, the cell is a yeast cell (e.g., Saccharomyces cerevisae). In another aspect, the invention features a recombinant polypeptide produced according to this aforementioned method.

In another aspect, the invention features a substantially pure antibody that specifically recognizes and binds to any one of the polypeptides described herein.

In yet another aspect, the invention features an antigenic composition including the aforementioned polypeptides and a pharmaceutically acceptable carrier, diluent, and/or excipient. In some embodiments, the composition further includes an adjuvant.

In another aspect, the invention features a method of inducing an immune response in a mammal against an antigen including administering

any of the aforementioned polypeptides, or the aforementioned antigenic composition to the mammal (e.g., a human), wherein the polypeptide or the composition induces an immune response against the antigen in the mammal. Typically, the mammal is administered a single dose of the polypeptide or the composition. In some embodiments, the mammal is administered a plurality of doses of the polypeptide or the composition. In some embodiments, the plurality of doses are administered at least one day apart (e.g., the plurality of doses are administered at least two weeks apart). In yet other embodiments, the composition is administered twice.

In another aspect, the invention features a vaccine including an immunogenic amount of any of the aforementioned polypeptides, and a pharmaceutically acceptable excipient. In some embodiments, the vaccine includes a mixture of distinct polypeptides of any one of the aforementioned polypeptides. In some embodiments, the vaccine further includes an adjuvant (Alhydrogel). The vaccine of the invention is useful for vaccination of a mammal (e.g., a human) against candidiasis, a bacterial infection such as one caused by Staphylococcus or Acinetobacter. Typically, the vaccine is to be administered by intramuscular, subcutaneous, or intradermal administration. The vaccine may also be administered by intramuscular administration. Vaccination may further includes administering a booster dose. Candidiasis may take many forms such as disseminated candidiasis (e.g., hematogenously disseminated candidiasis) or mucosal candidiasis. Candidiasis is caused, for example, by *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, or *Candida tropicalis*. In some embodiments, vaccination is against *Acinetobacter* or *Staphylococcus*.

In other aspects, the invention features a method of producing a chimeric vaccine including the steps of: (a) providing a phage, yeast, or virus; (b) inserting into the phage, yeast, or virus a nucleic acid molecule that encodes any of the aforementioned polypeptides; (c) allowing expression of the polypeptide in the phage, yeast, or virus; (d) isolating the phage, yeast, or virus of step (c) including the expressed polypeptide; and (e) adding a pharmaceutically acceptable

excipient to the isolated phage, yeast, or virus of step (d). In some embodiments, the polypeptide is displayed on the surface of the phage, yeast, or virus following step (c).

In other aspects, the invention features an isolated monoclonal antibody that binds to any of the aforementioned polypeptides or fusion polypeptides. Typically, the antibody is human or humanized. The antibody may also be chimeric. The antibody may also be produced recombinantly. A diagnostic composition including these antibodies is within the invention.

Another aspect of the invention relates to a pharmaceutical composition including any of the aforementioned antibodies alone or in combination, and a pharmaceutically acceptable excipient. In some embodiments, the pharmaceutical composition includes a mixture of antibodies with a plurality of distinct specificities.

In still another aspect, the invention features a pharmaceutical composition including polyclonal antibodies that bind to the any of the polypeptides or fusion polypeptides described herein, or that bind to a mixture of distinct polypeptides of such described polypeptides. In some embodiments, the pharmaceutical composition is for use in the passive immunization of a mammal (e.g., a mammal) against candidiasis or a bacterial infection. Typically, the pharmaceutical composition is administered by intramuscular, subcutaneous, or intradermal administration. In some embodiments, the pharmaceutical composition is administered by intramuscular administration. In some embodiments, the candidiasis is disseminated candidiasis, for example, hematogenously disseminated candidiasis. In other embodiments, the candidiasis is mucosal candidiasis. In some embodiments, candidiasis is caused by *Candida albicans, Candida glabrata, Candida krusei, Candida parapsilosis,* or *Candida tropicalis*. In some embodiments, passive immunization is against *Acinetobacter* or *Staphylococcus*.

In another aspect, the invention features a method of passive immunization of a mammal (e.g., a human) against candidiasis or a bacterial infection such as one caused by *Staphyloccocus* or *Acinetobacter* including administering to the mammal an effective amount of any of the pharmaceutical compositions disclosed herein,

thereby passively immunizing the mammal against the candidiasis. In some embodiments, the pharmaceutical composition is administered by intramuscular, subcutaneous, or intradermal administration. In other embodiments, the pharmaceutical composition is administered by intramuscular administration. In some embodiments, the candidiasis is disseminated candidiasis, for example, hematogenously disseminated candidiasis. In some embodiments, the candidiasis is mucosal candidiasis. In some embodiments, the candidiasis is caused by *Candida albicans, Candida glabrata, Candida krusei, Candida parapsilosis,* or *Candida tropicalis*.

In another aspect, the invention features an isolated polypeptide including a sequence having substantial identity to the amino acid sequence

A-B-X-C-D-E (SEQ ID NO: 31),

wherein A is absent or is SEQ ID NO: 2;
wherein B is absent or is SEQ ID NO: 3;
wherein X is absent or is a linker peptide;
wherein C is absent or is SEQ ID NO: 5;
wherein D is absent or is SEQ ID NO: 6; and
wherein E is absent or is SEQ ID NO: 10,
provided that two or more of A, B, C, D and E are present in the polypeptide.

In some embodiments, the polypeptide is A-B-C-D-E (SEQ ID NO: 32); A-B-X-C-D (SEQ ID NO: 11); A-B-C-D (SEQ ID NO: 12); A-X-C-D-E (SEQ ID NO: 25);A-C-D-E (SEQ ID NO: 26); A-X-C-D (SEQ ID NO: 13); A-C-D (SEQ ID NO: 14); A-X-D-E (SEQ ID NO: 27); A-D-E (SEQ ID NO: 28); A-X-D (SEQ ID NO: 15); or A-D (SEQ ID NO: 16).

In another aspect, the invention features an isolated polypeptide including a sequence having substantial identity to the amino acid sequence

C-D-E-X-A-B (SEQ ID NO: 29),

wherein C is absent or is SEQ ID NO: 5;
wherein D is absent or is SEQ ID NO: 6;
wherein E is absent or is SEQ ID NO: 10;
wherein X is absent or is a linker peptide;

wherein A is absent or is SEQ ID NO: 2;
wherein B is absent or is SEQ ID NO: 3,
provided that two or more of C, D, E, A, and B are present in the polypeptide.

In some embodiments, the polypeptide is C-D-E-A-B (SEQ ID NO: 30); C-D-X-A-B (SEQ ID NO: 17); C-D-A-B (SEQ ID NO: 18); D-X-A-B (SEQ ID NO: 21); D-A-B (SEQ ID NO: 22): D-X-A (SEQ ID NO: 23); or D-A (SEQ ID NO: 24).

In still other aspects, the invention features compositions and methods as disclosed herein that are based, at least in part, on the proposition that an immune response, such as antibodies and other mechanisms, that target the a *Candida* HYR1 polypeptide and confer protection from *Acinetobacter* infection such as *Acinetobacter baumannii*. Active or passive immunization approaches using the a HYR1 polypeptide fragment or an Als3/Hyr1 polypeptide fusion proteins disclosed herein are useful to protect against infections caused by gram negative rod bacteria, including, but not limited to, *Acinetobacter baumannii*. Some uses of the compositions and methods disclosed herein include passive vaccination of acutely at-risk patients with a dose of anti-HYR1 or anti-Als/Hyr1 antibody to prevent the acquisition of *Acinetobacter baumannii* infection. Additionally, patients with active *Acinetobacter baumannii* infection can be treated with the antibody alone or combined with other antibacterial agents. Alternatively, patients who are at risk of developing such infections, such as, for example, military personnel, can be actively vaccinated with Hyr1 or Als3/Hyr1 polypeptides or specific *Acinetobacter baumannii* polypeptides disclosed herein to prevent such infections.

In addition to vaccination of subjects susceptible to *Acinetobacter* or *Candida* infections or both, the vaccine compositions of the present invention can also be used to treat, immunotherapeutically, subjects suffering from a variety of gram negative bacterial infections. Accordingly, vaccines that contain one or more of the polypeptides and/or antibody compositions described herein in combination with adjuvants, can act for the purposes of prophylactic or therapeutic treatment of infections from gram negative

bacteria. In one embodiment, vaccines of the present invention will induce the body's own immune system to seek out and inhibit gram negative bacteria or *Candida* or both.

Vaccines according to the invention refer to a composition that can be administered to an individual to protect the individual against an infectious disease. Vaccines protect against diseases by inducing or increasing an immune response in an animal against the infectious disease. An exemplary infectious disease amenable to treatment with the vaccines of the invention include severe pneumonia, infections of the urinary tract, infections of the bloodstream and infections of other parts of the body. The vaccine-mediated protection can be humoral and/or cell mediated immunity induced in host when a subject is challenged with, for example, or an immunogenic portion of a polypeptide or protein described herein.

Accordingly, in some embodiments, the invention provides a method of treating or preventing an infection from gram negative bacteria in a subject in need thereof by administering a therapeutically effective amount of a pharmaceutical composition as disclosed herein or a vaccine composition as disclosed herein. For example, the invention provides methods of treating or preventing infections caused by one or more gram negative bacteria including bacteria of the *Acinetobacter* genus, such as *A. baumannii, A. iwoffii, A. haemolyticus, A. calcoaceticus, A. johnsonii, A. radioresistens,* and *A. junii,* bacteria of the *Haemophilus* genus, such as *H. aegyptius, H. aphrophilus, H. avium, H. ducreyi, H. felis, H. haemolyticus, H. influenza, H. parainfluenzae, H. paracuniculus, H. parahaemolyticus, H. pittmaniae,* and *H. somnus*, bacteria of the *Bordetella* genus, such as *B. ansorpii, B avium, B. bronchiseptica, B. hinzii, B. holmesii, B. parapertussis, B. pertussis, B. petrii,* and *B. trematum,* bacteria of the *Salmonella* genus, such as *S. typhimurium, S. bongori, S. enterica subsp. enterica, S. enterica subsp. salamae, S. arizonae, S. enterica subsp. diarizonae, S. enterica subsp. houtenae,* and *S. enterica subsp. indica*, bacteria of the *Yersina* genus, such as *Yersina pseudotuber, Y. aldovae, Y. aleksiciae, Y. bercovieri, Y. enterocolitica, Y. frederiksenii, Y. intermedia, Y. kristensenii, Y. mollaretii, Y.*

pestis, Y. pseudotuberculosis, Y. rohdei, and Y. ruckeri, bacteria of the *Escherichia* genus, such as *E. albertii, E. blattae, E. coli, E. fergusonii, E. hermannii* and *E. vulneris*, bacteria of the Pedobacter genus, such as *P. heparinus, P. roseus sp. nov.* and *P. aquatilis sp. nov*, bacteria of the *Pseudomonas* genus, such as *P. aeruginosa, P. alcaligenes, P. mendocina, P. fluorescens, P. monteilii, P. oryzihabitans, P. luteola, P. putida, P. cepacia, P. stutzeri, P. maltophilia, P. putrefaciens, P. mallei* and *P. pseudomallei*, or bacteria of the *Klebsiella* genus, such as *K. pneumoniae, K. planticola K. oxytoca* and *K. rhinoscleromatis.* In other embodiments, *Candida* species as disclosed herein may be treated or prevented.

By "adjuvant" is meant one or more substances that cause stimulation of the immune system. In this context, an adjuvant is used to enhance an immune response to one or more vaccine antigens or antibodies. An adjuvant may be administered to a subject before, in combination with, or after administration of the vaccine or antibody. Examples of chemical compounds used as adjuvants include, but are not limited to, aluminum compounds (e.g., alum, Alhydrogel), oils, block polymers, immune stimulating complexes, vitamins and minerals (e.g., vitamin E, vitamin A, selenium, and vitamin B12), Quil A (saponins), bacterial and fungal cell wall components (e.g., lipopolysaccarides, lipoproteins, and glycoproteins), hormones, cytokines, and co-stimulatory factors.

By "antibody" is meant whole antibodies, immunoglobulins, or any antigen-binding fragment or single chains thereof. Antibodies, as used herein, can be mammalian (e.g., human or mouse), humanized, chimeric, recombinant, synthetically produced, or naturally isolated, and can be, e.g., monoclonal or polyclonal. In most mammals, including humans, whole antibodies have at least two heavy (H) chains and two light (L) chains connected by disulfide bonds. Each heavy chain consists of a heavy chain variable region (abbreviated herein as V_H) and a heavy chain constant region. The heavy chain constant region consists of three domains, C_H1, C_H2, and C_H3 and a hinge region between C_H1 and C_H2. Each light chain consists of a light chain variable region (abbreviated herein as V_L) and a light chain constant region. The light chain constant region consists of

one domain, C_L. The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (Clq) of the classical complement system.

Antibodies of the present invention include all known forms of antibodies and other protein scaffolds with antibody-like properties. For example, the antibody can be a human antibody, a humanized antibody, a bispecific antibody, a chimeric antibody, or a protein scaffold with antibody-like properties, such as fibronectin or ankyrin repeats. The antibody also can be a Fab, Fab'2, scFv, SMIP, diabody, nanobody, aptamers, or a domain antibody. The antibody can have any of the following isotypes: IgG (e.g., IgG1, IgG2, IgG3, and IgG4), IgM, IgA (e.g., IgA1, IgA2, and IgAsec), IgD, or IgE.

The term "antibody fragment," as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen. The antigen-binding function of an antibody can be performed by fragments of a full-length antibody, which include but are not limited to: (i) a Fab fragment, a monovalent fragment consisting of the V_L, V_H, C_L, and C_H1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the V_H and C_H1 domains; (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (v) a dAb including V_H and V_L domains; (vi) a dAb fragment (Ward *et al., Nature* 341:544-546 (1989)), which consists of a V_H domain; (vii) a dAb which consists of a V_H or a V_L domain; (viii) an isolated complementarity determining region (CDR); and (ix) a combination of two or more isolated CDRs which may optionally be joined by a synthetic

linker. Furthermore, although the two domains of the Fv fragment, V_L and V_H, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single chain Fv (scFv); see *e.g.*, Bird *et al.*, *Science* 242:423-426 (1988) and Huston *et al.*, *Proc. Natl. Acad. Sci. USA* 85:5879-5883 (1988)). These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies. Antibody fragments can be produced by recombinant DNA techniques, or by enzymatic or chemical cleavage of intact immunoglobulins.

By "antigen" is meant a molecule to which an antibody can selectively bind. The target antigen may be a protein (e.g., an antigenic peptide), carbohydrate, nucleic acid, lipid, hapten, or other naturally occurring or synthetic compound. The target antigen may be a polypeptide or peptide mimic. An antigen may also be administered to an animal to generate an immune response in the animal.

By "carrier" in the context of a conjugate is meant a moiety or particle, e.g., KLH, CRM197, tetanus toxoid, a phage, a yeast, a virus, a virosome, or a recombinant virus-like particle, that is suitable for being linked to or displaying a polypeptide as described herein.

By "chimeric antibody" is meant an immunoglobulin or antibody whose variable regions derive from a first species and whose constant regions derive from a second species. Chimeric antibodies can be constructed, for example, by genetic engineering, from immunoglobulin gene segments belonging to different species (e.g., from a mouse and a human).

By "chimeric vaccine" is meant a vaccine that includes at least two distinct antigens, e.g., joined covalently. An example of a chimeric vaccine is a composition that includes a polypeptide displayed, e.g., on the surface of a particle such as a phage, virus, yeast, virosome, or recombinant virus-like particle.

By “conjugate” is meant a compound that includes a polypeptide of the invention linked to another moiety or particle, e.g., KLH, CRM197, tetanus toxoid, a phage, a yeast, a virus, a virosome, or a recombinant virus-like particle.

By “conservative substitution” in an amino acid sequence is meant replacement of an amino acid for another within a family of amino acids that are related in the chemical nature of their side chains.

Genetically encoded amino acids can be divided into four families: acidic (aspartate, glutamate); basic (lysine, arginine, histidine); nonpolar (alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan); and uncharged polar (glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine). Phenylalanine, tryptophan, and tyrosine are sometimes grouped as aromatic amino acids. In similar fashion, the amino acids can also be separated into the following groups: acidic (aspartate, glutamate); basic (lysine, arginine, histidine); alipathic (glycine, alanine, valine, leucine, isoleucine, serine, threonine), with serine and threonine optionally grouped separately as alipathic-hydroxyl; aromatic (phenylalanine, tyrosine, tryptophan); amide (asparagine, glutamine); and sulfur-containing (cysteine, methionine).

Whether a change in the amino acid sequence results in a functional variant can be determined by assessing the ability of the variant polypeptide to function in a fashion similar to the wild-type polypeptide using standard methods such as those described herein.

By “diagnostic composition” is meant a composition containing a polypeptide, conjugate, vaccine, or antibody of the invention, formulated for use in conjunction with a diagnostic method.

By “effective amount” in the context of passive immunization using a pharmaceutical composition, e.g., comprising an antibody, is meant the amount of the pharmaceutical composition required to passively immunize in a clinically relevant manner. An effective amount of pharmaceutical composition used to practice the methods of passive immunization described herein varies depending upon the manner of administration, the age, body weight, and general health of the

subject. Ultimately, the prescribers will decide the appropriate amount and dosage regimen.

By “flanking amino acid” is meant an amino acid in a polypeptide sequence that is immediately adjacent to the N- or C-terminus of a particular defined sequence. Desirably, a flanking amino acid is present on the N- and/or C-terminus of the amino acid sequence of SEQ ID NO: 1 or 2 or a fragment thereof; and more desirably, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 flanking amino acids are present at the N- and/or C-terminus of the amino acid sequence of SEQ ID NO: 1 or 2, or fragment thereof.

By “fusion protein” is meant a protein that includes a polypeptide of the invention, e.g., a peptide fragment or variant, and a fusion partner.

By “fusion partner” is meant a heterologous sequence that can be fused to a polypeptide or peptide of the invention , e.g., one or more of Peptide 3-11 or variants thereof. Examples of fusion partners are described herein and include detection markers, stabilizing domains, sequences which aid in production or purification of the protein, or domains which increase the antigenicity of the polypeptide.

By “immunogenic” is meant any substance that is capable of inducing an immune response in a subject.

By “immunogenic amount” in the context of a vaccine is meant an amount of the vaccine required to induce an immune response in a subject in a clinically relevant manner. An immunogenic amount of vaccine used to practice the methods of vaccination as described herein varies depending upon the manner of administration, the age, body weight, and general health of the subject. Ultimately, the prescribers will decide the appropriate amount and dosage regimen.

By “isolated” or “purified” is meant separated from other naturally accompanying components. Typically, a compound (e.g., nucleic acid, polypeptide, antibody, or small molecule) is substantially isolated when it is at least 60%, by weight, free from the proteins and/or naturally occurring organic molecules with which it is naturally associated. The definition also extends, e.g., to a polypeptide or nucleic acid molecule separated from its flanking

sequences (e.g., for an amino acid sequence, isolated refers to a sequence that is free from the flanking amino acids with which the sequence is naturally associated in a polypeptide). In some instances, the compound is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight, isolated. An isolated compound, e.g., polypeptide, may be obtained by standard techniques, for example, by extraction from a natural source (e.g., purification from a cell infected with *Candida*); by expression of a recombinant nucleic acid encoding an Als3 or CNA fragment or variant, or a fusion protein thereof; or by chemically synthesizing the polypeptide. Purity can be measured by any appropriate method, e.g., by column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

By "linked to" or "conjugated to" in the context of a conjugate is meant a covalent or non-covalent interaction between the polypeptide and the carrier or fusion partner. Non-covalent interactions include, but are not limited to, hydrogen bonding, ionic interactions among charged groups, electrostatic binding, van der Waals interactions, hydrophobic interactions among non-polar groups, lipophobic interactions, and LogP-based attractions.

By "linker" as used herein is meant an amino acid sequence of one or more amino acids in length, e.g., that is not cleavable, for example, by auto-cleavage, enzymatic, or chemical cleavage. The linker can include nonpolar, polar, and/or charged amino acids. In some embodiments, linkers include or consist of flexible portions, e.g., regions without significant fixed secondary or tertiary structure. Exemplary flexible linkers are glycine-rich linkers, e.g., containing at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or even 100% glycine residues. Linkers may also contain, e.g., serine residues. In some cases, the amino acid sequence of linkers consists only of glycine and serine residues. A linker can be, for example, 1 to 100 amino acids in length, for example, 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acids in length.

By "monoclonal antibody" is meant an antibody obtained from a population of substantially homogeneous antibodies, i.e., the

individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. Monoclonal antibodies can be prepared using any art recognized technique and those described herein such as, for example, a hybridoma method, as described by Kohler *et al.*, *Nature* 256:495 (1975), a transgenic animal (*e.g.*, Lonberg *et al.*, *Nature* 368(6474):856-859 (1994)), recombinant DNA methods (*e.g.*, U.S. Pat. No. 4,816,567), or using phage, yeast, or synthetic scaffold antibody libraries using the techniques described in, for example, Clackson *et al.*, *Nature* 352:624-628 (1991) and Marks *et al.*, *J. Mol. Biol.* 222:581-597 (1991).

By "nucleic acid molecule" is meant a molecule, e.g., RNA or DNA, having a sequence of two or more covalently bonded, naturally occurring or modified nucleotides. The nucleic acid molecule may be, e.g., single or double stranded, and may include modified or unmodified nucleotides, or mixtures or combinations thereof. Various salts, mixed salts, and free acid forms are also included.

By "patient" or "subject" is meant a mammal, including, but not limited to, a human or non-human mammal, such as a bovine, equine, canine, ovine, or feline.

The terms "peptide," "polypeptide," and "protein" are used interchangeably and refer to any chain of two or more natural or unnatural amino acids, regardless of post-translational modification (e.g., glycosylation or phosphorylation), constituting all or part of a naturally-occurring or non-naturally occurring polypeptide or peptide, as is described herein. Such polypeptides typically are continuous and unbranched peptide. A peptide is a short polymer of amino acid monomers. "Proteins" are intended to include one or more polypeptides arranged in a biologically functional way. The amino acids comprising the polypeptides of the invention may be linked by

peptide bonds or other bonds, for example, ester or ether bonds. The amino acids comprising the polypeptides of the invention can include non-genetically coded amino acids that either occur naturally or are chemically synthesized.

A polypeptide of the invention can also encompass one or more conservative substitutions. Conservative substitutions of encoded amino acids include, for example, amino acids that belong within the following groups: (1) non-polar amino acids (Gly, Ala, Val, Leu, and Ile); (2) polar neutral amino acids (Cys, Met, Ser, Thr, Asn, and Gln); (3) polar acidic amino acids (Asp and Glu); (4) polar basic amino acids (Lys, Arg and His); and (5) aromatic amino acids (Phe, Trp, Tyr, and His). Other minor modifications are also included within polypeptides of the invention so long as the polypeptide retains some or all of its function as described herein.

The invention polypeptides can also include derivatives, analogues and functional mimetics thereof, provided that such polypeptide retains some or all of its function as disclosed herein. For example, derivatives can include chemical modifications of the polypeptide such as alkylation, acylation, carbamylation, iodination, or any modification that derivatizes the polypeptide. Such derivatized molecules include, for example, those molecules in which free amino groups have been derivatized to form amine hydrochlorides, p-toluene sulfonyl groups, carbobenzoxy groups, t-butyloxycarbonyl groups, chloroacetyl groups or formyl groups. Free carboxyl groups can be derivatized to form salts, methyl and ethyl esters or other types of esters or hydrazides. Free hydroxyl groups can be derivatized to form O-acyl or O-alkyl derivatives. The imidazole nitrogen of histidine can be derivatized to form N-im-benzylhistidine. Also included as derivatives or analogues are those peptides which contain one or more naturally occurring amino acid derivatives of the twenty standard amino acids, for example, 4-hydroxyproline, 5-hydroxylysine, 3-methylhistidine, homoserine, ornithine or carboxyglutamate, and can include amino acids that are not linked by peptide bonds. Polypeptides of the present invention also include any polypeptide having one or more additions and/or deletions of residues,

relative to the sequence of a polypeptide whose sequence is shown herein, so long as immunogenic activity as disclosed herein is maintained.

The invention polypeptides can be isolated by a variety of methods well-known in the art, for example, recombinant expression systems, precipitation, gel filtration, ion-exchange, reverse-phase and affinity chromatography, and the like. Other well-known methods are described in Deutscher et al., *Guide to Protein Purification: Methods in Enzymology* Vol. 182, (Academic Press, (1990)). Alternatively, the isolated polypeptides of the present invention can be obtained using well-known recombinant methods (see, for example, Ausubel et al., "Immunology," *Short Protocols in Molecular Biology, John Wiley & Sons, Inc*. Chapter 11. Page 11.1-11.29 (1999); Sambrook and Russell, "Molecular Cloning: A Laboratory Manual," *Cold Spring Harbor Laboratory* (2001)). The methods and conditions for biochemical purification of a polypeptide of the invention can be chosen by those skilled in the art, and purification monitored, for example, by an immunological assay or a functional assay.

An example of the means for preparing an invention polypeptide is to express nucleic acids encoding a polypeptide of the invention in a suitable host cell, such as a bacterial cell, a yeast cell, an amphibian cell such as an oocyte, or a mammalian cell, using methods well known in the art, and recovering the expressed polypeptide, again using well-known purification methods, so described herein. Invention polypeptides can be isolated directly from cells that have been transformed with expression vectors as described herein. The invention polypeptides can also be produced by chemical synthesis. Methods for chemically synthesizing polypeptides are well known in the art and are commercially available.

Recombinantly expressed polypeptides of the invention can also be expressed as fusion proteins with appropriate fusion partners. An appropriate fusion partner can be an amino acid sequence that is not normally connected to the amino acid sequence such as an heterologous sequence, which serves a particular function or provides additional characteristic to the polypeptides of the invention. Non-limiting

examples of suitable heterologous sequences include a detectable marker, a stabilizing domain, a carrier protein for the generation of antibodies, a linker sequence and a sequence that aids in the purification of the polypeptide. Sequences that can aid in the purification of the invention polypeptides include affinity tags, such as glutathione S transferase (GST) or poly His. Thus, in some aspects, the invention provide a fusion protein having a polypeptide as disclosed herein fused to a heterologous sequence, a carrier protein, an affinity tag or a linker sequence or other polypeptides as disclosed herein.

As used herein, a natural amino acid is a natural α-amino acid having the L-configuration, such as those normally occurring in natural polypeptides. Unnatural amino acid refers to an amino acid that normally does not occur in polypeptides, e.g., an epimer of a natural α-amino acid having the L configuration, that is to say an amino acid having the unnatural D-configuration; or a (D,L)-isomeric mixture thereof; or a homolog of such an amino acid, for example, a β-amino acid, an α,α-disubstituted amino acid, or an α-amino acid wherein the amino acid side chain has been shortened by one or two methylene groups or lengthened to up to 10 carbon atoms, such as an α-amino alkanoic acid with 5 up to and including 10 carbon atoms in a linear chain, an unsubstituted or substituted aromatic (α-aryl or α-aryl lower alkyl), for example, a substituted phenylalanine or phenylglycine.

The terms “pharmaceutically acceptable carrier” and “pharmaceutically acceptable excipient” are used interchangeably and mean a carrier or excipient that is physiologically acceptable to the treated patient while retaining the therapeutic properties of the compound with which it is administered. One exemplary pharmaceutically acceptable carrier substance is physiological saline. Other physiologically acceptable carriers and their formulations are known to those skilled in the art and described, for example, in Remington’s Pharmaceutical Sciences, (20^{th} edition), ed. A. Gennaro, 2000, Lippincott, Williams & Wilkins, Philadelphia, PA.

By “pharmaceutical composition” is meant a composition containing a polypeptide, conjugate, vaccine, or antibody of the invention, formulated with a pharmaceutically acceptable excipient, and manufactured or sold with the approval of a governmental regulatory agency as part of a therapeutic regimen for the treatment or prevention of a disease or event in a mammal. Pharmaceutical compositions can be formulated, for example, for intravenous administration (e.g., as a sterile solution free of particulate emboli and in a solvent system suitable for intravenous use), for oral administration (e.g., a tablet, capsule, caplet, gelcap, or syrup), or any other formulation described herein, e.g., in unit dosage form.

By “specifically binds” is meant the preferential association of a binding moiety (e.g., an antibody, antibody fragment, receptor, ligand, or small molecule portion of an agent as described herein) to a target molecule (e.g., a polypeptide or conjugate including same) or to a cell or tissue bearing the target molecule (e.g., a cell surface antigen, such as a receptor or ligand) and not to non-target molecules, cells, or tissues lacking the target molecule. It is recognized that a certain degree of non-specific interaction may occur between a binding moiety and a non-target molecule (present alone or in combination with a cell or tissue). Nevertheless, specific binding may be distinguished as mediated through specific recognition of the target molecule. Specific binding results in a stronger association between the binding moiety (e.g., an antibody) and the target molecule (e.g., a polypeptide or conjugate including same) than between the binding moiety and, e.g., non-target molecules or other compositions lacking the target molecule. Specific binding typically results in greater than 2-fold, preferably greater than 5-fold, more preferably greater than 10-fold and most preferably greater than 100-fold increase in amount of bound binding moiety (per unit time) to e.g., a cell or tissue bearing the target molecule or marker as compared to a cell or tissue lacking that target molecule or marker. Binding moieties bind to the target molecule or marker with a dissociation constant of e.g., less than 10^{-6}M, less than 10^{-7}M, 10^{-8}M, 10^{-9}M, 10^{-10}M, 10^{-11}M, or 10^{-12}M, or even less than 10^{-13}M, 10^{-14}M, or 10^{-15}M. Specific

binding to a protein under such conditions requires a binding moiety that is selected for its specificity for that particular protein. A variety of assay formats are appropriate for selecting binding moieties (e.g., antibodies) capable of specifically binding to a particular target molecule. For example, solid-phase ELISA immunoassays are routinely used to select monoclonal antibodies specifically immunoreactive with a protein. See Harlow & Lane, Antibodies, A Laboratory Manual, Cold Spring Harbor Publications, New York (1988), for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity.

By "substantially identical" is meant an amino acid sequence or nucleic acid sequence that exhibits at least 50% identity to a reference sequence. Such a sequence is generally at least, e.g., 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical at the amino acid level or nucleic acid level to a reference sequence. In general, for polypeptides, the length of comparison sequences can be at least five amino acids, e.g., 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 125, 150, 175, 200, 250, 300, or more amino acids, up to the entire length of the polypeptide. For nucleic acids, the length of comparison sequences can generally be at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 125, 150, 175, 200, 250, 300, 400, 500, 600, 700, 800, 900, or more nucleotides, up to the entire length of the nucleic acid molecule. It is understood that for the purposes of determining sequence identity when comparing a DNA sequence to an RNA sequence, a thymine nucleotide is equivalent to a uracil nucleotide.

As used herein, when a polypeptide or nucleic acid sequence is referred to as having "at least X% sequence identity" to a reference sequence, it is meant that at least X percent of the amino acids or nucleotides in the polypeptide or nucleic acid are identical to those of the reference sequence when the sequences are optimally aligned. An optimal alignment of sequences can be determined in various ways that are within the skill in the art, for instance, the Smith Waterman alignment algorithm (Smith et al., J. Mol. Biol. 147:195-7, 1981) and BLAST (Basic Local Alignment Search Tool; Altschul et al., J. Mol. Biol. 215: 403-10, 1990). These and other alignment algorithms are

accessible using publicly available computer software such as "Best Fit" (Smith and Waterman, Advances in Applied Mathematics, 482-489, 1981) as incorporated into GeneMatcher PlusTM (Schwarz and Dayhof, Atlas of Protein Sequence and Structure, Dayhoff, M.O., Ed pp 353-358, 1979), BLAST, BLAST-2, BLAST-P, BLAST-N, BLAST-X, WU-BLAST-2, ALIGN, ALIGN-2, CLUSTAL, or Megalign (DNASTAR). In addition, those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve optimal alignment over the length of the sequences being compared.

By "*Staphylococcus aureus* skin or soft tissue infection", "*Staphylococcus aureus* SSTI", "*Staphylococcus aureus* skin/skin structure infection", and "*Staphylococcus aureus* SSSI" are used interchangeably herein and refer to a skin or soft tissue infection (e.g. cellulitis, soft tissue abscess, dermonecrosis, myositis, or other infections) resulting from *S. aureus* entering the body at a site where a cut, scrape, bite, or other wound has broken the skin. In some instances, *S. aureus* SSSI is the result of *S. aureus* living on the body, and may occur spontaneously in the absence of a visible site of skin injury or wound. Such infections may affect the layers of the skin or deeper tissues, such as muscle and connective tissue (the interlacing framework of tissue that forms ligaments, tendons, and other supporting structures of the body). Skin abscesses may also occur in areas of the skin where the body has been fighting a *S. aureus* infection. The more important strains of *S. aureus* responsible for skin or soft tissue infections are the antibiotic-resistant *Staphylococcus* known as methicillin-resistant *Staphylococcus aureus* (MRSA); vancomycin-resistant and daptomycin-resistant strains of *S. aureus* may also cause SSSI. MRSA is resistant to commonplace antibiotics. *Staphylococcus aureus* SSSIs may also be caused by methicillin-sensitive *Staphylococcus aureus* (MSSA).

Mammals which are at risk of developing a *S. aureus* skin or soft tissue infection can be treated in a prophylactic mode. Alternatively, mammals may be treated when presenting with symptoms of a *S. aureus* skin or soft tissue infection. Vaccination as described herein will reduce the severity, delay, or prevent the development of symptoms.

Mammals are at elevated risk of infection if they are hospitalized or living in an institutionalized community, antibiotic treated, or immunosuppressed including children having HIV/AIDS or other diseases that compromise immune function, individuals having frequent contact with the healthcare system, having a chronic illness such as diabetes, cancer, HIV/AIDS, being very young or very old, frequent use of antibiotics, having an open wound, dermatitis or skin lesions, poor nutrition or poor hygiene. Other mammals at risk include those living in crowded living conditions, military personnel, especially deployed troops, athletes, and prison inmates. Still others at risk of developing a *S. aureus* skin or soft tissue infection are those individuals previously having such infections or individuals scheduled for or having had a surgical or invasive medical procedure.

A "target molecule" or "target cell" is meant a molecule (e.g., a polypeptide, epitope, antigen, receptor, or ligand) or cell to which a binding moiety (e.g., an antibody) can specifically bind. In some instances, target molecules are exposed on the exterior of a target cell (e.g., a cell surface or secreted protein), but target molecules may alternately or also be present in the interior of a target cell.

By "treating" or "treatment" is meant the medical management of a patient with the intent to cure, ameliorate, stabilize, reduce the likelihood of, or prevent a disease, pathological condition, disorder, or event, by administering a pharmaceutical composition. This term includes active treatment, that is, treatment directed specifically toward the improvement or associated with the cure of a disease, pathological condition, disorder, or event, and also includes causal treatment, that is, treatment directed toward removal of the cause of the associated disease, pathological condition, disorder, or event. In addition, this term includes palliative treatment, that is, treatment designed for the relief of symptoms rather than the curing of the disease, pathological condition, disorder, or event; symptomatic treatment, that is, treatment directed toward constitutional symptoms of the associated disease, pathological condition, disorder, or event; preventative treatment, that is, treatment directed to minimizing or partially or completely inhibiting

the development of the associated disease, pathological condition, disorder, or event, e.g., in a patient who is not yet ill, but who is susceptible to, or otherwise at risk of, a particular disease, pathological condition, disorder, or event; and supportive treatment, that is, treatment employed to supplement another specific therapy directed toward the improvement of the associated disease, pathological condition, disorder, or event.

By "vaccine," as used herein, is meant a composition that elicits an immune response in a subject to which it is administered.

By "vaccinate," as used herein, is meant to treat a patient by administering a vaccine, e.g., to prevent or ameliorate a disease, pathological condition, disorder, or event.

By "variant" in the context of a polypeptide or portion thereof as described herein, or a nucleic acid molecule encoding same, is meant to include substitutions or alterations in the amino acid sequence or nucleic acid sequence, e.g., resulting in a substantially identical sequence. A polypeptide having a variant sequence may maintain at least one biological activity of the original polypeptide, e.g., immunogenic activity. The term "variant" includes, e.g., amino acid insertional derivatives such as amino and/or carboxylterminal fusions, as well as intrasequence insertions of single or multiple amino acids. Insertional amino acid variants are those in which one or more amino acid residues are introduced into a predetermined site in the protein. Random insertion is also possible with suitable screening of the resulting product. Deletional variants are characterized by removal of one or more amino acids from the sequence. Substitutional amino acid variants are those in which at least one residue inserted in its place. Where the protein is derivatized by amino acid substitution, amino acids are generally replaced by conservative substitutions, e.g., other amino acids having similar physical chemical properties such as hydrophobicity, hydrophilicity, electronegativity, bulky sidechains and the like.

For purposes of the present invention, variants also include single or multiple substitutions, deletions and/or additions of any component(s) naturally or artificially associated with the portion of

a naturally occurring protein from which the polypeptide may be derived, such as carbohydrate, lipid and/or other proteinaceous moieties. All such molecules are encompassed by the term "variant."

By "variant sequence" is meant the amino acid or nucleic acid sequence of a variant as defined herein.

Other features and advantages of the invention will be apparent from the following Detailed Description and the claims.

DETAILED DESCRIPTION OF THE INVENTION

The identification of the Als3 and Hyr1 polypeptide fragments and Als3/Hyr1 fusion polypeptides and other compositions described herein allow, e.g., for the effective treatment of and vaccination against candidiasis or bacterial infections such as those caused by any of those disclosed herein.

The invention provides polypeptides, e.g., derived from Als3 or Hyr1 or Als3/Hyr1 fusion polypeptides, conjugates, vaccines, antibodies, compositions, methods of vaccination using same, and methods of production of same, as described in further detail below.

Polypeptides

The invention features polypeptides derived from Als3 or Hyr1. The amino acid sequence of rAls3 protein is as follows:

```
1    KTITGVFNSFNSLTWSNAAT  YNYKGPGTPTWNAVLGWSLD  GTSASPGDTFTLNMPCVFKF
61   TTSQTSVDLTAHGVKYATCQ  FQAGEEFMTFSTLTCTVSNT  LTPSIKALGTVTLPLAFNVG
121  GTGSSVDLEDSKCFTAGTNT  VTFNDGGKKISINVDFERSN  VDPKGYLTDSRVIPSLNKVS
181  TLFVAPQCANGYTSGTMGFA  NTYGDVQIDCSNIHVGITKG  LNDWNYPVSSESFSYTKTCS
241  SNGIFITYKNVPAGYRPFVD  AYISATDVNSYTLSYANEYT  CAGGYWQRAPFTLRWTGYRN
301  SDAGSNGIVIVATTRTVTDS  TTAVTTLPFDPNRDKTKTIE  ILKPIPTTTITTSYVGVTTS
361  YSTKTAPIGETATVIVDIPY  HTTTTVTSKWTGTITSTTTH  TNPTDSIDTVIVQVP
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The amino acid sequence of the rHyr1 protein is as follows:

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1    TSRIDRGGIQGFHGDVKVHS GATWAILGTTLCSFFGGLEV EKGASLFIKSDNGPVLALNV
61   ALSTLVRPVINNGVISLNSK SSTSFSNFDIGGSSFTNNGE IYLDSSGLVKSTAYLYAREW
121  TNNGLIVAYQNQKAAGNIAF GTAYQTITNNGQICLRHQDF VPATKIKGTGCVTADEDTWI
181  KLGNTILSVEPTHNFYLKDS KSSLIVHAVSSNQTFTVHGF GNGNKLGLTLPLTGNRDHFR
241  FEYYPDTGILQLRADALPQY FKIGKGYDSKLFRIVNSRGL KNAVTYDGPVPNNEIPAVCL
301  IPCTNGPSAPESESDLNTPT TSSIETSSYSSAATESSVVS ESSSAVDSLTSSSLSSKSES
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361 SDVVSSTTNIESSSTAIETT MNSESSTDAGSSSISQSESS STAITSSSETSSSESMSASS
401 TTASNTSIETDSGIVSQSES SSNAL

The invention features polypeptides having substantial identity to any of the polypeptides described herein, including the following.

Als3

A= KTITGVFNSFNSLTWSNAAT YNYKGPGTPTWNAVLGWSLD GTSASPGDTFTLNMPCVFKF
TTSQTSVDLTAHGVKYATCQ FQAGEEFMTFSTLTCTVSNT LTPSIKALGTVTLPLAFNVG
GTGSSVDLEDSKCFTAGTNT VTFNDGGKKISINVDFERSN VDPKGYLTDSRVIPSLNKVS
TLFVAPQCANGYTSGTMGFA NTYGDVQIDCSNIHVGITKG LNDWNYPVSSESFSYTKTCS
SNGIFITYKNVPAGYRPFVD AYISATDVNSYTLSYANEYT CAGGYWQRAPFTLRWTGYRN
SDAGSNG (SEQ ID NO: 2).

B= IVIVATTRTVTDS TTAVTTLPFDPNRDKTKTIE ILKPIPTTTITTSYVGVTTS
YSTKTAPIGETATVIVDIPY HTTTTVTSKWTGTITSTTTH TNPTDSIDTVIVQVP (SEQ ID
NO: 3)

Hyr1

C= TSRIDRGGIQGFHGDVKVHS GATWAILGTTLCSFFGGLEV EKGASLFIKSDNGPVLALNV
ALSTLVRPVINNGVISLNSK SSTSFSNFDIGGSSFTNNGE IYLDSSGLVKSTAYLYAREW
TNNGLIVAY (SEQ ID NO: 5)

D= QNQKAAGNIAF GTAYQTITNNGQICLRHQDF VPATKIKGTGCVTADEDTWI
KLGNTILSVEPTHNFYLKDS KSSLIVHAVSSNQTFTVHGF GNGNKLGLTLPLTGNRDHFR
FEYYPDTGILQLRADALPQY FKIGKGYDSKLFRIVNSRGL KNAVTYDGPVPNNEIPAVCL
IPCTNGPSAPESESDLNTPT TSSIET (SEQ ID NO: 6)

X= is present or absent (-X), wherein X is a linker peptide.

E1=	A-B-X-C-D (SEQ ID NO: 11)	E1(-X)=	A-B-C-D (SEQ ID NO: 12)
E2=	A-X-C-D (SEQ ID NO: 13)	E2(-X)=	A-C-D (SEQ ID NO: 14)
E3=	A-X-D (SEQ ID NO: 15)	E3(-X)=	A-D (SEQ ID NO: 16)
E4=	C-D-X-A-B (SEQ ID NO: 17)	E4(-X)=	C-D-A-B (SEQ ID NO: 18)
E5=	C-D-X-A (SEQ ID NO: 19)	E5(-X)=	C-D-A (SEQ ID NO: 20)
E6=	D-X-A-B (SEQ ID NO: 21)	E6(-X)=	D-A-B (SEQ ID NO: 22)
E7=	D-X-A (SEQ ID NO: 23)	E7(-X)=	D-A (SEQ ID NO: 24)

Als3

A= KTITGVFNSFNSLTWSNAAT YNYKGPGTPTWNAVLGWSLD GTSASPGDTFTLNMPCVFKF
TTSQTSVDLTAHGVKYATCQ FQAGEEFMTFSTLTCTVSNT LTPSIKALGTVTLPLAFNVG
GTGSSVDLEDSKCFTAGTNT VTFNDGGKKISINVDFERSN VDPKGYLTDSRVIPSLNKVS
TLFVAPQCANGYTSGTMGFA NTYGDVQIDCSNIHVGITKG LNDWNYPVSSESFSYTKTCS
SNGIFITYKNVPAGYRPFVD AYISATDVNSYTLSYANEYT CAGGYWQRAPFTLRWTGYRN
SDAGSNG (SEQ ID NO: 2)

B= IVIVATTRTVTDS TTAVTTLPFDPNRDKTKTIE ILKPIPTTTITTSYVGVTTS
YSTKTAPIGETATVIVDIPY HTTTTVTSKWTGTITSTTTH TNPTDSIDTVIVQVP (SEQ ID NO 3)

Hyr1

C= TSRIDRGGIQGFHGDVKVHS GATWAILGTTLCSFFGGLEV EKGASLFIKSDNGPVLALNV
ALSTLVRPVINNGVISLNSK SSTSFSNFDIGGSSFTNNGE IYLDSSGLVKSTAYLYAREW
TNNGLIVAY (SEQ ID NO: 5)

D= QNQKAAGNIAF GTAYQTITNNGQICLRHQDF VPATKIKGTGCVTADEDTWI
KLGNTILSVEPTHNFYLKDS KSSLIVHAVSSNQTFTVHGF GNGNKLGLTLPLTGNRDHFR
FEYYPDTGILQLRADALPQY FKIGKGYDSKLFRIVNSRGL KNAVTYDGPVPNNEIPAVCL
IPCTNGPSAPESESDLNTPT TSSIET (SEQ ID NO: 6)

E= S SYSSAATESSVVS ESSSAVDSLTSSSLSSKSES
SDVVSSTTNIESSSTAIETT MNSESSTDAGSSSISQSESS STAITSSSETSSSESMSASS
TTASNTSIETDSGIVSQSES SSNAL (SEQ ID NO: 10)

X= is present or absent (-X), wherein X is a linker peptide.

S1=	A-B-X-C-D (SEQ ID NO: 11)	S1(-X)=	A-B-C-D (SEQ ID NO: 12)
S2=	A-X-C-D-E (SEQ ID NO: 25)	S2(-X)=	A-C-D-E ((SEQ ID NO: 26)
S3=	A-X-D-E (SEQ ID NO: 27)	S3(-X)=	A-D-E (SEQ ID NO: 28)
S4=	C-D-E-X-A-B (SEQ ID NO: 29)	S4(-X)=	C-D-E-A-B (SEQ ID NO: 30)
S5=	C-D-X-A-B (SEQ ID NO: 17)	S5(-X)=	C-D-A-B (SEQ ID NO: 18)
S6=	D-X-A-B (SEQ ID NO: 21)	S6(-X)=	D-A-B (SEQ ID NO: 22)
S7=	D-X-A (SEQ ID NO: 23)	S7(-X)=	D-A (SEQ ID NO: 24)

In some instances, a modification to a polypeptide as described herein does not substantially reduce the biological activity, e.g., immunogenic activity, of the polypeptide. The modified polypeptide may have or may optimize a characteristic of a polypeptide, such as *in vivo* stability, bioavailability, toxicity, immunological activity, immunological identity, or conjugation properties.

Modifications include those by natural processes, such as posttranslational processing, or by chemical modification techniques known in the art. Modifications may occur anywhere in a polypeptide including the polypeptide backbone, the amino acid side chains, and the amino- or carboxy-terminus. The same type of modification may be present in the same or varying degrees at several sites in a given polypeptide, and a polypeptide may contain more than one type of modification.

A variant or otherwise modified polypeptide can also include one or more amino acid insertions, deletions, or substitutions, either conservative or non-conservative (e.g., D-amino acids, desamino acids) in the polypeptide sequence. For example, the addition of one or more cysteine residues to the amino or carboxy terminus of any of the polypeptides of the invention can facilitate conjugation of these polypeptides. Exemplary polypeptides having an N- or C-terminal cysteine.

Amino acid substitutions can be conservative (i.e., wherein a residue is replaced by another of the same general type or group) or non-conservative (i.e., wherein a residue is replaced by an amino acid of another type). In addition, a non-naturally occurring amino acid can be substituted for a naturally occurring amino acid (i.e., non-naturally occurring conservative amino acid substitution or a non-naturally occurring non-conservative amino acid substitution).

Polypeptides made synthetically, e.g., using methods known in the art, can include substitutions of amino acids not naturally encoded by DNA (e.g., non-naturally occurring or unnatural amino acid). Examples of non-naturally occurring amino acids include D-amino acids, an amino acid having an acetylaminomethyl group attached to a sulfur atom of a cysteine, a pegylated amino acid, the omega amino acids of the formula

$NH_2(CH_2)_nCOOH$ wherein n is 2-6, neutral nonpolar amino acids, such as sarcosine, t-butyl alanine, t-butyl glycine, N-methyl isoleucine, and norleucine. Phenylglycine may substitute for Trp, Tyr, or Phe; citrulline and methionine sulfoxide are neutral nonpolar, cysteic acid is acidic, and ornithine is basic. Proline may be substituted with hydroxyproline and retain the conformation conferring properties.

Variants may be generated by substitutional mutagenesis and retain or even increase the biological activity, e.g., immunogenic activity, of the original polypeptide.

The polypeptides described herein can be obtained, e.g., by chemical synthesis using a commercially available automated peptide synthesizer. The synthesized protein or polypeptide can be precipitated and further purified, for example by high performance liquid chromatography (HPLC). Alternatively, the proteins and polypeptides can be obtained by recombinant methods, e.g., that are well-known in the art.

Conjugates

Polypeptides of the invention may be conjugated to another moiety or particle.

Protein moieties

In some instances, it may be useful to conjugate the polypeptide to a protein that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin (KLH), CRM197, tetanus toxoid, diptheria toxoid, serum albumin, bovine thyroglobulin, soybean trypsin inhibitor, or a polycation (poly-L-Lysine or poly-L-arginine), e.g., using a bifunctional or derivatizing agent as known in the art, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, or succinic anhydride.

In some instances, the conjugate may be a recombinant fusion protein, e.g., to facilitate expression and purification of the polypeptide.

Particles for Conjugation or Display of Polypeptides

In some instances, polypeptides are conjugated to or displayed on a particle, e.g., a phage, a yeast, a virus, a virosome, or a recombinant virus-like particle.

For example, one or more polypeptides may be conjugated to a phage, a yeast, or a virus particle, e.g., to the surface of the particle. In one embodiment, a nucleic acid molecule encoding the polypeptide is inserted into the phage, yeast, or virus particle, resulting in expression of the polypeptide in the phage, yeast, or virus, e.g., at the surface of the particle. The phage, yeast, or virus population containing the polypeptide may then be isolated and prepared, e.g., as a vaccine, by adding a pharmaceutically acceptable excipient.

In some embodiments, polypeptides as described herein are conjugated to a virosome or virus-like particle (VLP). Virosomes and VLPs generally contain one or more proteins from a virus optionally combined or formulated with a phospholipid. They are generally non-pathogenic, non-replicating and generally do not contain any of the native viral genome. The viral proteins may be recombinantly produced or isolated from whole viruses. Viral proteins suitable for use in virosomes or VLPs include proteins derived from influenza virus (such as HA or NA), Hepatitis B virus (such as core or capsid proteins), Hepatitis E virus, measles virus, Sindbis virus, Rotavirus, Foot-and-Mouth Disease virus, Retrovirus, Norwalk virus, human Papilloma virus, HIV, RNA-phages, Qβ-phage (such as coat proteins), GA-phage, fr-phage, AP205 phage, and Ty (such as retrotransposon Ty protein p 1). Virosomes are discussed further in, e.g., Gluck et al. (2002), *Vaccine* 20:B10-B16, which is incorporated by reference in its entirety.

VLPs are discussed further, e.g., in Niikura et al. (2002), *Virology* 293:273-280; Lenz et al. (2001), *J Immunol* 166:5346-5355; Pinto et al. (2003), *J Infect Dis* 188:327-338; Gerber et al. (2001), *Viral* 75:4752-4760; WO03/024480; and WO03/024481, each of which is incorporated by reference in its entirety.

Antibodies

The invention features monoclonal and polyclonal antibodies that bind to the polypeptides or conjugates described herein.

Monoclonal Antibodies

Monoclonal antibodies may be made, e.g., using the hybridoma method first described by Kohler et al., *Nature* 256:495, 1975, or may be made by recombinant DNA methods (see, e.g., U.S. Patent No. 4,816,567). In the hybridoma method, a mouse or other appropriate host animal, such as a hamster or macaque monkey, is immunized, e.g., using a polypeptide or conjugate described herein, to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the polypeptide or conjugate used for immunization. Alternatively, lymphocytes may be immunized *in vitro*. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, Monoclonal Antibodies: Principles and Practice, pp. 59-103, Academic Press, 1986).

The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that can contain one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

Exemplary myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these, particular myeloma cell lines that may be considered for use are murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, CA, USA, and SP-2 or X63-Ag8-653 cells available from the American Type Culture Collection, Manassas, VA, USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies

(Kozbor, *J. Immunol.* 133:3001, 1984; Brodeur et al., Monoclonal Antibody Production Techniques and Applications, pp. 51-63, Marcel Dekker, Inc., New York, 1987).

Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. The binding specificity of monoclonal antibodies produced by hybridoma cells can be determined by immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA).

After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, Monoclonal Antibodies: Principles and Practice, pp. 59-103, Academic Press, 1986). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown *in vivo* as ascites tumors in an animal.

The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the monoclonal antibodies). The hybridoma cells serve as a source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Recombinant production of antibodies will be described in more detail below.

In a further embodiment, antibodies or antibody fragments can be isolated from antibody phage libraries generated using the techniques

described, for example, in McCafferty et al., *Nature* 348:552-554, 1990.

Clackson et al., *Nature* 352:624-628, 1991 and Marks et al., *J. Mol. Biol.* 222:581-597, 1991, describe the isolation of murine and human antibodies, respectively, using phage libraries. Subsequent publications describe the production of high affinity (nM range) human antibodies by chain shuffling (Marks et al., *Bio/Technology* 10:779-783, 1992), as well as combinatorial infection and in vivo recombination as a strategy for constructing very large phage libraries (Waterhouse et al., *Nucl. Acids. Res.* 21:2265-2266, 1993). Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies.

The DNA also may be modified, for example, by substituting the coding sequence for human heavy- and light-chain constant domains in place of the homologous murine sequences (U.S. Patent No. 4,816,567; Morrison et al., *Proc. Natl. Acad. Sci. U.S.A.* 81:6851, 1984), or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide.

Typically, such non-immunoglobulin polypeptides are substituted for the constant domains of an antibody, or they are substituted for the variable domains of one antigen-combining site of an antibody to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for an antigen and another antigen-combining site having specificity for a different antigen.

Polyclonal Antibodies

Polyclonal antibodies are typically raised in animals by multiple injections, e.g., subcutaneous or intraperitoneal injections, of the relevant antigen and an adjuvant. In some instances, it may be useful to conjugate the polypeptide to a protein that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin (KLH), CRM197, tetanus toxoid, diptheria toxoid, serum albumin, bovine thyroglobulin, soybean trypsin inhibitor, or a polycation (poly-L-Lysine or poly-L-arginine), e.g., using a bifunctional or derivatizing agent as known in the art, for example, maleimidobenzoyl

sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, or succinic anhydride.

Vaccines and Antibody-Containing Pharmaceutical Compositions

Formulations for vaccines and antibody-containing pharmaceutical compositions (collectively "compositions") as described herein can be prepared using standard pharmaceutical formulation chemistries and methodologies that are readily available to the reasonably skilled artisan. For example, polypeptides, conjugates, or antibodies as described herein can be combined with one or more pharmaceutically acceptable excipients or vehicles. Auxiliary substances, such as wetting or emulsifying agents, pH buffering substances and the like, may be present in the excipient or vehicle. These excipients, vehicles and auxiliary substances are generally pharmaceutical agents that do not induce an immune response in the individual receiving the composition, and which may be administered without undue toxicity. Pharmaceutically acceptable excipients include, but are not limited to, liquids such as water, saline, polyethyleneglycol, hyaluronic acid, glycerol and ethanol. Pharmaceutically acceptable salts can also be included therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. A thorough discussion of pharmaceutically acceptable excipients, vehicles and auxiliary substances is available in Remington's Pharmaceutical Sciences (Mack Pub. Co., N.J. 1991).

Such compositions may be prepared, packaged, or sold in a form suitable for bolus administration or for continuous administration. Injectable compositions may be prepared, packaged, or sold in unit dosage form, such as in ampoules or in multi-dose containers containing a preservative. Compositions may include, but are not limited to, suspensions, solutions, emulsions in oily or aqueous vehicles, pastes, and implantable sustained-release or biodegradable formulations. Such compositions may further comprise one or more

additional ingredients including, but not limited to, suspending, stabilizing, or dispersing agents. In one embodiment of a composition for parenteral administration, the active ingredient is provided in dry (for e.g., a powder or granules) form for reconstitution with a suitable vehicle (e.g., sterile pyrogen-free water) prior to parenteral administration of the reconstituted composition. The compositions may be prepared, packaged, or sold in the form of a sterile injectable aqueous or oily suspension or solution. This suspension or solution may be formulated according to the known art, and may comprise, in addition to the active ingredient, additional ingredients such as the dispersing agents, wetting agents, or suspending agents described herein. Such sterile injectable formulations may be prepared using a non-toxic parenterally-acceptable diluent or solvent, such as water or 1,3-butane diol, for example. Other acceptable diluents and solvents include, but are not limited to, Ringer's solution, isotonic sodium chloride solution, and fixed oils such as synthetic mono- or di-glycerides.

Other parentally-administrable compositions that are useful include those which comprise the active ingredient in microcrystalline form, in a liposomal preparation, or as a component of a biodegradable polymer systems. Compositions for sustained release or implantation may comprise pharmaceutically acceptable polymeric or hydrophobic materials such as an emulsion, an ion exchange resin, a sparingly soluble polymer, or a sparingly soluble salt.

Alternatively, the polypeptides, conjugates, and antibodies described herein may be encapsulated, adsorbed to, or associated with particulate carriers. Suitable particulate carriers include those derived from polymethyl methacrylate polymers, as well as PLG microparticles derived from poly(lactides) and poly(lactide-co-glycolides). See, e.g., Jeffery et al. (1993) Pharm. Res. 10:362-368. Other particulate systems and polymers can also be used, for example, polymers such as polylysine, polyarginine, polyornithine, spermine, spermidine, as well as conjugates of these molecules.

The formulated compositions will include an amount of one or more polypeptides or conjugates described herein that is sufficient to

mount an immunological response. An immunogenic amount can be readily determined by one of skill in the art. Such an amount will fall in a relatively broad range that can be determined through routine trials. The compositions may contain from about 0.1% to about 99.9% of the polypeptides, conjugates, or antibodies, and can be administered directly to the subject or, alternatively, delivered *ex vivo*, to cells derived from the subject, using methods known to those skilled in the art.

Compositions can include a mixture of distinct polypeptides, conjugates, or antibodies as described herein. For example, vaccines may include, e.g., 2, 3, 4, 5, 6, 7, 8, or more distinct polypeptides or conjugates as described herein, e.g., containing or consisting of the amino acid sequences disclosed herein, or a variant sequence thereof having up to three substitutions, deletions, or additions to the amino acid sequence of any one of amino acid sequences disclosed herein. In one embodiment, a vaccine includes eight distinct polypeptides, wherein the amino acid sequence of the eight polypeptides consist of the sequence of the amino acid sequences disclosed herein. In another embodiment, antibody-containing pharmaceutical compositions may include a mixture of monoclonal or polyclonal antibodies, e.g., having distinct specificities to polypeptides or conjugates as described herein.

Substances that stimulate the immune response, e.g., adjuvants, may be included in the compositions, e.g., in vaccines. Examples of chemical compounds used as adjuvants include, but are not limited to, aluminum compounds (e.g., alum, Alhydrogel), oils, block polymers, immune stimulating complexes, vitamins and minerals (e.g., vitamin E, vitamin A, selenium, and vitamin B12), Quil A (saponins), bacterial and fungal cell wall components (e.g., lipopolysaccarides, lipoproteins, and glycoproteins), hormones, cytokines, and co-stimulatory factors.

Methods of Treatment

The invention features methods of vaccinating a mammal against candidiasis including administering to the animal a vaccine as

described herein, thereby vaccinating the mammal against candidiasis. Additionally, the invention features methods of passive immunization of a mammal against candidiasis including administering to the mammal an effective amount of a pharmaceutical composition as described herein, thereby passively immunizing the mammal against candidiasis. Candidiasis may include, e.g., disseminated candidiasis, e.g., hematogenously disseminated candidiasis, or mucosal candidiasis. In some instances, the candidiasis is caused, e.g., by *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, or *Candida tropicalis*. Other *Candida* species include *Candida lusitaniae* and *Candida stellatoidea*.

Additionally, the compositions and methods described herein may be used, e.g., to vaccinate a human at risk for the development of a *S. aureus* systemic infection or a skin or soft tissue infection against *S. aureus*. First, a human at risk for the development of a *S. aureus* infection or a *S. aureus* SSSI is identified. Second, the human is administered an immunogenic amount of a vaccine comprising a polypeptide described herein, in a pharmaceutically acceptable medium with or without an adjuvant. For example, the human is administered between one and three doses of a polypeptide disclosed herein containing between 3 and 1000 µg of the polypeptide per dose, with multiple doses occurring at intervals of two weeks to six months.

It is expected that, following administration of the vaccine, the human is at decreased risk for the development of a *S. aureus* infection or an *S. aureus* SSSI for a period lasting from one month to several years or more.

Likewise, a human who is identified as having an *S. aureus* infection or an *S. aureus* SSSI may be treated by administration of an immunogenic amount of a pharmaceutical composition comprising a Peptide 1 in a pharmaceutically acceptable medium with or without an adjuvant. For example, the human is administered between one and three doses of a polypeptide disclosed herein containing between 3 and 1000 µg of the polypeptide per dose, with multiple doses occurring at intervals of two weeks to six months.

Again, it is expected that, following administration of the pharmaceutical composition, the *S. aureus* SSSI of the human is decreased in severity.

The compositions and methods described herein may be used, e.g., to vaccinate a bovine species at risk for the development of a systemic S. aureus infection or even *S. aureus* skin or soft tissue infection against *Staphylococcus aureus*. In particular, the bovine species may be at risk of developing bovine mastitis caused by *S. aureus*. First, a bovine species at risk for the development of an *S. aureus* SSSI, e.g., bovine mastitis, is identified. For example, any milk-producing bovine may be considered to be at risk of developing bovine mastitis caused by *S. aureus*. Second, the bovine species is administered an immunogenic amount of a vaccine comprising one or more of the polypeptides disclosed herein in a pharmaceutically acceptable medium with or without an adjuvant. For example, the bovine species is administered between one and three doses of a polypeptide disclosed herein containing between 3 and 1000 µg of the polypeptide per dose, with multiple doses occurring at intervals of two weeks to six months.

It is expected that, following administration of the vaccine, the bovine species is at decreased risk for the development of an *S. aureus* SSSI, e.g., bovine mastitis.

Likewise, a bovine species identified as having an *S. aureus* SSSI, e.g., bovine mastitis, may be treated by administration of an immunogenic amount of a pharmaceutical composition comprising one or more polypeptides disclosed herein in a pharmaceutically acceptable medium with or without an adjuvant. For example, the bovine species is administered between one and three doses of the polypeptide containing between 3 and 1000 µg of the polypeptide per dose, with multiple doses occurring at intervals of two weeks to six months.

It is expected that, following administration of the pharmaceutical composition, the *S. aureus* SSSI, e.g., bovine mastitis, of the bovine species is decreased in severity.

Vaccines and antibody-containing pharmaceutical compositions (collectively “compositions”) as described herein can be administered prophylactically or therapeutically on their own or in combination

with other art-known compositions that induce protective responses against pathogens (e.g., viral, bacterial, fungal, or parasitic pathogens), tumors or cancers, allergens, autoimmune disorders, or graft rejection. For example, the compositions can be administered simultaneously, separately, or sequentially, e.g., with another immunization vaccine, such as a vaccine for, e.g., influenza, malaria, tuberculosis, smallpox, measles, rubella, mumps, or any other vaccines known in the art.

Compositions as described herein can be delivered to a mammalian subject (e.g., a human or other mammal described herein) using a variety of known routes and techniques. For example, a composition can be provided as an injectable solution, suspension, or emulsion, and administered via intramuscular, subcutaneous, intradermal, intracavity, parenteral, epidermal, intraarterial, intraperitoneal, or intravenous injection using a conventional needle and syringe, or using a liquid jet injection system. Compositions can also be administered topically to skin or mucosal tissue, such as nasally, intratracheally, intestinal, rectally or vaginally, or provided as a finely divided spray suitable for respiratory or pulmonary administration. Other modes of administration include oral administration, suppositories, and active or passive transdermal delivery techniques.

The compositions described herein can be administered to a mammalian subject (e.g., a human or other mammal described herein) in an amount that is compatible with the dosage formulation and that will be prophylactically and/or therapeutically effective. An appropriate effective amount will fall in a relatively broad range but can be readily determined by one of skill in the art by routine trials. The "Physicians Desk Reference" and "Goodman and Gilman's The Pharmacological Basis of Therapeutics" are useful for the purpose of determining the amount needed.

Prophylaxis or therapy can be accomplished by a single direct administration at a single time point or by multiple administrations, optionally at multiple time points. Administration can also be delivered to a single or to multiple sites. Those skilled in the art

can adjust the dosage and concentration to suit the particular route of delivery. In one embodiment, a single dose is administered on a single occasion. In an alternative embodiment, a number of doses are administered to a subject on the same occasion but, for example, at different sites. In a further embodiment, multiple doses are administered on multiple occasions. Such multiple doses may be administered in batches, i.e. with multiple administrations at different sites on the same occasion, or may be administered individually, with one administration on each of multiple occasions (optionally at multiple sites). Any combination of such administration regimes may be used.

In one embodiment, different compositions of the invention may be administered at different sites or on different occasions as part of the same treatment regime.

Different administrations may be performed on the same occasion, on the same day, one, two, three, four, five or six days apart, or one, two, three, four or more weeks apart. In some instances, administrations are 1 to 5 weeks apart, e.g., 2 to 4 weeks apart, such as 2 weeks, 3 weeks or 4 weeks apart. The schedule and timing of such multiple administrations can be optimised for a particular vaccine or pharmaceutical composition by one of skill in the art by routine trials.

Dosages

An adequate dose of the vaccines or antibody-containing pharmaceutical compositions described herein may vary depending on such factors as preparation method, administration method, age, body weight and sex of the patient, severity of symptoms, administration time, administration route, rate of excretion, and responsivity. A physician of ordinary skill in the art will easily determine and diagnose the administration dose effective for treatment.

Compositions may be prepared into unit-dose or multiple-dose preparations by those skilled in the art using a pharmaceutically acceptable carrier and/or excipient according to a method known in the art.

Vectors

The invention also provides vectors containing the nucleic acids encoding the polypeptides disclosed herein. Suitable expression vectors are well-known in the art and include vectors capable of expressing a nucleic acid operatively linked to a regulatory sequence or element such as a promoter region or enhancer region that is capable of regulating expression of the nucleic acid. Appropriate expression vectors include vectors that are replicable in eukaryotic cells and/or prokaryotic cells and vectors that remain episomal or integrate into the host cell genome.

The terms “vector”, “cloning vector” and “expression vector” mean the vehicle by which a nucleic acid can be introduced into a host cell. The vector can be used for propagation or harboring a nucleic acid or for polypeptide expression of an encoded sequence. A wide variety of vectors are known in the art and include, for example, plasmids, phages and viruses. Exemplary vectors can be found described in, for example, Sambrook et al., “Molecular Cloning: A Laboratory Manual,” 3rd Edition. Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y., 2001; and Ausubel et al., “Current Protocols in Molecular Biology,” John Wiley and Sons, Baltimore, MD (1999)).

Promoters or enhancers, depending upon the nature of the regulation, can be constitutive or regulated. The regulatory sequences or regulatory elements are operatively linked to a nucleic acid of the invention such that the physical and functional relationship between the nucleic acid and the regulatory sequence allows transcription of the nucleic acid.

Vectors useful for expression in eukaryotic cells can include, for example, regulatory elements including the SV40 early promoter, the cytomegalovirus (CMV) promoter, the mouse mammary tumor virus (MMTV) steroid-inducible promoter, Moloney murine leukemia virus (MMLV) promoter, and the like. The vectors of the invention are useful for subcloning and amplifying a nucleic acid molecule and for recombinantly expressing a polypeptide as disclosed herein. A vector of the invention can include, for example, viral vectors such as a bacteriophage, a baculovirus or a retrovirus; cosmids or plasmids;

and, particularly for cloning large nucleic acid molecules, bacterial artificial chromosome vectors (BACs) and yeast artificial chromosome vectors (YACs). Such vectors are commercially available, and their uses are well known in the art. One skilled in the art will know or can readily determine an appropriate promoter for expression in a particular host cell.

The invention additionally provides recombinant cells containing nucleic acids of the invention. The recombinant cells are generated by introducing into a host cell a vector containing a nucleic acid molecule. The recombinant cells are transducted, transfected or otherwise genetically modified. Exemplary host cells that can be used to express recombinant molecules include mammalian primary cells; established mammalian cell lines, such as COS, CHO, HeLa, NIH3T3, HEK 293 and PC12 cells; amphibian cells, such as *Xenopus* embryos and oocytes; and other vertebrate cells. Exemplary host cells also include insect cells such as *Drosophila*, yeast cells such as *Saccharomyces cerevisiae*, *Saccharomyces pombe*, or *Pichia pastoris*, and prokaryotic cells such as *Escherichia coli*.

Embodiments of the present invention also provide specific Als3 or Hyr1 polypeptides or Als3/Hyr1 polypeptides that can act as antigens for generating an immune response to *Candida*, gram negative bacteria including bacteria of the *Acinetobacter* genus, for example, *Acinetobacter baumannii*, as well as a staphylococcal bacterium.

In some aspects of the invention, the polypeptides of the invention include substantially the same amino acid sequence set forth in any one of the amino acid sequences described herein. For example, the amino acid sequence can have at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% sequence identity to any one of SEQ ID NOS: 1-33. In other aspects, such polypeptides are immunogenic and capable of eliciting production of an anti-Als3 antibody, anti-HYR1 antibody, and Als3/Hyr1 antibody or immunogenic response in a subject.

As described herein, the polypeptides of the invention can encompass substantially similar amino acid sequences having at least about 65% identity with respect to the reference amino acid sequence, and retaining comparable functional and biological activity

characteristic of the reference amino acid sequence. In one aspect, polypeptides having substantially the same amino acid sequence will have at least 50% or 60% identity, at least 65% identity, at least 70% identity, at least 75% identity, at least 80% identity, at least 85% identity, at least 90% identity, at least 95% identity, at least 98% identity, or at least 99% identity. It is recognized, however, that polypeptides, or encoding nucleic acids, containing less than the described levels of sequence identity arising as splice variants or that are modified by conservative amino acid substitutions, or by substitution of degenerate codons are also encompassed within the scope of the present invention.

ASSESSMENT

The following examples are intended to illustrate the invention. These are not meant to limit the invention in any way.

Methods and materials for evaluating treatment of candidiasis

Candida strains and growth conditions

C. albicans 15663, *C. glabrata* 31028, *C. parapsilosis* 22019 and *C. tropicalis* 4243 are clinical bloodstream isolates collected from Harbor-UCLA Medical Center. *C. krusei* 91-1159 was generously provided by Michael Rinaldi, San Antonio, TX. *C. albicans* strains CAAH-31 and THE31 are as described in the literature. All tested strains were routinely grown in YPD (2% Bacto Peptone, 1% yeast extract, 2% dextrose). Cell densities were determined by counting in a hemacytometer.

Recombinant polypeptides and rabbit polyclonal antibodies

Recombinant polypeptides are generated according to standard methods. To generate antibodies, the peptides may be purified and conjugated to keyhole limpet hemocyanin (KLH) before raising rabbit antiserum individually using a standard immunization protocol. Total IgG from pooled serum is affinity purified using Pierce Protein A plus Agarose (Thermo Scientific, Rockford, IL) prior to administering in passive immunization studies.

Immunization protocol and animal studies

All active vaccinations are conducted according to standard methods. In brief, juvenile (10-12 week) Balb/C mice are vaccinated subcutaneously with 30 µg of a polypeptide mixed with alum (2% Alhydrogel; Brenntag Biosector, Frederikssund, Denmark) as an adjuvant in phosphate buffered saline (PBS) on day 0, boosted with the same dose on day 21, then infected via the tail vein on day 35. Control mice are vaccinated with alum alone.

To test the efficacy of the vaccine in immunocompromised mice, mice are vaccinated as above prior to inducing neutropenia by intraperitoneal injection of 200 mg/kg of cyclophosphamide on day -2 followed by another dose of 100 mg/kg on day +7 relative to infection. This regimen results in approximately 10 days of leucopenia with reduction in neutrophil, lymphocyte and monocyte counts according to standard methods. For both immunocompetent and neutropenic mice differences in survival between vaccinated and adjuvant vaccinated mice are compared by the Log Rank test.

For passive immunization, immune IgG is administered intraperitoneally to naïve mice 2 h before infecting i.v. with *C. albicans*. Control mice are given isotype matching IgG (Innovative Research, USA). IgG doses are repeated 3 days after infection, and survival of mice was monitored twice daily.

Quantitative culturing of kidneys from vaccinated or control mice to be infected with different species of *Candida* is performed according to standard methods. In brief, mice are infected through tail veins. Kidneys are harvested 3 day post infection, homogenized, serially diluted in 0.85% saline, and quantitatively cultured on YPD that contained 50 µg/mL chloramphenicol. Colonies are counted after incubation of the plates at 37°C for 24 to 48 h, and results are expressed as log CFU per gram of infected organ.

Concomitant with the fungal burden experiment, kidneys are removed aseptically from two mice per group for histopathological examination. Kidneys are immersed in zinc formalin fixative until examination. Fixed organs are dehydrated in graded alcohol solutions, embedded in paraffin, and cut into 6-µm-thick sections. Mounted

sections are stained with Gomori methenamine silver and examined by light microscopy (Davis et al. (2000) Infect Immun 68: 5953-5959).

Enzyme-linked immunosorbent assay (ELISA)

To test if a polypeptide induces an immune response, antibody titers of serum samples are collected from vaccinated and control mice are determined by ELISA in 96-well plates as previously described (Ibrahim et al. (2005) Infect Immun 73: 999-1005). Wells are coated at 100 µl per well with a peptide (e.g., one of more of peptide 2-11) at 5 µg/ml in PBS. Mouse sera are incubated for 1 h at room temperature following a blocking step with Tris-buffered saline (TBS; 0.01 M Tris HCl [pH 7.4], 0.15 M NaCl) containing 3% bovine serum albumin. The wells are then washed three times with TBS containing 0.05% Tween 20, followed by another three washes with TBS. Goat anti-mouse secondary antibody conjugated with horseradish peroxidase (Sigma) is added at a final dilution of 1:5000, and the plate is further incubated for 1 h at room temperature. Wells are then washed with TBS and incubated with substrate containing 0.1 M citrate buffer (pH 5.0), 50 mg of *o*-phenylenediamine (Sigma), and 10 µl of 30% H_2O_2. The color is allowed to develop for 30 min, after which the reaction is terminated by addition of 10% H_2SO_4 and the optical density (OD) at 490 nm is determined in a microtiter plate reader. Negative control wells received only diluent, and background absorbance is subtracted from the test wells to obtain final OD readings. The ELISA titer is taken as the reciprocal of the last serum dilution that gave a positive OD reading (i.e., more than the mean OD of negative control samples plus 2 standard deviations).

F(ab')$_2$ *blocking assay*

To study the mechanism of protection mediated by anti-polypetides (e.g., one described herein) antibodies in phagocyte-mediated killing of *C. albicans,* HL-60 cells that have been differentiated to neutrophil-like phenotype are employed (Luo et al., (2010) J Infect Dis 201: 1718-1728). A killing assay is conducted in the presence of anti-peptide IgG or F(ab')$_2$ fragments as described before (Luo (2010) J Infect Dis 201: 1718-1728). In brief, HL-60 cells are induced with 2.5 µM of retinoic acid and 1.3% DMSO for three days at 37°C with

5% CO_2. Immune anti-Als3 or anti-Hyr1 or anti-Als3/Hyr1 polypeptide sera are, if desired, pooled and total IgG is isolated using protein A agarose (Thermo Scientific). Serum collected from the same rabbits prior to immunization with the polypeptides serves as control serum. The $F(ab')_2$ fragments from immune or control IgG is purified with Pierce $F(ab')_2$ Preparation Kit according to the manufacturer's instruction. SDS-PAGE analysis is utilized to indicate >95% of Fc fragment is digested. Next, *C. albicans* cells overexpressing or suppressing Als3 is incubated with 50 µg/ml of vaccinated or control $F(ab')_2$ fragments on ice for 45 min. *C. albicans* cocultured with the $F(ab')_2$ fragments is incubated with HL-60 derived neutrophils for 1 h at 37°C with 5% CO_2 prior to sonication and quantitative culturing on YPD plates. % killing is calculated by dividing the number of CFU after coculturing with HL-60 derived neutrophils by the number of CFU from *C. albicans* incubated with media without neutrophil-like cells.

Statistical analysis

The nonparametric log rank test is used to determine differences in the survival times of the mice. Neutrophil killing assay, titers of antibody, and tissue fungal burden is compared by the Mann-Whitney U test for unpaired comparisons. Correlations are calculated with the Spearman rank sum test. *P* values of <0.05 are considered significant.

Expected Results

Peptides that significantly improved survival and decreased fungal burden in immunocompetent mice challenged i.v. with *C. albicans* are taken as being useful in the invention. Similarly, polypeptides that statistically protect immunocompromised mice against candidiasis are useful in the invention. Mice protected from fungal infection after receiving purified IgG targeting a polypeptide disclosed herein in a dose specific manner are not only taken as an indication of the usefulness of passive immunization strategies for treating candidiasis but also for the usefulness of the polypeptide antigen used to raise an immune response. Polypeptide vaccines that substantially reduce tissue fungal burden in BALB/c mice challenged with several non-albicans species of Candida are likewise taken as being useful in the invention.

Methods and materials for evaluating treatment of an Acinteobacter infection

Recombinant polypeptides disclosed herein are produces according to standard methods, for example, using *E. coli* expression system. The recombinant polypeptide is then used to actively vaccinate mice. Mice, for example, are immunized with aluminum hydroxide alone or the recombinant polypeptide mixed with aluminum hydroxide (n=9) on day 0, and boosted on day 21. The vaccinated mice are subsequently infected with *A. baumannii* on day 35. Polypeptide vaccines providing statistically significant survival compared to control mice are taken as useful in the invention. Additionally, measurement of bacterial burden in the tissue of mice vaccinated and infected similarly are examined. The bacterial burden as measured by the number of colony forming units per gram of tissue showing that tissue isolates from kidney, lung and spleen have a lower bacterial burden as compared to control tissue samples are also taken as indicative of useful polypeptide vaccines.

In another working example, overall passive immunization against *Acinetobacter baumannii* infection may also be assayed in diabetic mice. Purified IgG from the eight different polyclonal antibodies are given to diabetic mice 2 hours prior to infection. Commercially available unrelated rabbit IgG is given to diabetic control mice. The mice are then infected with a lethal dose of *Acinetobacter baumannii* via tail vein injection. Mice identified as significantly surviving longer after receiving a single dose of the appropriate IgG than mice receiving the control IgG (e.g., – 80% survival in the anti-polypeptide IgG vs. 0 % in the control arm, $p<0.0001$ by Log Rank test) are taken as evidence of the effectiveness of the polypeptide antigen.

Methods and materials for evaluating treatment of a staphylococcal infection

Briefly, to determine whether a polypeptide described herein protects against *S. aureus*, female Balb/c mice are vaccinated with complete Freund's Adjuvant according to standard methods with a regimen on day 0, followed by a booster dose in Incomplete Freund's

Adjuvant at 3 weeks. Two weeks following vaccination, mice are infected via the tail-vein with a lethal dose of *S. aureus* strain 67-0, which is methicillin-resistant and is known to be virulent in animal models. Polypeptides mediating improved long-term survival in these infected mice are taken as being useful in the invention.

Polypeptides may also be tested in the following murine model of skin or soft tissue infection. Polypeptide vaccination is evaluated across a dose range using a regimen of alhydrogel adjuvant. Doses of 3, 10, 30, 100, or 300µg (IM) are studied in parallel. Primary vaccination (day 0) is followed by an identical boost on study day 21. Mice are infected with *S. aureus* 14 days after boost (study day 35). A subcutaneous skin / soft tissue abscess model is modified from Ding et al. (*J. Bacteriol*. 2008 190:7123-9) and/or Voyich et al. (.*J Infect. Dis*. 2006 194:1761-1770) for these studies. On study day 35, mice are anesthesized, flanks were shaved and sterilized, and 2x107 CFU inocula (without beads or matrix) is introduced into the subcutaneous compartment by injection (100 µl). A minimum of 20 mice per control or vaccine-regimen groups is used in each study. Abscess area / volume is then measured in each mouse flank during the study period up to 14 days post-challenge. To do so, mice are anesthetized, and the lesion site length (l) and width (w) is assessed to quantify abscess or dermonecrosis area (cm2). Abscess volume (cm^3) is calculated per the formula for a spherical ellipsoid: [v = (π/6) x l x w2]. For quantitative culture analyses, at pre-selected times post-infection, mice were humanely sacrificed and processed for quantitative culture of abscesses. Each flank are aseptically dissected, the abscess removed and prepared for culture. Abscesses are individually homogenized, and serially diluted in sterile PBS for quantitative culture onto sheep blood agar plates. Cultures are incubated (37°C) for 24 hours, and resulting colonies enumerated. For statistical analyses, differences in experimental results are compared based on power estimates indicating that 16-20 mice per group yields > 85% power to detect 1 log difference in CFU per gram tissue, or 2 mm abscess area (a = 0.05; Mann-Whitney U test. *P* values are defined according to standard methods.

Expected Results

Polypeptide vaccines that significantly reduce the abscess area, volume, or CFU densities in the murine model of MRSA skin or soft tissues assay are taken as being useful in the invention. Such results are taken to indicate that the polypeptide vaccine tested is useful as a means to prevent or mitigate MRSA skin infection or abscesses or both in mammals.

Additional assessment utilizing human PMBCs

Useful polypeptide antigens described herein are also identified using standard human PMBCs. PMBCs are obtained from individuals vaccinated using an Als3 or Hyr1 at various time points following vaccination. Collected PMBCs are stored at -80°C and thawed before use.

For an assay, ELISpot plates coated with antibodies to specific human cytokines or chemokines, e.g. IFN-γ, IL-17A, IL-4, or GRO are used. PBMC samples are then activated in culture for 48 h and are distributed in 96-well ELISpot plates at ~200,000 cells per well. Specific polypeptides and/or combinations of polypeptides are added to triplicate wells and incubated for 48-96 h and then the supernatants from each well are removed for analysis. The ELISpot plates are developed to reveal the spot forming units per well reflecting the number or cells in the well that produce the compound of interest. Peptides or combination of peptides having an increase in cytokine or chemokine production relative to unstimulated PMBCs are taken as being useful in the invention.

Other Embodiments

All publications, patents, and patent applications mentioned in the above specification are hereby incorporated by reference. Various modifications and variations of the described methods of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific embodiments, it should be understood that the invention as claimed should not be unduly limited

to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention that are obvious to those skilled in the art are intended to be within the scope of the invention.

Other embodiments are in the claims.

What is claimed is:

Claims

Als3 (18-324)

1. An isolated polypeptide optionally fused to a heterologous fusion partner, wherein the amino acid sequence of said polypeptide consists of an amino acid sequence having at least 95% identity to

KTI TGVFNSFNSLTWSNAATYNY KGPGTPTWNAVLGWSLDGTS ASPGDTFTLNMPCVFKFTTS QTSVDLTAHGVKYATCQFQA GEEFMTFSTLTCTVSNTLTP SIKALGTVTLPLAFNVGGTG SSVDLEDSKCFTAGTNTVTF NDGGKKISINVDFERSNVDP KGYLTDSRVIPSLNKVSTLF VAPQCANGYTSGTMGFANTY GDVQIDCSNIHVGITKGLND WNYPVSSESFSYTKTCSSNG IFITYKNVPAGYRPFVDAYI SATDVNSYTLSYANEYTCAG GYWQRAPFTLRWTGYRNSDA GSNG (SEQ ID NO: 2).

Als3 (Ser/Thr-rich sequence)

2. An isolated polypeptide optionally fused to a heterologous fusion partner, wherein the amino acid sequence of said polypeptide consists of an amino acid sequence having at least 95% identity to

IVIVATTRTVTDSTTA VTTLPFDPNRDKTKTIEILK PIPTTTITTSYVGVTTSYST KTAPIGETATVIVDIPYHTT TTVTSKWTGTITSTTTHTNP TDSIDTVIVQVP (SEQ ID NO: 3).

Hyr1 (hydrophobic sequence)

3. An isolated polypeptide optionally fused to a heterologous fusion partner, wherein the amino acid sequence of said polypeptide consists of an amino acid sequence having at least 95% identity to

TSRIDRGGIQGFHGDVKVHS GATWAILGTTLCSFFGGLEV EKGASLFIKSDNGPVLALNV ALSTLVRPVINNGVISLNSK SSTSFSNFDIGGSSFTNNGE IYLDSSGLVKSTAYLYAREW TNNGLIVAY (SEQ ID NO: 5).

Hyr1 (154-350)

4. An isolated polypeptide optionally fused to a heterologous fusion partner, wherein the amino acid sequence of said polypeptide consists of an amino acid sequence having at least 95% identity to

QNQKAAG NIAFGTAYQTITNNGQICLR
HQDFVPATKIKGTGCVTADE DTWIKLGNTILSVEPTHNFY LKDSKSSLIVHAVSSNQTFT
VHGFGNGNKLGLTLPLTGNR DHFRFEYYPDTGILQLRADA LPQYFKIGKGYDSKLFRIVN
SRGLKNAVTYDGPVPNNEIP AVCLIPCTNGPSAPESESDL NTPTTSSIET (SEQ ID NO: 6).

Hyr1 (201-350)

5. An isolated polypeptide optionally fused to a heterologous fusion partner, wherein the amino acid sequence of said polypeptide consists of an amino acid sequence having at least 95% identity to

DTWIKLGNTILSVEPTHNFY LKDSKSSLIVHAVSSNQTFT
VHGFGNGNKLGLTLPLTGNR DHFRFEYYPDTGILQLRADA LPQYFKIGKGYDSKLFRIVN
SRGLKNAVTYDGPVPNNEIP AVCLIPCTNGPSAPESESDL NTPTTSSIET (SEQ ID NO: 7).

Hyr1 (25-469)

6. An isolated polypeptide optionally fused to a heterologous fusion partner, wherein the amino acid sequence of said polypeptide consists of an amino acid sequence having at least 95% identity to

TSRIDRGGIQGFHGDV KVHSGATWAILGTTLCSFFG
GLEVEKGASLFIKSDNGPVL ALNVALSTLVRPVINNGVIS LNSKSSTSFSNFDIGGSSFT
NNGEIYLDSSGLVKSTAYLY AREWTNNGLIVAYQNQKAAG NIAFGTAYQTITNNGQICLR
HQDFVPATKIKGTGCVTADE DTWIKLGNTILSVEPTHNFY LKDSKSSLIVHAVSSNQTFT
VHGFGNGNKLGLTLPLTGNR DHFRFEYYPDTGILQLRADA LPQYFKIGKGYDSKLFRIVN
SRGLKNAVTYDGPVPNNEIP AVCLIPCTNGPSAPESESDL NTPTTSSIETSSYSSAATES
SVVSESSSAVDSLTSSSLSS KSESSDVVSSTTNIESSSTA IETTMNSESSTDAGSSSISQ
SESSSTAITSSSETSSSESM SASSTTASNTSIETDSGIVS QSESSSNAL (SEQ ID NO: 8).

Hyr1 (201-469)

7. An isolated polypeptide optionally fused to a heterologous fusion partner, wherein the amino acid sequence of said polypeptide consists of an amino acid sequence having at least 95% identity to

DTWIKLGNTILSVEPTHNFY LKDSKSSLIVHAVSSNQTFT
VHGFGNGNKLGLTLPLTGNR DHFRFEYYPDTGILQLRADA LPQYFKIGKGYDSKLFRIVN
SRGLKNAVTYDGPVPNNEIP AVCLIPCTNGPSAPESESDL NTPTTSSIETSSYSSAATES
SVVSESSSAVDSLTSSSLSS KSESSDVVSSTTNIESSSTA IETTMNSESSTDAGSSSISQ

SESSSTAITSSSETSSSESM SASSTTASNTSIETDSGIVS QSESSSNAL (SEQ ID NO: 9).

Hyr1 (Ser/Thr-rich sequence)

8. An isolated polypeptide optionally fused to a heterologous fusion partner, wherein the amino acid sequence of said polypeptide consists of an amino acid sequence having at least 95% identity to

SSYSSAATESSVVS ESSSAVDSLTSSSLSSKSES
SDVVSSTTNIESSSTAIETT MNSESSTDAGSSSISQSESS STAITSSSETSSSESMSASS
TTASNTSIETDSGIVSQSES SSNAL (SEQ ID NO: 10).

Hyr1 (154-469)

9. An isolated polypeptide optionally fused to a heterologous fusion partner, wherein the amino acid sequence of said polypeptide consists of an amino acid sequence having at least 95% identity to

QNQKAAG NIAFGTAYQTITNNGQICLR
HQDFVPATKIKGTGCVTADE DTWIKLGNTILSVEPTHNFY LKDSKSSLIVHAVSSNQTFT
VHGFGNGNKLGLTLPLTGNR DHFRFEYYPDTGILQLRADA LPQYFKIGKGYDSKLFRIVN
SRGLKNAVTYDGPVPNNEIP AVCLIPCTNGPSAPESESDL NTPTTSSIETSSYSSAATES
SVVSESSSAVDSLTSSSLSS KSESSDVVSSTTNIESSSTA IETTMNSESSTDAGSSSISQ
SESSSTAITSSSETSSSESM SASSTTASNTSIETDSGIVS QSESSSNAL (SEQ ID NO: 33).

E. coli expressed Als3/Hyr1 fusion proteins

E1= A-B-X-C-D

10. An isolated polypeptide comprising a sequence having substantial identity to the amino acid sequence

A-B-X-C-D (SEQ ID NO: 11),

wherein A is SEQ ID NO: 2;
wherein B is SEQ ID NO: 3;
wherein X is absent or is a linker peptide;
wherein C is SEQ ID NO: 5; and
wherein D is SEQ ID NO: 6.

11. The isolated polypeptide of claim 10, wherein said polypeptide is substantially identical to A-B-C-D (SEQ ID NO: 12).

12. The isolated polypeptide of claim 10, wherein said polypeptide is A-B-C-D (SEQ ID NO: 12).

E2= A-X-C-D

13. An isolated polypeptide comprising a sequence having substantial identity to the amino acid sequence

A-X-C-D (SEQ ID NO: 13),

wherein A is SEQ ID NO: 2;
wherein X is absent or is a linker peptide;
wherein C is SEQ ID NO: 5; and
wherein D is SEQ ID NO: 6.

14. The isolated polypeptide of claim 13, wherein said polypeptide is substantially identical to A-C-D (SEQ ID NO: 14).

15. The isolated polypeptide of claim 13, wherein said polypeptide is A-C-D (SEQ ID NO: 14).

E3= A-X-D

16. An isolated polypeptide comprising a sequence having substantial identity to the amino acid sequence

A-X-D (SEQ ID NO: 15),

wherein A is SEQ ID NO: 2;
wherein X is absent or is a linker peptide; and
wherein D is SEQ ID NO: 6.

17. The isolated polypeptide of claim 16, wherein said polypeptide is substantially identical to A-D (SEQ ID NO: 16).

18. The isolated polypeptide of claim 16, wherein said polypeptide is A-D (SEQ ID NO: 16).

E4= C-D-X-A-B

19. An isolated polypeptide comprising a sequence having substantial identity to the amino acid sequence

C-D-X-A-B (SEQ ID NO: 17),

wherein C is SEQ ID NO: 5;

wherein D is SEQ ID NO: 6;

wherein X is absent or is a linker peptide;

wherein A is SEQ ID NO: 2; and

wherein B is SEQ ID NO: 3.

20. The isolated polypeptide of claim 19, wherein said polypeptide is substantially identical to C-D-A-B (SEQ ID NO: 18).

21. The isolated polypeptide of claim 19, wherein said polypeptide is C-D-A-B (SEQ ID NO: 18).

E5= C-D-X-A

22. An isolated polypeptide comprising a sequence having substantial identity to the amino acid sequence

C-D-X-A (SEQ ID NO: 19),

wherein C is SEQ ID NO: 5;

wherein D is SEQ ID NO: 6;

wherein X is absent or is a linker peptide; and

wherein A is SEQ ID NO: 2.

23. The isolated polypeptide of claim 22, wherein said polypeptide is substantially identical to C-D-A (SEQ ID NO: 20).

24. The isolated polypeptide of claim 22, wherein said polypeptide is C-D-A (SEQ ID NO: 20).

E6= D-X-A-B

25. An isolated polypeptide comprising a sequence having substantial identity to the amino acid sequence

D-X-A-B (SEQ ID NO: 21),

wherein D is SEQ ID NO: 6;

wherein X is absent or is a linker peptide;

wherein A is SEQ ID NO: 2; and
wherein B is SEQ ID NO: 3.

26. The isolated polypeptide of claim 25, wherein said polypeptide is substantially identical to D-A-B (SEQ ID NO: 22).

27. The isolated polypeptide of claim 25, wherein said polypeptide is D-A-B (SEQ ID NO: 22).

E7 D-X-A

28. An isolated polypeptide comprising a sequence having substantial identity to the amino acid sequence

D-X-A (SEQ ID NO: 23),

wherein D is SEQ ID NO: 6;
wherein X is absent or is a linker peptide; and
wherein A is SEQ ID NO: 2.

29. The isolated polypeptide of claim 28, wherein said polypeptide is substantially identical to D-A (SEQ ID NO: 24).

30. The isolated polypeptide of claim 28, wherein said polypeptide is D-A (SEQ ID NO: 24).

S. cerevisiae expressed Als3/Hyr1 fusion proteins

S1= A-B-X-C-D

31. An isolated polypeptide comprising a sequence having substantial identity to the amino acid sequence

A-B-X-C-D (SEQ ID NO: 11),

wherein A is SEQ ID NO: 2;
wherein B is SEQ ID NO: 3;
wherein X is absent or is a linker peptide;
wherein C is SEQ ID NO: 5; and
wherein D is SEQ ID NO: 6.

32. The isolated polypeptide of claim 31, wherein said polypeptide is substantially identical to A-B-C-D (SEQ ID NO: 12).

33. The isolated polypeptide of claim 31, wherein said polypeptide is A-B-C-D (SEQ ID NO: 12).

S2= A-X-C-D-E

34. An isolated polypeptide comprising a sequence having substantial identity to the amino acid sequence

A-X-C-D-E (SEQ ID NO: 25),

wherein A is SEQ ID NO: 2;
wherein X is absent or is a linker peptide;
wherein C is SEQ ID NO: 5;
wherein D is SEQ ID NO: 6; and
wherein E is SEQ ID NO: 10.

35. The isolated polypeptide of claim 34, wherein said polypeptide is substantially identical to A-C-D-E (SEQ ID NO: 26).

36. The isolated polypeptide of claim 34, wherein said polypeptide is A-C-D-E (SEQ ID NO: 26).

S3= A-X-D-E

37. An isolated polypeptide comprising a sequence having substantial identity to the amino acid sequence

A-X-D-E (SEQ ID NO: 27),

wherein A is SEQ ID NO: 2;
wherein X is absent or is a linker peptide;
wherein D is SEQ ID NO: 6; and
wherein E is SEQ ID NO: 10.

38. The isolated polypeptide of claim 37, wherein said polypeptide is substantially identical to A-D-E (SEQ ID NO: 28).

39. The isolated polypeptide of claim 37, wherein said polypeptide is A-D-E (SEQ ID NO: 28).

S4= C-D-E-X-A-B

40. An isolated polypeptide comprising a sequence having substantial identity to the amino acid sequence

C-D-E-X-A-B (SEQ ID NO: 29),

wherein C is SEQ ID NO: 5;
wherein D is SEQ ID NO: 6;
wherein E is SEQ ID NO: 10;
wherein X is absent or is a linker peptide;
wherein A is SEQ ID NO: 2; and
wherein B is SEQ ID NO: 3.

41. The isolated polypeptide of claim 40, wherein said polypeptide is substantially identical to C-D-E-A-B (SEQ ID NO: 30).

42. The isolated polypeptide of claim 40, wherein said polypeptide is C-D-E-A-B (SEQ ID NO: 30).

S5= C-D-X-A-B

43. An isolated polypeptide comprising a sequence having substantial identity to the amino acid sequence

C-D-X-A-B (SEQ ID NO: 17),

wherein C is SEQ ID NO: 5;
wherein D is SEQ ID NO: 6;
wherein X is absent or is a linker peptide;
wherein A is SEQ ID NO: 2; and
wherein B is SEQ ID NO: 3.

44. The isolated polypeptide of claim 43, wherein said polypeptide is substantially identical to C-D-A-B (SEQ ID NO: 18).

45. The isolated polypeptide of claim 43, wherein said polypeptide is C-D-A-B (SEQ ID NO: 18).

S6= D-X-A-B

46. An isolated polypeptide comprising a sequence having substantial identity to the amino acid sequence

D-X-A-B (SEQ ID NO: 21),

wherein D is SEQ ID NO: 6;
wherein X is absent or is a linker peptide;
wherein A is SEQ ID NO: 2; and
wherein B is SEQ ID NO: 3.

47. The isolated polypeptide of claim 46, wherein said polypeptide is substantially identical to D-A-B (SEQ ID NO: 22).

48. The isolated polypeptide of claim 46, wherein said polypeptide is D-A-B (SEQ ID NO: 22).

S7= D-X-A

49. An isolated polypeptide comprising a sequence having substantial identity to the amino acid sequence

D-X-A (SEQ ID NO: 23),

wherein D is SEQ ID NO: 6;
wherein X is absent or is a linker peptide; and
wherein A is SEQ ID NO: 2.

50. The isolated polypeptide of claim 49, wherein said polypeptide is substantially identical to D-A (SEQ ID NO: 24).

51. The isolated polypeptide of claim 49, wherein said polypeptide is D-A (SEQ ID NO: 24).

52. An isolated nucleic acid molecule which encodes a polypeptide of any one of claims 1-51, 59, or 118-137.

53. An isolated nucleic acid molecule comprising a nucleic acid sequence which is substantially identical to the isolated nucleic acid molecule of claim 52.

54. A vector comprising the nucleic acid molecule of claim 52 or 53.

55. A cell comprising the nucleic acid molecule of claim 52 or 53.

56. A method of producing a recombinant polypeptide, said method comprising the steps of:

(a) providing a cell transformed with the nucleic acid molecule of claim 52 or 53 encoding an polypeptide positioned for expression in the cell;

(b) culturing the transformed cell under conditions for expressing the nucleic acid molecule, wherein said culturing results in expression of said recombinant polypeptide; and

(c) isolating the recombinant polypeptide.

57. The method of claim 56, wherein said cell is a bacterium.

58. The method of claim 57, wherein said cell is a yeast.

59. A recombinant polypeptide produced according to the method of claim 56.

60. A substantially pure antibody that specifically recognizes and binds to any one of the polypeptides of claims 1-51, 59, or 118-137.

61. An antigenic composition comprising the polypeptide of any one of claims 1-51, 59, or 118-137, and a pharmaceutically acceptable carrier, diluent, and/or excipient.

62. The composition of claim 61, further comprising an adjuvant.

63. A method of inducing an immune response in a mammal against an antigen comprising administering a polypeptide of any one of claims 1-51, 59, or 118-137, or the antigenic composition of claim 61 to said mammal, wherein said polypeptide or said composition induces an immune response against said antigen in said mammal.

64. The method of claim 63, wherein the mammal is administered a single dose of said polypeptide or said composition.

65. The method of claim 63, wherein the mammal is administered a plurality of doses of said polypeptide or said composition.

66. The method of claim 63, wherein said plurality of doses are administered at least one day apart.

67. The method of claim 65, wherein said composition is administered twice.

68. The method of claim 65, wherein said plurality of doses are administered at least two weeks apart.

69. The method of any one of claims 63-68, wherein said mammal is a human.

70. A vaccine comprising an immunogenic amount of the polypeptide of any one of claims 1-51, 59, or 118-137, and a pharmaceutically acceptable excipient.

71. The vaccine of claim 70, comprising a mixture of distinct polypeptides of any one of claims 1-51, 59, or 118-137.

72. The vaccine of claim 70 or 71, further comprising an adjuvant.

73. The vaccine of claim 72, wherein said adjuvant is Alhydrogel.

74. The vaccine of any one of claims 70-73 for use in the vaccination of a mammal against candidiasis or a gram negative bacterium or *S. aureus*.

75. The vaccine of claim 74, wherein said mammal is a human.

76. The vaccine of claim 74 or 75, wherein said vaccine is to be administered by intramuscular, subcutaneous, or intradermal administration.

77. The vaccine of claim 76, wherein said vaccine is to be administered by intramuscular administration.

78. The vaccine of any one of claims 70-77, wherein said vaccination further comprises administering a booster dose.

79. The vaccine of any one of claims 74-77, wherein said candidiasis is disseminated candidiasis.

80. The vaccine of claim 79, wherein said disseminated candidiasis is hematogenously disseminated candidiasis.

81. The vaccine of any one of claims 74-77, wherein said candidiasis is mucosal candidiasis.

82. The vaccine of any one of claims 74-81, wherein said candidiasis is caused by *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, or *Candida tropicalis*.

83. A method of vaccinating a mammal against candidiasis comprising administering to said mammal the vaccine of claim 70, thereby vaccinating said mammal against candidiasis or a gram negative bacterium or *S. aureus*.

84. The method of claim 83, wherein said mammal is a human.

85. The method of claim 83 or 84, wherein said vaccine is administered by intramuscular, subcutaneous, or intradermal administration.

86. The method of claim 83 or 84, wherein said vaccine is administered by intramuscular administration.

87. The method of any one of claims 83-86, wherein said administering further comprises administering a booster dose.

88. The method of any one of claims 83-87, wherein said candidiasis is disseminated candidiasis.

89. The method of claim 88, wherein said disseminated candidiasis is hematogenously disseminated candidiasis.

90. The method of any one of claims 83-87, wherein said candidiasis is mucosal candidiasis.

91. The method of any one of claims 83-90, wherein said candidiasis is caused by *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, or *Candida tropicalis*.

92. A method of producing a chimeric vaccine comprising the steps of:

(a) providing a phage, yeast, or virus;

(b) inserting into said phage, yeast, or virus a nucleic acid molecule that encodes the polypeptide of any one of claims 1-51, 59, or 118-137;

(c) allowing expression of said polypeptide in said phage, yeast, or virus;

(d) isolating said phage, yeast, or virus of step (c) comprising said expressed polypeptide; and

(e) adding a pharmaceutically acceptable excipient to said isolated phage, yeast, or virus of step (d).

93. The method of claim 92, wherein said polypeptide is displayed on the surface of said phage, yeast, or virus following step (c).

94. An isolated monoclonal antibody that binds to the polypeptide of any one of claims 1-51, 59, or 118-137.

95. The antibody of claim 94, wherein said antibody is human or humanized.

96. The antibody of claim 94, wherein said antibody is chimeric.

97. The antibody of any one of claims 94 and 96, wherein said antibody is produced recombinantly.

98. A diagnostic composition comprising the antibody of any one of claims 94-97.

99. A pharmaceutical composition comprising the antibody of any one of claims 94-97 and a pharmaceutically acceptable excipient.

100. The pharmaceutical composition of claim 99, comprising a mixture of antibodies of any one of claims 94-97 with a plurality of distinct specificities.

101. A pharmaceutical composition comprising polyclonal antibodies that bind to the polypeptide of any one of claims 1-51, 59, or 118-137, or that bind to a mixture of distinct polypeptides of any one of claims 1-51, 59, or 118-137.

102. The pharmaceutical composition of any one of claims 98-100 for use in the passive immunization of a mammal against candidiasis or a gram negative bacterium or *S. aureus*.

103. The composition of claim 102, wherein said mammal is a human.

104. The composition of claim 102 or 103, wherein said pharmaceutical composition is administered by intramuscular, subcutaneous, or intradermal administration.

105. The composition of claim 104, wherein said pharmaceutical composition is administered by intramuscular administration.

106. The composition of any one of claims 102-105, wherein said candidiasis is disseminated candidiasis.

107. The composition of claim 106, wherein said disseminated candidiasis is hematogenously disseminated candidiasis.

108. The composition of any one of claims 102-105, wherein said candidiasis is mucosal candidiasis.

109. The composition of any one of claims 102-108, wherein said candidiasis is caused by *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, or *Candida tropicalis*.

110. A method of passive immunization of a mammal against candidiasis comprising administering to said mammal an effective amount of the pharmaceutical composition of claim 102, thereby passively immunizing said mammal against said candidiasis or a gram negative bacterium or *S. aureus*.

111. The method of claim 110, wherein said mammal is a human.

112. The method of claim 110 or 111, wherein said pharmaceutical composition is administered by intramuscular, subcutaneous, or intradermal administration.

113. The method of claim 112, wherein said pharmaceutical composition is administered by intramuscular administration.

114. The method of any one of claims 110-113, wherein said candidiasis is disseminated candidiasis.

115. The method of claim 114, wherein said disseminated candidiasis is hematogenously disseminated candidiasis.

116. The method of any one of claims 110-113, wherein said candidiasis is mucosal candidiasis.

117. The method of any one of claims 110-116, wherein said candidiasis is caused by *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, or *Candida tropicalis*.

118. An isolated polypeptide comprising a sequence having substantial identity to the amino acid sequence

A-B-X-C-D-E (SEQ ID NO: 31),

wherein A is absent or is SEQ ID NO: 2;
wherein B is absent or is SEQ ID NO: 3;
wherein X is absent or is a linker peptide;
wherein C is absent or is SEQ ID NO: 5;
wherein D is absent or is SEQ ID NO: 6; and
wherein E is absent or is SEQ ID NO: 10,
provided that two or more of A, B, C, D and E are present in said polypeptide.

119. The polypeptide of claim 118, wherein said polypeptide is A-B-C-D-E (SEQ ID NO: 32).

120. The polypeptide of claim 118, wherein said polypeptide is A-B-X-C-D (SEQ ID NO: 11).

121. The polypeptide of claim 120, wherein said polypeptide is A-B-C-D (SEQ ID NO: 12).

122. The polypeptide of claim 118, wherein said polypeptide is A-X-C-D-E (SEQ ID NO: 25).

123. The polypeptide of claim 122, wherein said polypeptide is A-C-D-E (SEQ ID NO: 26).

124. The polypeptide of claim 118, wherein said polypeptide is A-X-C-D (SEQ ID NO: 13).

125. The polypeptide of claim 124, wherein said polypeptide is A-C-D (SEQ ID NO: 14).

126. The polypeptide of claim 118, wherein said polypeptide is A-X-D-E (SEQ ID NO: 27).

127. The polypeptide of claim 126, wherein said polypeptide is A-D-E (SEQ ID NO: 28).

128. The polypeptide of claim 118, wherein said polypeptide is A-X-D (SEQ ID NO: 15).

129. The polypeptide of claim 128, wherein said polypeptide is A-D (SEQ ID NO: 16).

130. An isolated polypeptide comprising a sequence having substantial identity to the amino acid sequence

C-D-E-X-A-B (SEQ ID NO: 29),

wherein C is absent or is SEQ ID NO: 5;
wherein D is absent or is SEQ ID NO: 6;
wherein E is absent or is SEQ ID NO: 10;
wherein X is absent or is a linker peptide;
wherein A is absent or is SEQ ID NO: 2;
wherein B is absent or is SEQ ID NO: 3,
provided that two or more of C, D, E, A, and B are present in said polypeptide.

131. The polypeptide of claim 130, wherein said polypeptide is C-D-E-A-B (SEQ ID NO: 30).

132. The polypeptide of claim 130, wherein said polypeptide is C-D-X-A-B (SEQ ID NO: 17).

133. The polypeptide of claim 132, wherein said polypeptide is C-D-A-B (SEQ ID NO: 18).

134. The polypeptide of claim 130, wherein said polypeptide is D-X-A-B (SEQ ID NO: 21).

135. The polypeptide of claim 134, wherein said polypeptide is D-A-B (SEQ ID NO: 22).

136. The polypeptide of claim 130, wherein said polypeptide is D-X-A (SEQ ID NO: 23).

137. The polypeptide of claim 136, wherein said polypeptide is D-A (SEQ ID NO: 24).

138. In any aforementioned claim, wherein the gram negative bacterium is *Acinetobacter*.

139. In any aforementioned claim, wherein *S. aureus* is MRSA.