

COMPOSITIONS AND METHODS FOR THE REMOVAL OF BIOFILMS

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Serial Nos. 61/318,743, filed March 29, 2010; 61/347,362, filed May 21, 2010, 61/397,891 filed June 16, 2010, and 61/454,972 filed March 21, 2011 the contents of each of which are incorporated by reference in their entirety into the current disclosure.

STATEMENT OF GOVERNMENT SUPPORT

This invention was made with government support under Contract No. 5R01DE013230 awarded by the National Institute of Dental and Craniofacial Research (NIDCR) at the National Institutes of Health. The government has certain rights in the invention.

FIELD OF THE INVENTION

This invention generally relates to the methods and compositions to lessen and/or cure clinical or industrial bacterial biofilms.

BACKGROUND

Bacteria persisting in a biofilm in the mammalian body cause about two-thirds of all chronic/recurrent diseases. These biofilms are comprised of bacteria protected by an outer “slime” that is often comprised primarily of DNA that prevents the innate and adaptive immune systems, antibiotics and other antibacterial agents from gaining access to the bacteria inside the biofilm, making it extremely difficult to clear the infection from the body. Furthermore, the biofilm can act as a reservoir for future acute infections often with lethal consequences.

At least one protein from the DNABII family of proteins are found in all known eubacteria and are naturally found outside of the bacterial cell. While they elicit a strong innate immune response, host subjects fail to naturally produce specific antibody to family members as a result of infection. The major problem with bacterial biofilms is the inability of the host immune system and/or antibiotics and other antimicrobials to gain access to the bacteria protected within the biofilm.

Biofilms are present in an industrial setting as well. For example, biofilms are implicated in a wide range of petroleum process problems, from the production field to the gas station storage tank. In the field, sulfate reducing biofilm bacteria produce hydrogen sulfide (soured

oil). In the process pipelines, biofilm activity develops slimes which impede filters and orifices. Biofilm and biofilm organisms also cause corrosion of pipeline and petroleum process equipment. These problems can be manifested throughout an oil or gas production facility to the point where fouling and corrosive biofilm organisms have even been found on the surfaces of final product storage tanks.

In the home, biofilms are found in or on any surface that supports microbial growth, e.g., in drains, on food preparation surfaces, in toilets, and in swimming pools and spas.

Biofilms are implicated in a wide range of water processes, both domestic and industrial.

They can grow on the surface of process equipment and impede the performance of the equipment, such as degradation of heat transfer or plugging of filters and membranes.

Biofilms growing on a cooling tower fill can add enough weight to cause collapse of the fill.

Biofilms cause corrosion of even highly specialized stainless steels. Biofilms in a water process can degrade the value of a final product such as biofilm contamination in a paper process or the attachment of even a single cell on a silicon chip. Biofilms growing in drinking water distribution systems can harbor potential pathogenic organisms, corrosive organisms or bacteria that degrade the aesthetic quality of the water.

Thus, a need exists to break through the protective barrier of biofilms to treat or kill the associated bacterial infections and clear them from surfaces and in water systems. This invention satisfies this need and provides related advantages as well.

SEQUENCE LISTING

SEQ. ID NO. 1

A1-A2-A3-A4-A5-A6-A7-A8-A9

wherein:

A1 is V or I;

A2 is any one of K, Q, E, A, V or Y;

A3 is any one of K, L, I, V or F;

A4 is any one of S, I, R or V;

A5 is any one of G or S;

A6 is F;

A7 is G;

A8 is any one of N or S or T or K; and

A9 is F.

SEQ. ID NO. 2 is VKKSGFGNF

SEQ ID NO. 3 is B1-B2-B3-B4-B5-B6-B7

wherein:

- B1 is absent or any one of G or K;
- B2 is absent or any one of R, I or K;
- B3 is N or V;
- B4 is P or I;
- B5 is any one of K, Q, S or G;
- B6 is any one of T, K or S; and
- B7 is any one of G, K, Q or D.

Seq. ID NO. 4 is NP(K/Q)TG

Seq. ID NO. 5 GRNP(K/Q)TG

Seq. ID NO. 6 Full Length Wild type (wt) 86-028NP *Haemophilus influenzae* IhfA;

Genbank accession No.: AAX88425.1, last accessed March 21, 2011:

MATITKLDIIEYLSDKYHLSK

QDTKNVVENFLEEIRLSLESGQDVKLSGFGNFELRDKSSRPGRNPKTGDVVPVSARR
VVTFKPGQKLRRARVEKTK

Seq. ID NO. 7 Full Length wt 86-028NP *Haemophilus influenzae* HU, Genbank accession

No.: YP_248142.1, last accessed March 21, 2011: MRFVTIFINHA FNSSQVRLSFAQFLR

QIRKDTFKESNFLFNRRYKFMNKTDLIDAIANA AELNKKQAKAALEATLDAITASLK
EGEPVQLIGFGTFKVNERRAARTGRNPQTGA EIQIAASKVPAFVSGKALKDAIK

Seq. ID NO. 8 Full Length wt R2846 *Haemophilus influenzae* IhfA, Genbank accession

No.: ADO96375, last accessed March 21, 2011:

MATITKLDIIEYLSDKYHLSKQDTKNVVENFL

EEIRLSLESGQDVKLSGFGNFELRDKSSRPGRNPKTGDVVPVSARRVVTFKPGQKLRR
ARVEKTK

Seq. ID NO. 9 Full Length wt Rd *Haemophilus influenzae* IhfA; Genbank accession No.:

AAC22959.1, last accessed March 21, 2011: MATITKLDIIEYLSDKYHLSKQDTK

NVVENFLEEIRLSLESGQDVKLSGFGNFELRDKSSRPGRNPKTGDVVPVSARRVVTF
KPGQKLRRARVEKTK;

Seq. ID NO. 10 Full Length wt *E. coli* K12 IhfA; Genbank accession No.: AAC74782.1, last accessed March 21, 2011: MALTKAEMSEYLFDKLGLSKRDAKELVELFFE
EIRRALENGEQVKLSGFGNFDLRDKNQRPGRNPKTGEDIPITARRVVT
FRPGQKLKSRVENASPKDE; DNA Genbank No. NC_000913

Seq. ID NO. 11 Full Length wt *P. aeruginosa* PA 01 IhfA; Genbank accession No.: AAG06126.1, last accessed March 21, 2011: MGALTKAEIAERLYEELGLNKREA
KELVELFFEEIRQALEHNEQVKLSGFGNFDLRDKRQRPGRNPKTGEEIPITARRVVTF
RPGQKLKARVEAYAGTKS

Seq ID NOS. 12 and 13: β -3 and α -3 portions of (IHF α) SEQ ID NO. 12: TFRPGQ and
SEQ ID NO. 13: KLKSRVENASPKDE

Seq ID NOS. 14 and 15: β -3 and α -3 portions of (IHF β) SEQ ID NO. 14: HFKPGK and
SEQ ID NO. 15: ELRDRANIYG

Seq ID NOS. 16 and 17: β -3 and α -3 portions of SEQ ID NOS. 6, SEQ ID NOS. 16:
TFKPGQ and SEQ ID NO. 17: KLRARVEKTK

SEQ ID NOS. 18 and 19: β -3 and α -3 portions of 2019 *Haemophilus influenzae* IhfA, SEQ
ID NO. 18: TFKPGQ and SEQ ID NO. 19: KLRARVENTK

SEQ ID NOS. 20 and 21: β -3 and α -3 portions of SEQ ID NO. 8, SEQ ID NO. 20:
TFKPGQ and SEQ ID NO. 21: KLRARVEKTK

SEQ ID NOS. 22 and 23: β -3 and α -3 portions of SEQ ID NO. 9, SEQ ID NO. 22:
TFKPGQ and SEQ ID NO. 23: KLRARVEKTK

SEQ ID NOS. 24 and 25: β -3 and α -3 portions of SEQ ID NO. 10, SEQ ID NO. 24:
TFRPGQ and SEQ ID NO. 25: KLKSRVENASPKDE

SEQ ID NOS. 26 and 27: β -3 and α -3 portions of SEQ ID NO. 11, SEQ ID NO. 26:
TFRPGQ and SEQ ID NO. 27: KLKARVEAYAGTKS

SEQ ID NO. 28: *E. coli* hupA, Genbank accession No.: AP_003818, Last accessed March
21, 2011: MNKTQLIDVIAEKAELSKTQAKAALESTLAAITESLKEGDAVQLVGFGTFK
VNHRAERTGRNPQTGKEIKIAAANVPAFVSGKALKDAVK

SEQ ID NO. 29: E. coli hupB, Genbank accession No.: AP_001090.1, Last accessed March 21, 2011: MNKSQLIDKIAAGADISKAAAGRALDAIIASVTESLKEGDDVALVGFG TFAVKERAARTGRNPQTGKEITIAAAKVPSFRAGKALKDAVN

SEQ ID NOS. 30 and 31: β -3 and α -3 portions of SEQ ID NO. 28, SEQ ID NO. 30: AFVSGK and SEQ ID NO. 31: ALKDAVK

SEQ ID NOS. 32 and 33: β -3 and α -3 portions of SEQ ID NO. 29, SEQ ID NO. 32: SFRAGK and SEQ ID NO. 33: ALKDAVN

SEQ. ID NO. 34: C-terminal 20 amino acids of IHF α : TFRPGQKLKSRVENASPKDE

SEQ. ID NO. 35: C-terminal 20 amino acids of IHF β : KYVPHFKPGKELRDRANIYG

SEQ. ID NO. 36: DNABII binding consensus sequence: WATCAANNNTTR wherein W is A or T, N is any base and R is a purine

SEQ. ID NO. 337: E. coli IHF α : GRNPKTGEDIPI

SEQ. ID NO. 338: E. coli IHF β : GRNPKTGDKVEL

SEQ. ID NO. 339: E. coli HU α : GRNPQTGKEIKI

SEQ. ID NO. 340: E. coli HU β : GRNPQTGKEITI

DESCRIPTION OF TABLES

Tables 1-5 are the results of an *in vitro* bioassay of the reversal of the biofilm in the indicated organism.

Table 6 is the scoring scheme of the relative amount of biomass within the middle ear in the Chinchilla model of otitis media (OM).

Table 7 is the results of an *in vitro* bioassay of the reversal of the biofilm upon treatment with DNase.

Table 8 is a non-limited summary of DNA binding proteins produced by gram(+) and gram (-) bacteria that can be used in the methods provided herein.

Table 9A is a sequence alignment of relevant portions of the DNA binding proteins of various embodiments of this invention. Bold letters indicate an exact match to consensus, light gray lettering indicates a conservative amino acid change, and lightly or darkly shaded

sequences are highly conserved across species. Gray shaded undefined sequences at the amino and/or carboxy-terminal are undefined amino acids that do not share consensus sequences.. Table 9A is based on information previously published in Obeto et al. (1994) *Biochimie* 76:901-908. Table 9B is a comparison of the 16 amino acid peptide motif to Liu et al. (2008) *Cell Microbiol.* 10(1):262-276.

Table 10 is a listing of α , β , and C-terminal portions of DNABII proteins from the indicated organism.

SUMMARY OF THE INVENTION

Within bacterial cells, the DNABII proteins are DNA binding proteins that necessarily bend DNA substrates upon binding. Similarly, DNA that is already in a bent conformation is a preferred substrate as the energy required for bending is rendered unnecessary.

The DNABII family of proteins is found outside of bacterial cells in the biofilm state. Applicants have shown that these proteins are in fact bound to the extracellular DNA at critical branched junctions. In one aspect, Applicants have shown that by immunizing the host with polypeptides and proteins that produce specific antibodies, the DNA-based lattice is sufficiently altered to now permit the host immune system to clear the biofilm.

Applicants have also demonstrated the removal of pre-formed non-typeable *Haemophilus influenza* biofilms in the middle ear of the chinchilla host by various modes of immunization with a DNABII family member (*E. coli* integration host factor, IHF). This chinchilla middle ear biofilm animal system has already been well documented as an excellent model for human otitis media (or middle ear infections).

The method for using this technology is straightforward. In one embodiment, the polypeptides of this invention are used to vaccinate individuals as a prophylactic to chronic/recurrent biofilm disease or as a therapeutic for those with an existing infection. The individual's immune system will then naturally generate antibodies which prevent or clear these bacteria from the host by interfering with the construction and or maintenance of a functional protective biofilm. Alternatively, antibodies to the polypeptides can be administered to treat or prevent infection. Bacteria that cannot form functional biofilms are more readily cleared by the remainder of the host's immune system.

Thus, in one aspect a method for inhibiting, competing or titrating the binding of a DNABII polypeptide or protein to a microbial DNA is provided by comprising, or alternatively consisting essentially of, or yet further consisting of contacting the DNABII polypeptide or

protein or the microbial DNA with an interfering agent, thereby inhibiting, competing or titrating the binding of the DNABII protein or polypeptide to the microbial DNA. The contacting can be performed in vitro or in vivo.

In another aspect, provided is a method for inhibiting, preventing or breaking down a microbial biofilm, comprising, or alternatively consisting essentially of, or yet further consisting of contacting the biofilm with an interfering agent, thereby inhibiting, preventing or breaking down the microbial biofilm. The contacting can be performed in vitro or in vivo.

In a further aspect, provided is a method of inhibiting, preventing or breaking down a biofilm in a subject, comprising, or alternatively consisting essentially of, or yet further consisting of administering to the subject an effective amount of an interfering agent, thereby inhibiting, preventing or breaking down the microbial biofilm.

In a yet further aspect, a method for inhibiting, preventing or treating a microbial infection that produces a biofilm in a subject is provided. The method comprises, or alternatively consists essentially of, or yet further consists of administering to the subject an effective amount of an interfering agent, thereby inhibiting, preventing or treating a microbial infection that produces the biofilm in the subject.

For the methods as described herein, any agent that interferes or impedes the binding of the microbial DNA to the DNABII protein or polypeptide is intended within the scope of this invention. Non-limiting examples of interfering agents include:

- (a) an isolated or recombinant integration host factor (IHF) polypeptide or a fragment or an equivalent of each thereof;
- (b) an isolated or recombinant histone-like protein from *E.coli* strain U93 (HU) polypeptide or a fragment or an equivalent of each thereof;
- (c) an isolated or recombinant protein or polypeptide identified in Table 8, Table 9A, Table 9B, Table 10 or a DNA binding peptide identified in Figure 6, or a fragment or an equivalent of each thereof;
- (d) an isolated or recombinant polypeptide of SEQ ID NO. 1 through 340, or a fragment or an equivalent of each thereof;
- (e) an isolated or recombinant C-terminal polypeptide of SEQ ID NO. 6 through 11, 28, 29, 42 through 100, Table 8 or those C-terminal polypeptides identified in Table 10 or a fragment or an equivalent of each thereof;

- (f) a polypeptide or polynucleotide that competes with an integration host factor on binding to a microbial DNA;
- (g) a four-way junction polynucleotide resembling a Holliday junction, a 3 way junction polynucleotide resembling a replication fork, a polynucleotide that has inherent flexibility or bent polynucleotide;
- (h) an isolated or recombinant polynucleotide encoding any one of (a) through (f) or an isolated or recombinant polynucleotide of SEQ ID NO. 36 or an equivalent of each thereof, or a polynucleotide that hybridizes under stringent conditions to the polynucleotide its equivalent or its complement;
- (i) an antibody or antigen binding fragment that specifically recognizes or binds any one of (a) through (f), or an equivalent or fragment of each antibody or antigen binding fragment thereof;
- (j) an isolated or recombinant polynucleotide encoding the antibody or antigen binding fragment of (i) or its complement; or
- (k) a small molecule that competes with the binding of a DNABII protein or polypeptide to a microbial DNA.

Also provided herein is a method for inducing an immune response in or conferring passive immunity on a subject in need thereof, comprising, or alternatively consisting essentially of, or yet further consisting of, administering to the subject an effective amount of one or more agents of the group:

- (a) an isolated or recombinant integration host factor (IHF) polypeptide, or a fragment or an equivalent of each thereof;
- (b) an isolated or recombinant histone-like protein from *E.coli* strain U93 (HU) polypeptide or a fragment or an equivalent of each thereof;
- (c) an isolated or recombinant protein polypeptide identified in Table 8, Table 9A, Table 9B, Table 10 or an DNA binding peptide identified in Figure 6, or a fragment or an equivalent of each thereof;
- (d) an isolated or recombinant polypeptide of SEQ ID NO. 1 through 340, or a fragment or an equivalent thereof;

(e) an isolated or recombinant C-terminal polypeptide of SEQ ID NO. 6 through 11, 28, 29, 42 through 100, Table 8 or those C-terminal polypeptides identified in Table 10 or a fragment or an equivalent of each thereof;

(f) an isolated or recombinant polynucleotide encoding any one of (a) through (e) or an isolated or recombinant polynucleotide of SEQ ID NO. 36 or an equivalent of each thereof, or a polynucleotide that hybridizes under stringent conditions to the polynucleotide, its equivalent or its complement;

(g) an antibody or antigen binding fragment that specifically recognizes or binds any one of (a) through (e), or an equivalent or fragment of each thereof

(h) an isolated or recombinant polynucleotide encoding the antibody or antigen binding fragment of (g).

(i) an antigen presenting cell pulsed with any one of (a) through (e); and

(j) an antigen presenting cell transfected with one or more polynucleotides encoding any one of (a) through (e).

Subjects in need of such immune response include those at risk of or suffering from an infection that produces a microbial biofilm.

Also provided herein are compositions for use in the above methods, non-limiting examples of which are discussed below.

In one aspect, provided is an isolated or recombinant polypeptide comprising, or alternatively consisting essentially of of an amino acid sequence selected from SEQ ID NO. 1 to 5 or 12 to 27, 30 to 35, 101-340 or a DNA binding peptide identified in Figure 6 or a fragment or an equivalent of each thereof.

In another aspect, provided is an isolated or recombinant polypeptide comprising, or alternatively consisting essentially of, or yet further consisting of, SEQ ID NO. 1 or 2, with the proviso that the polypeptide is none of SEQ ID NO. 6 to 11, 28, 29, or 42 through 100.

In one aspect, provided is an isolated or recombinant polypeptide comprising, or alternatively consisting essentially of, or yet further consisting of, SEQ ID NO. 3, 4 or 5, with the proviso that the polypeptide is none of SEQ ID NO. 6 to 11, 28, 29, or 42 through 100.

In one aspect, provided is an isolated or recombinant polypeptide comprising, or alternatively consisting essentially of, or yet further consisting of, SEQ ID NO. 12, 14, 16, 18, 20, 22, 24,

26, 30 or 32, with the proviso that the polypeptide is none of SEQ ID NO. 6 to 11, 28, 29, or 42 through 100.

In one aspect, provided is an isolated or recombinant polypeptide comprising, or alternatively consisting essentially of, or yet further consisting of SEQ ID NO. 13, 15, 17, 19, 21, 23, 25, 27, 31 or 33, with the proviso that the polypeptide is none of SEQ ID NO. 6 to 11, 28, 29, or 42 through 100.

In one aspect, provided is an isolated or recombinant polypeptide comprising, or alternatively consisting essentially of, or yet further consisting of SEQ ID NO. 337, 338, 339, or 340, with the proviso that the polypeptide is none of SEQ ID NO. 6 to 11, 28, 29, or 42 through 100.

In one aspect, provided is an isolated or recombinant polypeptide comprising, or alternatively consisting essentially of, or yet further consisting of, SEQ ID NO. 12 and 13 or 14 and 15 or 16 and 17 or 18 and 19 or 20 and 21 or 22 and 23 or 24 and 25, or 26 and 27 or 30 and 31 or 32 and 33, with the proviso that the polypeptide is none of SEQ ID NO. 6 to 11, 28, 29, or 42 through 100.

In one aspect, provided is an isolated or recombinant polypeptide comprising, or alternatively consisting essentially of, or yet further consisting of, the C-terminal region containing at least 10, or alternatively at least 15, or alternatively at least 20, or alternatively at least 25, or alternatively at least 30, C-terminal amino acids of a polypeptide of the group of a DNABII polypeptide, an IHF polypeptide, an HU polypeptide, SEQ ID NO. 6 through 11, 28, 29 or those identified in Table 8, Table 10 or a fragment or an equivalent of each thereof.

In one aspect, provided is an isolated or recombinant polypeptide of the group of:

a polypeptide comprising SEQ ID NO. 12 and 13;

a polypeptide comprising SEQ ID NO. 14 and 15;

a polypeptide comprising SEQ ID NO. 16 and 17;

a polypeptide comprising SEQ ID NO. 18 and 19;

a polypeptide comprising SEQ ID NO. 20 and 21;

a polypeptide comprising SEQ ID NO. 23 and 24;

a polypeptide comprising SEQ ID NO. 25 and 26;

a polypeptide comprising SEQ ID NO. 30 and 31;

- a polypeptide comprising SEQ ID NO. 32 and 33;
- a polypeptide comprising SEQ ID NO. 34 and 35;
- a polypeptide comprising SEQ ID NO. 337 and 338; or
- a polypeptide comprising SEQ ID NO. 339 and 340;

with the proviso that the polypeptide is none of wild-type of any one of IHF alpha, IHF beta or SEQ ID NO. 6 to 11, 28, 29, or 42 through 100.

[0001] In one aspect, provided is an isolated or recombinant polypeptide of the group of:

- a polypeptide consisting essentially of SEQ ID NO. 12 and 13;
- a polypeptide consisting essentially of SEQ ID NO. 14 and 15;
- a polypeptide consisting essentially of SEQ ID NO. 16 and 17;
- a polypeptide consisting essentially of SEQ ID NO. 18 and 19;
- a polypeptide consisting essentially of SEQ ID NO. 20 and 21;
- a polypeptide consisting essentially of SEQ ID NO. 23 and 24;
- a polypeptide consisting essentially of SEQ ID NO. 25 and 26;
- a polypeptide consisting essentially of SEQ ID NO. 30 and 31;
- a polypeptide consisting essentially of SEQ ID NO. 32 and 33;
- a polypeptide consisting essentially of SEQ ID NO. 34 and 35;
- a polypeptide consisting essentially of SEQ ID NO. 337 and 338; or
- a polypeptide consisting essentially of SEQ ID NO. 339 and 340;

with the proviso that the polypeptide is none of wild-type of any one of IHF alpha, IHF beta or SEQ ID NO. 6 to 11, 28, 29, or 42 through 100.

Also provided are isolated or recombinant polypeptides comprising, or alternatively consisting essentially of, or yet further consisting of, two or more, or three or more or four or more, or multiples of the above-identified isolated polypeptides, including fragments and equivalents thereof. Examples of such include isolated polypeptides comprising SEQ ID NO. 1 through 4 and/or 12 through 29, and/or 30 through 33, and/or 30 through 35 e.g., SEQ ID NO. 1 and 2, or alternatively 1 and 3 or alternatively 1 and 4, or alternatively 2 and 3, or alternatively SEQ ID NO. 1, 2 and 3 or alternatively, 2, 3 and 4, or alternatively 1, 3 and 4 or

equivalent polypeptides, examples of which are shown in Table 9. The polypeptides can be in any orientation, e.g., SEQ ID NO. 1, 2, and 3 or SEQ ID NO. 3, 2 and 1 or alternatively SEQ ID NO. 2, 1 and 3, or alternatively, 3, 1 and 2, or alternatively 11 and 12, or alternatively 1 and 12, or alternatively 2 and 12, or alternatively, 1 and 12, or alternatively 2 and 13, or alternatively 12, 16 and 1, or alternatively 1, 16 and 12.

In another aspect, this invention provides an isolated or recombinant polypeptide comprising SEQ ID NO. 1 or 2 and 3 or 4 or a polypeptide or recombinant polypeptide comprising, or alternatively consisting essentially of, or yet further consisting of an amino acid corresponding to fragments of a DNABII protein such as the β -3 and/or α -3 fragments of a *Haemophilus influenzae* IHFa or IHF β microorganism, non-limiting examples of which include SEQ ID NO. 12 through 27, or a fragment or an equivalent of each of the polypeptides, examples of which are shown in Table 9. In one aspect, isolated wildtype polypeptides are specifically excluded, e.g. that the polypeptide is none of SEQ ID NO. 6 through 11 or a wildtype sequence identified in Table 8. In this embodiment, SEQ ID NO. 1 or 2 or a polypeptide comprising, or alternatively consisting essentially of, or yet further consisting of an amino acid corresponding to the β -3 and/or α -3 fragments of an IHFa or IHF β microorganism, non-limiting examples of which include SEQ ID NO. 12 through 27 and 30 through 33 or an equivalent of each thereof is located upstream or amino terminus from SEQ ID NO. 3 or 4 or a fragment or an equivalent thereof. In another aspect, the isolated polypeptide comprises SEQ ID NO. 3 or 4 or a polypeptide comprising, or alternatively consisting essentially of, or yet further consisting of an amino acid corresponding to the β -3 and/or α -3 fragments of an IHFa or IHF β microorganism, non-limiting examples of which include SEQ ID NO. 12 through 27, or an equivalent thereof located upstream or amino terminus to SEQ ID. NO. 1 or 2 or an equivalent thereof.

In any of the above embodiments, a peptide linker can be added to the N-terminus or C-terminus of the polypeptide, fragment or equivalent thereof. In one aspect, the linker joins the polypeptides of this invention, e.g., SEQ ID NO. 1 to 4, 28, 29, 34, or 35 or 30 to 33, 34, or 35 or a polypeptide comprising, or alternatively consisting essentially of, or yet further consisting of an amino acid corresponding to the β -3 and/or α -3 fragments of a *Haemophilus influenzae* IHFa or IHF β microorganism, non-limiting examples of which include SEQ ID NO. 12 through 27 or an equivalent of each thereof. A “linker” or “peptide linker” refers to a peptide sequence linked to either the N-terminus or the C-terminus of a polypeptide sequence. In one aspect, the linker is from about 1 to about 20 amino acid residues long or

alternatively 2 to about 10, about 3 to about 5 amino acid residues long. An example of a peptide linker is Gly-Pro-Ser-Leu-Lys-Leu (SEQ ID NO: 37).

Further provided is a fragment or an equivalent of the isolated or recombinant polypeptide of any one of polypeptides identified above as well as an isolated or recombinant polypeptide comprising, or alternatively consisting essentially of, or yet further consisting of, two or more of the isolated or recombinant polypeptides identified above.

Yet further provided is a polynucleotide that interferes with the binding of the microbial DNA with a polypeptide or fragment or equivalent thereof, e.g., SEQ ID 36, or a four-way junction polynucleotide resembling a Holliday junction, a 3 way junction polynucleotide resembling a replication fork, a polynucleotide that has inherent flexibility or bent polynucleotide; an isolated or recombinant polynucleotide encoding a polypeptide described above or an antibody or fragment thereof, which can be operatively linked to regulatory elements necessary for the expression and/or replication of the polynucleotide. The polynucleotide can be contained within a vector.

Also provided is an isolated host cell comprising, or alternatively consisting essentially of, or yet further consisting of an isolated or recombinant polypeptide described above, a four-way junction polynucleotide resembling a Holliday junction, a 3 way junction polynucleotide resembling a replication fork, a polynucleotide that has inherent flexibility or bent polynucleotide; an isolated or recombinant polynucleotide as described above, or a vector as described above.

In one aspect the cell is an isolated antigen presenting cell comprising the isolated or recombinant polypeptide. In a further aspect, the polypeptide is present on the surface of the cell, such as a dendritic cell. In a further aspect, the antigen presenting cell is transfected with one or more polynucleotides encoding the polypeptide.

Yet further provided is an antibody or antigen binding fragment that specifically recognizes and binds the isolated or recombinant polypeptide as describe above, including a fragment or an equivalent of the polypeptide. Non-limiting examples of antibodies include a polyclonal antibody, a monoclonal antibody, a humanized antibody, a human antibody, an antibody derivative, a veneered antibody, a diabody, a chimeric antibody, an antibody derivative, a recombinant human antibody, or an antibody fragment. In a particular aspect, the antibody is a monoclonal antibody. Yet further provided is a hybridoma cell line that produces the monoclonal antibody.

This invention also provides isolated or recombinant polynucleotides encoding one or more of the above-identified isolated or recombinant polypeptides or antibodies or a fragment thereof. Vectors comprising the isolated polynucleotides are further provided. In one aspect where more than one isolated polypeptide of this invention, the isolated polynucleotides can be contained within a polycistronic vector.

Isolated host cells comprising one or more of isolated or recombinant polypeptides or isolated or recombinant polynucleotides or the vectors, described herein are further provided. In one aspect the isolated host cell is a prokaryotic cell or eukaryotic cell such as antigen presenting cell, e.g. a dendritic cell.

The polynucleotides, polypeptides, antibodies, antigen binding fragment, vectors or host cells can further comprise a detectable label.

Compositions comprising a carrier and one or more of an isolated or recombinant polypeptide of the invention, an isolated or recombinant polynucleotide of the invention, a vector of the invention, an isolated host cell of the invention, or an antibody of the embodiments are also provided. The carriers can be one or more of a solid support, a medical device like a stent or dental implant, or a liquid such as a pharmaceutically acceptable carrier. The compositions can further comprise an adjuvant, an antimicrobial or an antigenic peptide.

The compositions can further comprise additional biologically active agents. A non-limiting example of such is a antimicrobial agent such as other vaccine components (i.e., antigenic peptides) such as surface antigens, e.g. an OMP P5, rsPilA, OMP 26, OMP P2, or Type IV Pilin protein (see Jurcisek and Bakaletz (2007) J. of Bacteriology 189(10):3868-3875 and Murphy, TF, Bakaletz, LO and Smeesters, PR (2009) The Pediatric Infectious Disease Journal, 28:S121-S126) and antimicrobial agents.

This invention also provides a method for producing an antigenic peptide by growing or culturing a host cell comprising an isolated polynucleotide encoding an antigenic peptide as described above under conditions that favor the expression of the polynucleotide. The polypeptide produced by this method can be isolating for further in vitro or in vivo use.

A kit is also provided for diagnostic or therapeutic use comprising a composition as described above and instructions for use. A kit is also provided to perform screens for new drugs and/or combination therapies as provided herein.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1A is a biofilm formed in the chinchilla middle ear by NTHI strain 86-028NP and labeled for NTHI Tfp pilin protein (appears as white or light gray speckles and small clusters in the background of this image), as well as with DAPI for labeling of the double stranded DNA (dsDNA) (appears as dark gray overlapping strands and bundles of material with intermittent clumps in the foreground of this image). This figure has been reproduced from Jurcisek and Bakaletz (2007) *J. of Bacteriology* 189(10):3868-3875. **Figure 1B** is immunolabeling of bronchoalveolar lavage (BAL) from the lung of a child with cystic fibrosis. The lung of a child with cystic fibrosis was washed out via BAL and the particulate matter from the wash was frozen and affixed to slides for immunolabeling. The frozen particulate matter was immunolabeled with the anti-IHF antibody. The presence of IHF positive foci in the biofilm of human cystic fibrosis patients exemplifies that the etiology of human cystic fibrosis includes a biofilm with IHF at the vertices of the dsDNA. **Figure 1C** shows secretions from human sinus collected at the time of sinus surgery and embedded in OCT freezing medium (Optimal Cutting Temperature medium, available commercially from Fisher Scientific Cat. No. 14-373-65). 10 μ m frozen sections were cut and labeled with anti-IHF (appearing as gray clusters). dsDNA within the sample was stained with a fluorescent stain, DAPI (4',6-diamidino-2-phenylindole, available commercially from Invitrogen). The presence of IHF positive foci in the biofilm of sinusitis patients exemplifies that the etiology of human sinusitis includes a biofilm with IHF at the vertices of the dsDNA.

Figure 2 is a immunohistochemical labeling of double stranded DNA (appears as white strands in this image) within an NTHI biofilm formed in the middle ear of chinchilla. Positive labeling for IHF (as indicated by arrows pointing to punctuate foci in the middle panel of this 3-panel image) was observed at nearly 100% of vertices formed by dsDNA. Mean distance between vertices was approximately 6 μ m, or approximately 18 kb between each vertex if one assumes 0.34 nm per base of DNA for B-form DNA. To the best of our knowledge, the only proteins that possess epitopes that cross-react with anti-IHF are HU and IHF. Therefore, based on these observations, it appeared that not only were there extracellular DNABII proteins within the NTHI biofilm matrix, but more importantly that these proteins appeared to be exclusively positioned on eDNA strands that resembled cruciform structures in conformation (see Fig. 1 Panel B, bottom section), thus strongly suggesting their role in mediating the resulting bent conformation of the eDNA.

Figure 3 shows that antibodies directed against IHF reversed an established NTHI biofilm formed in a chamber slide. **Figure 3A** shows a biofilm treated with non specific antibody.

Figure 3B shows a biofilm treated with naïve rabbit serum. Note the robust NTHI biofilm with abundant towers (appear as white to light gray clustered areas) and water channels (black spaces). Figure 3C is a biofilm treated with anti-IHF. Note the eradication of biofilm structure after treatment with an anti-IHF. Individual NTHI (appear as small punctuate white to light gray spots) and sparse, short towers (appear as denser white to light gray clustered areas) remain. Figure 3D and 3E further depict that antibodies directed against IHF reverse an established NTHI biofilm. As shown in Figure 3 (Panel E), the biofilm showed a dramatic loss of 3-dimensional structure when compared to a biofilm incubated with naïve serum (Fig. 3, Panel D). Via COMSTAT analysis of multiple replicate assays, the measured parameters of biofilm height, biomass and biofilm thickness were all diminished by a mean of greater than 80% upon incubation with anti-IHF.

Figure 4A and Figure 4B are graphs showing treatment of an established biofilm formed by NTHI with anti-IHF results in more NTHI released into the supernatant. Sixteen (16) hour NTHI biofilms grown in chamber slides were sham treated with sterile medium (sBHI) or treated with naïve rabbit serum or rabbit anti-IHF. Six hours later (Figure 4A) or 10 hours later (Figure 4B), supernatants were collected and analyzed. Note the greater number of NTHI in supernatant after treatment with anti-IHF. Incubation with anti-IHF resulted in a marked increase in planktonic bacteria available for culture from the medium within the chamber slide within approximately 6 hrs, and increasing notably at 10 hours of incubation. These results suggested release of bacteria from the biofilm matrix.

Figure 5 shows the results of a transcutaneous immunization with IHF reduced an established biofilm in the middle ear. Note that bullae were blindly ranked onto a 0 to 4+ scale of relative remaining biofilm mass.

Figure 6A is a map indicating the amino acid residues of IHF that interact with or bind to another IHF in an IHF-IHF dimer (indicated by triangles at the upper level) or interact with or bind DNA (indicated by triangles at the lower level). The peptide is divided by the short vertical bars into regions containing 3 amino acids.

Figure 6B graphically depicts the interaction of microbial DNA with an IHF.

Figure 7 depicts the reduction of biofilms formed by *S. aureus*, *N. gonorrhoeae* and *P. aeruginosa* upon incubation with rabbit anti-IHF compared to incubation with naïve rabbit serum.

Figure 8 shows the effect of incubation of biofilms formed *in vitro* by *E. coli* with either naïve serum or with anti-IHF serum. Representative images of biofilms are shown in Panels A-F with height of individual biofilms shown to the right of each image, whereas mean values in terms of percent reduction in biofilm jheight, biomass and mean thickness as mediated by incubation with anti-IHF are shown in the tables at the end of each row. Panels A & B – parental strain MG1655; Panels C&D – HU-deficient *hupA*, *hupB* double mutant; Panels E&F – IHF-deficient *himD*, *himA* double mutant. Note the ability of anti-IHF serum to reduce biomass induced by either the parental isolate or the HU-deficient mutant, but not the IHF-deficient strain, as expected.

Figure 9A demonstrates that immunization with IHF via a transcutaneous delivery route induced the formation of antibodies that significantly reduced the biomass of an NTHI-induced biofilm resident within the middle ears of chinchillas ($p < 0.001$).

Figure 9B depicts representative images of biomasses that remained in the ears of animals immunized with adjuvant alone versus those immunized with IHF + adjuvant. Last column in Fig 9B shows images of biomasses at the extremes of the scoring system used here. Top image is that of a middle ear that contains a biomass that would receive a score of 4+, whereas lower image is a healthy middle ear that would receive a score of 0, indicating no biomass.

Figure 10A shows H&E staining of a frozen section of a biofilm recovered from the middle ear of a chinchilla that had been immunized via TCI with adjuvant alone versus immunization with IHF + adjuvant. Note condensed and collapsed appearance of biofilm recovered from the animal immunized with IHF + adjuvant compared to that immunized with adjuvant alone. Images shown in Panel A are shown at identical magnifications to illustrate the differences in height and density between the two representative biomasses.

Figure 10B demonstrates that there is a significant reduction of bacterial load present in the middle ears of animals immunized with adjuvant alone versus those immunized with IHF + adjuvant ($p < 0.05$).

Figure 11 depicts an electrophoretic mobility shift assay which demonstrated that IHF formed specific complexes with dsDNA under conditions used to immunize chinchillas.

Figure 12 graphically represents transcutaneous immunization with native IHF + adjuvant induced the formation of antibodies that significantly reduced the biomass resident within the middle ears of chinchillas compared to receipt of either adjuvant alone ($p < 0.017$), dsDNA

alone ($p < 0.003$) or IHF to which dsDNA was already bound + adjuvant ($p < 0.001$). This outcome suggested that binding of dsDNA to native IHF masked protective epitopes of this DNABII family member.

Figure 13 depicts the recognition of IHF (arrows) by antibody in serum after subcutaneous immunization with IHF or with IHF to which dsDNA was bound.

Figure 14. is a demonstration of synergism between a sub-optimal concentration of anti-IHF serum (1:200) and DNaseI individually, then mixed (Figure 14A); a sub-optimal concentration of anti-IHF (1:100) and anti-outer membrane protein P5 serum (OMP P5) individually, then mixed (Figure 14B); or that of an effective dilution of anti-IHF (in terms of debulking a biofilm but *not* inducing bacterial cell death) and amoxicillin individually, then mixed (Figure 14C). In each of these situations, when any agent was combined with anti-IHF, the biofilm debulking and/or killing effect observed was greater than that noted when any single agent was used alone. Note: biofilm height (in microns) is indicated under each image.

Figure 15 is a comparison of *E. coli* treated with either naive rabbit serum or rabbit anti-IHF serum (top row of images); with naive rat serum or rat anti-HNS serum (middle row of images); or with naive mouse serum or with mouse anti-DPS serum (bottom row of images). Note marked reduction in biomass following treatment with anti-IHF serum, however neither anti-HNS nor anti-DPS serum induced a reduction in biomass as used herein. Treatment with anti-IHF resulted in a 48.2% reduction in the biofilm height, an 81% reduction in the biofilm average thickness, and a 64.5% reduction in the biomass. In contrast treatment with the anti-HNS or anti-DPS resulted in a nominal height reduction of 0.7% and 4.2%, respectively, a reduction in the average thickness of 5.8% and 6.4% respectively, and a reduction in the biomass of 0.3% and -17.4% respectively.

Figure 16 shows *in situ* hybridization to demonstrate relative spatial distribution of DNA from either a host organism or from the bacterium when organized within a biofilm. In each circumstance, DNA appears as brighter, whiter areas within the foreground of these black and white images. DNA from the host is more densely labeled within the upper right hand image, demonstrating its more heavy distribution on the outer periphery of the biofilm, whereas DNA from the bacterium is more densely labeled in the lower left hand image of this 4-panel composite, thereby demonstrating its more dense distribution within the inner reaches of the biofilm.

DETAILED DESCRIPTION OF THE INVENTION

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. All nucleotide sequences provided herein are presented in the 5' to 3' direction. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods, devices, and materials are now described. All technical and patent publications cited herein are incorporated herein by reference in their entirety. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of tissue culture, immunology, molecular biology, microbiology, cell biology and recombinant DNA, which are within the skill of the art. See, e.g., Sambrook and Russell eds. (2001) *Molecular Cloning: A Laboratory Manual*, 3rd edition; the series Ausubel et al. eds. (2007) *Current Protocols in Molecular Biology*; the series *Methods in Enzymology* (Academic Press, Inc., N.Y.); MacPherson et al. (1991) *PCR 1: A Practical Approach* (IRL Press at Oxford University Press); MacPherson et al. (1995) *PCR 2: A Practical Approach*; Harlow and Lane eds. (1999) *Antibodies, A Laboratory Manual*; Freshney (2005) *Culture of Animal Cells: A Manual of Basic Technique*, 5th edition; Gait ed. (1984) *Oligonucleotide Synthesis*; U.S. Patent No. 4,683,195; Hames and Higgins eds. (1984) *Nucleic Acid Hybridization*; Anderson (1999) *Nucleic Acid Hybridization*; Hames and Higgins eds. (1984) *Transcription and Translation; Immobilized Cells and Enzymes* (IRL Press (1986)); Perbal (1984) *A Practical Guide to Molecular Cloning*; Miller and Calos eds. (1987) *Gene Transfer Vectors for Mammalian Cells* (Cold Spring Harbor Laboratory); Makrides ed. (2003) *Gene Transfer and Expression in Mammalian Cells*; Mayer and Walker eds. (1987) *Immunochemical Methods in Cell and Molecular Biology* (Academic Press, London); and Herzenberg et al. eds (1996) *Weir's Handbook of Experimental Immunology*.

All numerical designations, e.g., pH, temperature, time, concentration, and molecular weight, including ranges, are approximations which are varied (+) or (-) by increments of 1.0 or 0.1, as appropriate or alternatively by a variation of +/- 15 %, or alternatively 10% or alternatively 5% or alternatively 2%. It is to be understood, although not always explicitly stated, that all numerical designations are preceded by the term "about". It also is to be

understood, although not always explicitly stated, that the reagents described herein are merely exemplary and that equivalents of such are known in the art.

As used in the specification and claims, the singular form “a”, “an” and “the” include plural references unless the context clearly dictates otherwise. For example, the term “a polypeptide” includes a plurality of polypeptides, including mixtures thereof.

As used herein, the term “comprising” is intended to mean that the compositions and methods include the recited elements, but do not exclude others. “Consisting essentially of” when used to define compositions and methods, shall mean excluding other elements of any essential significance to the combination for the intended use. Thus, a composition consisting essentially of the elements as defined herein would not exclude trace contaminants from the isolation and purification method and pharmaceutically acceptable carriers, such as phosphate buffered saline, preservatives, and the like. “Consisting of” shall mean excluding more than trace elements of other ingredients and substantial method steps for administering the compositions of this invention. Embodiments defined by each of these transition terms are within the scope of this invention.

A “biofilm” intends an organized community of microorganisms that at times adhere to the surface of a structure, that may be organic or inorganic, together with the polymers such as DNA that they secrete and/or release. The biofilms are very resistant to microbials and antimicrobial agents. They live on gingival tissues, teeth and restorations, causing caries and periodontal disease, also known as periodontal plaque disease. They also cause chronic middle ear infections. Biofilms can also form on the surface of dental implants, stents, catheter lines and contact lenses. They grow on pacemakers, heart valve replacements, artificial joints and other surgical implants. The Centers for Disease Control) estimate that over 65% of nosocomial (hospital-acquired) infections are caused by biofilms. They cause chronic vaginal infections and lead to life-threatening systemic infections in people with hobbled immune systems. Biofilms also are involved in numerous diseases. For instance, cystic fibrosis patients have *Pseudomonas* infections that often result in antibiotic resistant biofilms.

The term “inhibiting, competing or titrating” intends a reduction in the formation of the DNA/protein matrix (for example as shown in Figure 1) that is a component of a microbial biofilm.

A “DNABII polypeptide or protein” intends a DNA binding protein or polypeptide that is composed of DNA-binding domains and thus have a specific or general affinity for microbial DNA. In one aspect, they bind DNA in the minor groove. Non-limiting examples of DNABII proteins are an integration host factor (IHF) protein and a histone-like protein from *E. coli* strain U93 (HU). Other DNA binding proteins that may be associated with the biofilm include DPS (Genbank Accession No.: CAA49169), H-NS (Genbank Accession No.: CAA47740), Hfq (Genbank Accession No.: ACE63256), CbpA (Genbank Accession No.: BAA03950) and CbpB (Genbank Accession No.: NP_418813).

An “integration host factor” or “IHF” protein is a bacterial protein that is used by bacteriophages to incorporate their DNA into the host bacteria. They also bind extracellular microbial DNA. The genes that encode the IHF protein subunits in *E. coli* are *himA* (Genbank accession No.: POA6X7.1) and *himD* (POA6Y1.1) genes. Homologs for these genes are found in other organisms, and peptides corresponding to these genes from other organisms can be found in Table 10.

“HMGB1” is an high mobility group box (HMGB) 1 protein that is reported to bind to and distort the minor groove of DNA and is an example of an interfering agent. Recombinant or isolated protein and polypeptide are commercially available from Atgenglobal, ProSpecBio, Protein1 and Abnova.

“HU” or “histone-like protein from *E. coli* strain U93” refers to a class of heterodimeric proteins typically associate with *E. coli*. HU proteins are known to bind DNA junctions. Related proteins have been isolated from other microorganisms. The complete amino acid sequence of *E. coli* HU was reported by Laine et al. (1980) Eur. J. Biochem. 103(3):447-481. Antibodies to the HU protein are commercially available from Abcam. The genes that encode the HU protein subunits in *E. coli* are *hupA* and *hupB* corresponding to SEQ ID Nos: 28 and 29, respectively. Homologs for these genes are found in other organisms, and peptides corresponding to these genes from other organisms can be found in Table 10.

The term “surface antigens” or “surface proteins” refers to proteins or peptides on the surface of cells such as bacterial cells. Examples of surface antigens are Outermembrane proteins such as OMP P5 (Genbank accession No.: YP_004139079.1), OMP P2 (Genbank accession No.: ZZX87199.1), OMP P26 (Genbank Accession No.: YP_665091.1), rsPilA or recombinant soluble PilA (Genbank accession No.: EFU96734.1) and Type IV Pilin (Genbank accession No.: YP_003864351.1).

The term “*Haemophilus influenzae*” refers to pathogenic bacteria that can cause many different infections such as, for example, ear infections, eye infections, and sinusitis. Many different strains of *Haemophilus influenzae* have been isolated and have an IhfA gene or protein. Some non-limiting examples of different strains of *Haemophilus influenzae* include Rd KW20, 86-028NP, R2866, PittGG, PittEE, R2846, and 2019.

“Microbial DNA” intends single or double stranded DNA from a microorganism that produces a biofilm.

“Inhibiting, preventing or breaking down” a biofilm intends the prophylactic or therapeutic reduction in the structure of a biofilm. An example of breaking down or reducing a biofilm is shown in Figure 5.

An “interfering agent” intends an agent that any one or more of competes, inhibits, prevents, titrates a DNABII polypeptide such as IHF to a microbial DNA or also breaks down a microbial biofilm. It can be any one or more of a chemical or biological molecule. For example, IHF can specifically bind, bend or distorted DNA structures such as DNA containing four-way junctions, cis-platinum adducts, DNA loop or base bulges. Examples of such agents, without limitation, include (1) small molecules that inhibit the DNA-binding activity of IHF, (2) small molecules such as polyamines and spermine that compete with IHF in DNA binding, (3) polypeptides such as peptide fragments of IHF that compete with IHF in DNA binding, (4) antibodies or fragments thereof directed to IHF, or (5) a four-way or bent polynucleotides or other types of polynucleotides containing bent or distorted DNA structures that compete in IHF-binding. A “small molecule that inhibits the binding of an IHF to a nucleic acid” refers to (1) or (2) above and include those that bind DNA in the minor groove, i.e., minor groove binding molecules. A “four-way polynucleotide” intends a polynucleotide that contains a four-way junction, also known as the Holliday junction, between four strands of DNA.

A “bent polynucleotide” intends a double strand polynucleotide that contains a small loop on one strand which does not pair with the other strand. In some embodiments, the loop is from 1 base to about 20 bases long, or alternatively from 2 bases to about 15 bases long, or alternatively from about 3 bases to about 12 bases long, or alternatively from about 4 bases to about 10 bases long, or alternatively has about 4, 5, or 6, or 7, or 8, or 9, or 10 bases.

“Polypeptides that compete with IHF in DNA binding” intend proteins or peptides that compete with IHF in binding bent or distorted DNA structures but do not form a biofilm with

the DNA. Examples, without limitation, include fragments of IHF that include one or more DNA binding domains of the IHF, or the biological equivalents thereof. DNA binding domains are shown in Figure 6.

A “subject” of diagnosis or treatment is a cell or an animal such as a mammal, or a human. Non-human animals subject to diagnosis or treatment and are those subject to infections or animal models, for example, simians, murines, such as, rats, mice, chinchilla, canine, such as dogs, leporids, such as rabbits, livestock, sport animals, and pets.

The term “protein”, “peptide” and “polypeptide” are used interchangeably and in their broadest sense to refer to a compound of two or more subunit amino acids, amino acid analogs or peptidomimetics. The subunits may be linked by peptide bonds. In another embodiment, the subunit may be linked by other bonds, e.g., ester, ether, etc. A protein or peptide must contain at least two amino acids and no limitation is placed on the maximum number of amino acids which may comprise a protein’s or peptide’s sequence. As used herein the term “amino acid” refers to either natural and/or unnatural or synthetic amino acids, including glycine and both the D and L optical isomers, amino acid analogs and peptidomimetics.

A “C-terminal polypeptide” intends at least the 10, or alternatively at least the 15, or alternatively at least 20, or at least the 25 C-terminal amino acids or alternatively half of a polypeptide. In another aspect, for polypeptides containing 90 amino acids, the C-terminal polypeptide would comprise amino acids 46 through 90. In one aspect, the term intends the C-terminal 20 amino acids from the carboxy terminus.

The terms “polynucleotide” and “oligonucleotide” are used interchangeably and refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides or analogs thereof. Polynucleotides can have any three-dimensional structure and may perform any function, known or unknown. The following are non-limiting examples of polynucleotides: a gene or gene fragment (for example, a probe, primer, EST or SAGE tag), exons, introns, messenger RNA (mRNA), transfer RNA, ribosomal RNA, RNAi, ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes and primers. A polynucleotide can comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure can be imparted before or after assembly of the polynucleotide. The sequence of nucleotides can be

interrupted by non-nucleotide components. A polynucleotide can be further modified after polymerization, such as by conjugation with a labeling component. The term also refers to both double- and single-stranded molecules. Unless otherwise specified or required, any embodiment of this invention that is a polynucleotide encompasses both the double-stranded form and each of two complementary single-stranded forms known or predicted to make up the double-stranded form.

A polynucleotide is composed of a specific sequence of four nucleotide bases: adenine (A); cytosine (C); guanine (G); thymine (T); and uracil (U) for thymine when the polynucleotide is RNA. Thus, the term “polynucleotide sequence” is the alphabetical representation of a polynucleotide molecule. This alphabetical representation can be input into databases in a computer having a central processing unit and used for bioinformatics applications such as functional genomics and homology searching.

The term “isolated” or “recombinant” as used herein with respect to nucleic acids, such as DNA or RNA, refers to molecules separated from other DNAs or RNAs, respectively that are present in the natural source of the macromolecule as well as polypeptides. The term “isolated or recombinant nucleic acid” is meant to include nucleic acid fragments which are not naturally occurring as fragments and would not be found in the natural state. The term “isolated” is also used herein to refer to polynucleotides, polypeptides and proteins that are isolated from other cellular proteins and is meant to encompass both purified and recombinant polypeptides. In other embodiments, the term “isolated or recombinant” means separated from constituents, cellular and otherwise, in which the cell, tissue, polynucleotide, peptide, polypeptide, protein, antibody or fragment(s) thereof, which are normally associated in nature. For example, an isolated cell is a cell that is separated from tissue or cells of dissimilar phenotype or genotype. An isolated polynucleotide is separated from the 3' and 5' contiguous nucleotides with which it is normally associated in its native or natural environment, e.g., on the chromosome. As is apparent to those of skill in the art, a non-naturally occurring polynucleotide, peptide, polypeptide, protein, antibody or fragment(s) thereof, does not require “isolation” to distinguish it from its naturally occurring counterpart.

It is to be inferred without explicit recitation and unless otherwise intended, that when the present invention relates to a polypeptide, protein, polynucleotide or antibody, an equivalent or a biologically equivalent of such is intended within the scope of this invention. As used herein, the term “biological equivalent thereof” is intended to be synonymous with “equivalent thereof” when referring to a reference protein, antibody, fragment, polypeptide or

nucleic acid, intends those having minimal homology while still maintaining desired structure or functionality. Unless specifically recited herein, it is contemplated that any polynucleotide, polypeptide or protein mentioned herein also includes equivalents thereof. In one aspect, an equivalent polynucleotide is one that hybridizes under stringent conditions to the polynucleotide or complement of the polynucleotide as described herein for use in the described methods. In another aspect, an equivalent antibody or antigen binding polypeptide intends one that binds with at least 70 %, or alternatively at least 75 %, or alternatively at least 80 %, or alternatively at least 85 %, or alternatively at least 90 %, or alternatively at least 95 % affinity or higher affinity to a reference antibody or antigen binding fragment. In another aspect, the equivalent thereof competes with the binding of the antibody or antigen binding fragment to its antigen under a competitive ELISA assay. In another aspect, an equivalent intends at least about 80 % homology or identity and alternatively, at least about 85 %, or alternatively at least about 90 %, or alternatively at least about 95 %, or alternatively 98 % percent homology or identity and exhibits substantially equivalent biological activity to the reference protein, polypeptide or nucleic acid. Examples of biologically equivalent polypeptides are provided in Table 9 which identify conservative amino acid substitutions to the preferred amino acid sequences.

A polynucleotide or polynucleotide region (or a polypeptide or polypeptide region) having a certain percentage (for example, 80%, 85%, 90%, or 95%) of “sequence identity” to another sequence means that, when aligned, that percentage of bases (or amino acids) are the same in comparing the two sequences. The alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in Current Protocols in Molecular Biology (Ausubel et al., eds. 1987) Supplement 30, section 7.7.18, Table 7.7.1. Preferably, default parameters are used for alignment. A preferred alignment program is BLAST, using default parameters. In particular, preferred programs are BLASTN and BLASTP, using the following default parameters: Genetic code = standard; filter = none; strand = both; cutoff = 60; expect = 10; Matrix = BLOSUM62; Descriptions = 50 sequences; sort by = HIGH SCORE; Databases = non-redundant, GenBank + EMBL + DDBJ + PDB + GenBank CDS translations + SwissProtein + SPupdate + PIR. Details of these programs can be found at the following Internet address: ncbi.nlm.nih.gov/cgi-bin/BLAST. Sequence identity and percent identity were determined by incorporating them into clustalW (available at the web address: [//align.genome.jp/](http://align.genome.jp/), last accessed on March 7, 2011).

“Homology” or “identity” or “similarity” refers to sequence similarity between two peptides or between two nucleic acid molecules. Homology can be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same base or amino acid, then the molecules are homologous at that position. A degree of homology between sequences is a function of the number of matching or homologous positions shared by the sequences. An “unrelated” or “non-homologous” sequence shares less than 40% identity, or alternatively less than 25% identity, with one of the sequences of the present invention.

“Homology” or “identity” or “similarity” can also refer to two nucleic acid molecules that hybridize under stringent conditions.

"Hybridization" refers to a reaction in which one or more polynucleotides react to form a complex that is stabilized via hydrogen bonding between the bases of the nucleotide residues. The hydrogen bonding may occur by Watson-Crick base pairing, Hoogsteen binding, or in any other sequence-specific manner. The complex may comprise two strands forming a duplex structure, three or more strands forming a multi-stranded complex, a single self-hybridizing strand, or any combination of these. A hybridization reaction may constitute a step in a more extensive process, such as the initiation of a PCR reaction, or the enzymatic cleavage of a polynucleotide by a ribozyme.

Examples of stringent hybridization conditions include: incubation temperatures of about 25°C to about 37°C; hybridization buffer concentrations of about 6x SSC to about 10x SSC; formamide concentrations of about 0% to about 25%; and wash solutions from about 4x SSC to about 8x SSC. Examples of moderate hybridization conditions include: incubation temperatures of about 40°C to about 50°C; buffer concentrations of about 9x SSC to about 2x SSC; formamide concentrations of about 30% to about 50%; and wash solutions of about 5x SSC to about 2x SSC. Examples of high stringency conditions include: incubation temperatures of about 55°C to about 68°C; buffer concentrations of about 1x SSC to about 0.1x SSC; formamide concentrations of about 55% to about 75%; and wash solutions of about 1x SSC, 0.1x SSC, or deionized water. In general, hybridization incubation times are from 5 minutes to 24 hours, with 1, 2, or more washing steps, and wash incubation times are about 1, 2, or 15 minutes. SSC is 0.15 M NaCl and 15 mM citrate buffer. It is understood that equivalents of SSC using other buffer systems can be employed.

As used herein, “expression” refers to the process by which polynucleotides are transcribed into mRNA and/or the process by which the transcribed mRNA is subsequently being translated into peptides, polypeptides, or proteins. If the polynucleotide is derived from genomic DNA, expression may include splicing of the mRNA in an eukaryotic cell.

The term “encode” as it is applied to polynucleotides refers to a polynucleotide which is said to “encode” a polypeptide if, in its native state or when manipulated by methods well known to those skilled in the art, it can be transcribed and/or translated to produce the mRNA for the polypeptide and/or a fragment thereof. The antisense strand is the complement of such a nucleic acid, and the encoding sequence can be deduced therefrom.

As used herein, the terms "treating," "treatment" and the like are used herein to mean obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disorder or sign or symptom thereof, and/or may be therapeutic in terms of a partial or complete cure for a disorder and/or adverse effect attributable to the disorder.

To prevent intends to prevent a disorder or effect in vitro or in vivo in a system or subject that is predisposed to the disorder or effect. An example of such is preventing the formation of a biofilm in a system that is infected with a microorganism known to produce one.

A “composition” is intended to mean a combination of active agent and another compound or composition, inert (for example, a detectable agent or label) or active, such as an adjuvant.

A “pharmaceutical composition” is intended to include the combination of an active agent with a carrier, inert or active, making the composition suitable for diagnostic or therapeutic use in vitro, in vivo or ex vivo.

“Pharmaceutically acceptable carriers” refers to any diluents, excipients, or carriers that may be used in the compositions of the invention. Pharmaceutically acceptable carriers include ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances, such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat. Suitable pharmaceutical carriers are described in Remington's Pharmaceutical Sciences, Mack

Publishing Company, a standard reference text in this field. They are preferably selected with respect to the intended form of administration, that is, oral tablets, capsules, elixirs, syrups and the like, and consistent with conventional pharmaceutical practices.

A “biologically active agent” or an active agent of this invention intends one or more of an isolated or recombinant polypeptide, an isolated or recombinant polynucleotide, a vector, an isolated host cell, or an antibody, as well as compositions comprising one or more of same.

“Administration” can be effected in one dose, continuously or intermittently throughout the course of treatment. Methods of determining the most effective means and dosage of administration are known to those of skill in the art and will vary with the composition used for therapy, the purpose of the therapy, the target cell being treated, and the subject being treated. Single or multiple administrations can be carried out with the dose level and pattern being selected by the treating physician. Suitable dosage formulations and methods of administering the agents are known in the art. Route of administration can also be determined and method of determining the most effective route of administration are known to those of skill in the art and will vary with the composition used for treatment, the purpose of the treatment, the health condition or disease stage of the subject being treated, and target cell or tissue. Non-limiting examples of route of administration include oral administration, nasal administration, injection, and topical application.

An agent of the present invention can be administered for therapy by any suitable route of administration. It will also be appreciated that the preferred route will vary with the condition and age of the recipient, and the disease being treated.

The term “effective amount” refers to a quantity sufficient to achieve a desired effect. In the context of therapeutic or prophylactic applications, the effective amount will depend on the type and severity of the condition at issue and the characteristics of the individual subject, such as general health, age, sex, body weight, and tolerance to pharmaceutical compositions. In the context of an immunogenic composition, in some embodiments the effective amount is the amount sufficient to result in a protective response against a pathogen. In other embodiments, the effective amount of an immunogenic composition is the amount sufficient to result in antibody generation against the antigen. In some embodiments, the effective amount is the amount required to confer passive immunity on a subject in need thereof. With respect to immunogenic compositions, in some embodiments the effective amount will depend on the intended use, the degree of immunogenicity of a particular antigenic

compound, and the health/responsiveness of the subject's immune system, in addition to the factors described above. The skilled artisan will be able to determine appropriate amounts depending on these and other factors.

In the case of an *in vitro* application, in some embodiments the effective amount will depend on the size and nature of the application in question. It will also depend on the nature and sensitivity of the *in vitro* target and the methods in use. The skilled artisan will be able to determine the effective amount based on these and other considerations. The effective amount may comprise one or more administrations of a composition depending on the embodiment.

The term "conjugated moiety" refers to a moiety that can be added to an isolated chimeric polypeptide by forming a covalent bond with a residue of chimeric polypeptide. The moiety may bond directly to a residue of the chimeric polypeptide or may form a covalent bond with a linker which in turn forms a covalent bond with a residue of the chimeric polypeptide.

A "peptide conjugate" refers to the association by covalent or non-covalent bonding of one or more polypeptides and another chemical or biological compound. In a non-limiting example, the "conjugation" of a polypeptide with a chemical compound results in improved stability or efficacy of the polypeptide for its intended purpose. In one embodiment, a peptide is conjugated to a carrier, wherein the carrier is a liposome, a micelle, or a pharmaceutically acceptable polymer.

"Liposomes" are microscopic vesicles consisting of concentric lipid bilayers. Structurally, liposomes range in size and shape from long tubes to spheres, with dimensions from a few hundred Angstroms to fractions of a millimeter. Vesicle-forming lipids are selected to achieve a specified degree of fluidity or rigidity of the final complex providing the lipid composition of the outer layer. These are neutral (cholesterol) or bipolar and include phospholipids, such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), and sphingomyelin (SM) and other types of bipolar lipids including but not limited to dioleoylphosphatidylethanolamine (DOPE), with a hydrocarbon chain length in the range of 14-22, and saturated or with one or more double C=C bonds. Examples of lipids capable of producing a stable liposome, alone, or in combination with other lipid components are phospholipids, such as hydrogenated soy phosphatidylcholine (HSPC), lecithin, phosphatidylethanolamine, lysolecithin, lysophosphatidylethanol-amine, phosphatidylserine, phosphatidylinositol, sphingomyelin, cephalin, cardiolipin, phosphatidic

acid, cerebroside, distearoylphosphatidylethanolamine (DSPE), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), palmitoyloleoylphosphatidylcholine (POPC), palmitoyloleoylphosphatidylethanolamine (POPE) and dioleoylphosphatidylethanolamine 4-(N-maleimido-methyl)cyclohexane-1-carboxylate (DOPE-mal). Additional non-phosphorous containing lipids that can become incorporated into liposomes include stearylamine, dodecylamine, hexadecylamine, isopropyl myristate, triethanolamine-lauryl sulfate, alkyl-aryl sulfate, acetyl palmitate, glycerol ricinoleate, hexadecyl stearate, amphoteric acrylic polymers, polyethoxylated fatty acid amides, and the cationic lipids mentioned above (DDAB, DODAC, DMRIE, DMTAP, DOGS, DOTAP (DOTMA), DOSPA, DPTAP, DSTAP, DC-Chol). Negatively charged lipids include phosphatidic acid (PA), dipalmitoylphosphatidylglycerol (DPPG), dioleoylphosphatidylglycerol (DOPG), dicetylphosphate that are able to form vesicles. Typically, liposomes can be divided into three categories based on their overall size and the nature of the lamellar structure. The three classifications, as developed by the New York Academy Sciences Meeting, "Liposomes and Their Use in Biology and Medicine," December 1977, are multi-lamellar vesicles (MLVs), small uni-lamellar vesicles (SUVs) and large uni-lamellar vesicles (LUVs). The biological active agents can be encapsulated in such for administration in accordance with the methods described herein.

A "micelle" is an aggregate of surfactant molecules dispersed in a liquid colloid. A typical micelle in aqueous solution forms an aggregate with the hydrophilic "head" regions in contact with surrounding solvent, sequestering the hydrophobic tail regions in the micelle center. This type of micelle is known as a normal phase micelle (oil-in-water micelle). Inverse micelles have the head groups at the center with the tails extending out (water-in-oil micelle). Micelles can be used to attach a polynucleotide, polypeptide, antibody or composition described herein to facilitate efficient delivery to the target cell or tissue.

The phrase "pharmaceutically acceptable polymer" refers to the group of compounds which can be conjugated to one or more polypeptides described here. It is contemplated that the conjugation of a polymer to the polypeptide is capable of extending the half-life of the polypeptide in vivo and in vitro. Non-limiting examples include polyethylene glycols, polyvinylpyrrolidones, polyvinylalcohols, cellulose derivatives, polyacrylates, polymethacrylates, sugars, polyols and mixtures thereof. The biological active agents can be conjugated to a pharmaceutically acceptable polymer for administration in accordance with the methods described herein.

A “gene delivery vehicle” is defined as any molecule that can carry inserted polynucleotides into a host cell. Examples of gene delivery vehicles are liposomes, micelles biocompatible polymers, including natural polymers and synthetic polymers; lipoproteins; polypeptides; polysaccharides; lipopolysaccharides; artificial viral envelopes; metal particles; and bacteria, or viruses, such as baculovirus, adenovirus and retrovirus, bacteriophage, cosmid, plasmid, fungal vectors and other recombination vehicles typically used in the art which have been described for expression in a variety of eukaryotic and prokaryotic hosts, and may be used for gene therapy as well as for simple protein expression.

A polynucleotide of this invention can be delivered to a cell or tissue using a gene delivery vehicle. “Gene delivery,” “gene transfer,” “transducing,” and the like as used herein, are terms referring to the introduction of an exogenous polynucleotide (sometimes referred to as a “transgene”) into a host cell, irrespective of the method used for the introduction. Such methods include a variety of well-known techniques such as vector-mediated gene transfer (by, e.g., viral infection/transfection, or various other protein-based or lipid-based gene delivery complexes) as well as techniques facilitating the delivery of “naked” polynucleotides (such as electroporation, “gene gun” delivery and various other techniques used for the introduction of polynucleotides). The introduced polynucleotide may be stably or transiently maintained in the host cell. Stable maintenance typically requires that the introduced polynucleotide either contains an origin of replication compatible with the host cell or integrates into a replicon of the host cell such as an extrachromosomal replicon (e.g., a plasmid) or a nuclear or mitochondrial chromosome. A number of vectors are known to be capable of mediating transfer of genes to mammalian cells, as is known in the art and described herein.

As used herein the term “eDNA” refers to extracellular DNA found as a component to pathogenic biofilms.

A “plasmid” is an extra-chromosomal DNA molecule separate from the chromosomal DNA which is capable of replicating independently of the chromosomal DNA. In many cases, it is circular and double-stranded. Plasmids provide a mechanism for horizontal gene transfer within a population of microbes and typically provide a selective advantage under a given environmental state. Plasmids may carry genes that provide resistance to naturally occurring antibiotics in a competitive environmental niche, or alternatively the proteins produced may act as toxins under similar circumstances.

“Plasmids” used in genetic engineering are called “plasmid vectors”. Many plasmids are commercially available for such uses. The gene to be replicated is inserted into copies of a plasmid containing genes that make cells resistant to particular antibiotics and a multiple cloning site (MCS, or polylinker), which is a short region containing several commonly used restriction sites allowing the easy insertion of DNA fragments at this location. Another major use of plasmids is to make large amounts of proteins. In this case, researchers grow bacteria containing a plasmid harboring the gene of interest. Just as the bacterium produces proteins to confer its antibiotic resistance, it can also be induced to produce large amounts of proteins from the inserted gene. This is a cheap and easy way of mass-producing a gene or the protein it then codes for.

A “yeast artificial chromosome” or “YAC” refers to a vector used to clone large DNA fragments (larger than 100 kb and up to 3000 kb). It is an artificially constructed chromosome and contains the telomeric, centromeric, and replication origin sequences needed for replication and preservation in yeast cells. Built using an initial circular plasmid, they are linearized by using restriction enzymes, and then DNA ligase can add a sequence or gene of interest within the linear molecule by the use of cohesive ends. Yeast expression vectors, such as YACs, YIps (yeast integrating plasmid), and YEps (yeast episomal plasmid), are extremely useful as one can get eukaryotic protein products with posttranslational modifications as yeasts are themselves eukaryotic cells, however YACs have been found to be more unstable than BACs, producing chimeric effects.

A “viral vector” is defined as a recombinantly produced virus or viral particle that comprises a polynucleotide to be delivered into a host cell, either in vivo, ex vivo or in vitro. Examples of viral vectors include retroviral vectors, adenovirus vectors, adeno-associated virus vectors, alphavirus vectors and the like. Infectious tobacco mosaic virus (TMV)-based vectors can be used to manufacture proteins and have been reported to express Griffithsin in tobacco leaves (O'Keefe et al. (2009) Proc. Nat. Acad. Sci. USA 106(15):6099-6104). Alphavirus vectors, such as Semliki Forest virus-based vectors and Sindbis virus-based vectors, have also been developed for use in gene therapy and immunotherapy. See, Schlesinger & Dubensky (1999) Curr. Opin. Biotechnol. 5:434-439 and Ying et al. (1999) Nat. Med. 5(7):823-827. In aspects where gene transfer is mediated by a retroviral vector, a vector construct refers to the polynucleotide comprising the retroviral genome or part thereof, and a therapeutic gene.

As used herein, “retroviral mediated gene transfer” or “retroviral transduction” carries the same meaning and refers to the process by which a gene or nucleic acid sequences are stably

transferred into the host cell by virtue of the virus entering the cell and integrating its genome into the host cell genome. The virus can enter the host cell via its normal mechanism of infection or be modified such that it binds to a different host cell surface receptor or ligand to enter the cell. As used herein, retroviral vector refers to a viral particle capable of introducing exogenous nucleic acid into a cell through a viral or viral-like entry mechanism.

Retroviruses carry their genetic information in the form of RNA; however, once the virus infects a cell, the RNA is reverse-transcribed into the DNA form which integrates into the genomic DNA of the infected cell. The integrated DNA form is called a provirus.

In aspects where gene transfer is mediated by a DNA viral vector, such as an adenovirus (Ad) or adeno-associated virus (AAV), a vector construct refers to the polynucleotide comprising the viral genome or part thereof, and a transgene. Adenoviruses (Ads) are a relatively well characterized, homogenous group of viruses, including over 50 serotypes. See, e.g., International PCT Application No. WO 95/27071. Ads do not require integration into the host cell genome. Recombinant Ad derived vectors, particularly those that reduce the potential for recombination and generation of wild-type virus, have also been constructed. See, International PCT Application Nos. WO 95/00655 and WO 95/11984. Wild-type AAV has high infectivity and specificity integrating into the host cell's genome. See, Hermonat & Muzyczka (1984) *Proc. Natl. Acad. Sci. USA* 81:6466-6470 and Lebkowski et al. (1988) *Mol. Cell. Biol.* 8:3988-3996.

Vectors that contain both a promoter and a cloning site into which a polynucleotide can be operatively linked are well known in the art. Such vectors are capable of transcribing RNA in vitro or in vivo, and are commercially available from sources such as Stratagene (La Jolla, CA) and Promega Biotech (Madison, WI). In order to optimize expression and/or in vitro transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the clones to eliminate extra, potential inappropriate alternative translation initiation codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites can be inserted immediately 5' of the start codon to enhance expression.

Gene delivery vehicles also include DNA/liposome complexes, micelles and targeted viral protein-DNA complexes. Liposomes that also comprise a targeting antibody or fragment thereof can be used in the methods of this invention. In addition to the delivery of polynucleotides to a cell or cell population, direct introduction of the proteins described

herein to the cell or cell population can be done by the non-limiting technique of protein transfection, alternatively culturing conditions that can enhance the expression and/or promote the activity of the proteins of this invention are other non-limiting techniques.

As used herein, the terms “antibody,” “antibodies” and “immunoglobulin” includes whole antibodies and any antigen binding fragment or a single chain thereof. Thus the term “antibody” includes any protein or peptide containing molecule that comprises at least a portion of an immunoglobulin molecule. The terms “antibody,” “antibodies” and “immunoglobulin” also include immunoglobulins of any isotype, fragments of antibodies which retain specific binding to antigen, including, but not limited to, Fab, Fab', F(ab)₂, Fv, scFv, dsFv, Fd fragments, dAb, VH, VL, VhH, and V-NAR domains; minibodies, diabodies, triabodies, tetrabodies and kappa bodies; multispecific antibody fragments formed from antibody fragments and one or more isolated. Examples of such include, but are not limited to a complementarity determining region (CDR) of a heavy or light chain or a ligand binding portion thereof, a heavy chain or light chain variable region, a heavy chain or light chain constant region, a framework (FR) region, or any portion thereof, at least one portion of a binding protein, chimeric antibodies, humanized antibodies, single-chain antibodies, and fusion proteins comprising an antigen-binding portion of an antibody and a non-antibody protein. The variable regions of the heavy and light chains of the immunoglobulin molecule contain a binding domain that interacts with an antigen. The constant regions of the antibodies (Abs) may mediate the binding of the immunoglobulin to host tissues. The term “anti-“ when used before a protein name, anti-IHF, anti-HU, anti-OMP P5, for example, refers to a monoclonal or polyclonal antibody that binds and/or has an affinity to a particular protein. For example, “anti-IHF” refers to an antibody that binds to the IHF protein. The specific antibody may have affinity or bind to proteins other than the protein it was raised against. For example, anti-IHF, while specifically raised against the IHF protein, may also bind other proteins that are related either through sequence homology or through structure homology.

The antibodies can be polyclonal, monoclonal, multispecific (e.g., bispecific antibodies), and antibody fragments, so long as they exhibit the desired biological activity. Antibodies can be isolated from any suitable biological source, e.g., murine, rat, sheep and canine.

As used herein, “monoclonal antibody” refers to an antibody obtained from a substantially homogeneous antibody population. Monoclonal antibodies are highly specific, as each monoclonal antibody is directed against a single determinant on the antigen. The antibodies

may be detectably labeled, e.g., with a radioisotope, an enzyme which generates a detectable product, a fluorescent protein, and the like. The antibodies may be further conjugated to other moieties, such as members of specific binding pairs, e.g., biotin (member of biotin-avidin specific binding pair), and the like. The antibodies may also be bound to a solid support, including, but not limited to, polystyrene plates or beads, and the like.

Monoclonal antibodies may be generated using hybridoma techniques or recombinant DNA methods known in the art. A hybridoma is a cell that is produced in the laboratory from the fusion of an antibody-producing lymphocyte and a non-antibody producing cancer cell, usually a myeloma or lymphoma. A hybridoma proliferates and produces a continuous supply of a specific monoclonal antibody. Alternative techniques for generating or selecting antibodies include *in vitro* exposure of lymphocytes to antigens of interest, and screening of antibody display libraries in cells, phage, or similar systems.

The term “human antibody” as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). However, the term “human antibody” as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. Thus, as used herein, the term “human antibody” refers to an antibody in which substantially every part of the protein (e.g., CDR, framework, C_L, C_H domains (e.g., C_{H1}, C_{H2}, C_{H3}), hinge, (VL, VH)) is substantially non-immunogenic in humans, with only minor sequence changes or variations. Similarly, antibodies designated primate (monkey, baboon, chimpanzee, etc.), rodent (mouse, rat, rabbit, guinea pig, hamster, and the like) and other mammals designate such species, sub-genus, genus, sub-family, family specific antibodies. Further, chimeric antibodies include any combination of the above. Such changes or variations optionally and preferably retain or reduce the immunogenicity in humans or other species relative to non-modified antibodies. Thus, a human antibody is distinct from a chimeric or humanized antibody. It is pointed out that a human antibody can be produced by a non-human animal or prokaryotic or eukaryotic cell that is capable of expressing functionally rearranged human immunoglobulin (e.g., heavy chain and/or light chain) genes. Further, when a human antibody is a single chain antibody, it can comprise a linker peptide that is not found in native human antibodies. For example, an Fv can comprise

a linker peptide, such as two to about eight glycine or other amino acid residues, which connects the variable region of the heavy chain and the variable region of the light chain. Such linker peptides are considered to be of human origin.

As used herein, a human antibody is “derived from” a particular germline sequence if the antibody is obtained from a system using human immunoglobulin sequences, e.g., by immunizing a transgenic mouse carrying human immunoglobulin genes or by screening a human immunoglobulin gene library. A human antibody that is “derived from” a human germline immunoglobulin sequence can be identified as such by comparing the amino acid sequence of the human antibody to the amino acid sequence of human germline immunoglobulins. A selected human antibody typically is at least 90% identical in amino acids sequence to an amino acid sequence encoded by a human germline immunoglobulin gene and contains amino acid residues that identify the human antibody as being human when compared to the germline immunoglobulin amino acid sequences of other species (e.g., murine germline sequences). In certain cases, a human antibody may be at least 95%, or even at least 96%, 97%, 98%, or 99% identical in amino acid sequence to the amino acid sequence encoded by the germline immunoglobulin gene. Typically, a human antibody derived from a particular human germline sequence will display no more than 10 amino acid differences from the amino acid sequence encoded by the human germline immunoglobulin gene. In certain cases, the human antibody may display no more than 5, or even no more than 4, 3, 2, or 1 amino acid difference from the amino acid sequence encoded by the germline immunoglobulin gene.

A “human monoclonal antibody” refers to antibodies displaying a single binding specificity which have variable and constant regions derived from human germline immunoglobulin sequences. The term also intends recombinant human antibodies. Methods to making these antibodies are described herein.

The term “recombinant human antibody”, as used herein, includes all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies isolated from an animal (e.g., a mouse) that is transgenic or transchromosomal for human immunoglobulin genes or a hybridoma prepared therefrom, antibodies isolated from a host cell transformed to express the antibody, e.g., from a transfectoma, antibodies isolated from a recombinant, combinatorial human antibody library, and antibodies prepared, expressed, created or isolated by any other means that involve splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and

constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies can be subjected to in vitro mutagenesis (or, when an animal transgenic for human Ig sequences is used, in vivo somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire in vivo. Methods to making these antibodies are described herein.

As used herein, chimeric antibodies are antibodies whose light and heavy chain genes have been constructed, typically by genetic engineering, from antibody variable and constant region genes belonging to different species.

As used herein, the term "humanized antibody" or "humanized immunoglobulin" refers to a human/non-human chimeric antibody that contains a minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a variable region of the recipient are replaced by residues from a variable region of a non-human species (donor antibody) such as mouse, rat, rabbit, or non-human primate having the desired specificity, affinity and capacity. Humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. The humanized antibody can optionally also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. a non-human antibody containing one or more amino acids in a framework region, a constant region or a CDR, that have been substituted with a correspondingly positioned amino acid from a human antibody. In general, humanized antibodies are expected to produce a reduced immune response in a human host, as compared to a non-humanized version of the same antibody. The humanized antibodies may have conservative amino acid substitutions which have substantially no effect on antigen binding or other antibody functions. Conservative substitutions groupings include: glycine-alanine, valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, serine-threonine and asparagine-glutamine.

The terms "polyclonal antibody" or "polyclonal antibody composition" as used herein refer to a preparation of antibodies that are derived from different B-cell lines. They are a mixture of immunoglobulin molecules secreted against a specific antigen, each recognizing a different epitope.

As used herein, the term "antibody derivative", comprises a full-length antibody or a fragment of an antibody, wherein one or more of the amino acids are chemically modified by alkylation, pegylation, acylation, ester formation or amide formation or the like, e.g., for linking the antibody to a second molecule. This includes, but is not limited to, pegylated antibodies, cysteine-pegylated antibodies, and variants thereof.

As used herein, the term "label" intends a directly or indirectly detectable compound or composition that is conjugated directly or indirectly to the composition to be detected, e.g., N-terminal histadine tags (N-His), magnetically active isotopes, e.g., ^{115}Sn , ^{117}Sn and ^{119}Sn , a non-radioactive isotopes such as ^{13}C and ^{15}N , polynucleotide or protein such as an antibody so as to generate a "labeled" composition. The term also includes sequences conjugated to the polynucleotide that will provide a signal upon expression of the inserted sequences, such as green fluorescent protein (GFP) and the like. The label may be detectable by itself (e.g. radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable. The labels can be suitable for small scale detection or more suitable for high-throughput screening. As such, suitable labels include, but are not limited to magnetically active isotopes, non-radioactive isotopes, radioisotopes, fluorochromes, chemiluminescent compounds, dyes, and proteins, including enzymes. The label may be simply detected or it may be quantified. A response that is simply detected generally comprises a response whose existence merely is confirmed, whereas a response that is quantified generally comprises a response having a quantifiable (e.g., numerically reportable) value such as an intensity, polarization, and/or other property. In luminescence or fluorescence assays, the detectable response may be generated directly using a luminophore or fluorophore associated with an assay component actually involved in binding, or indirectly using a luminophore or fluorophore associated with another (e.g., reporter or indicator) component. Examples of luminescent labels that produce signals include, but are not limited to bioluminescence and chemiluminescence. Detectable luminescence response generally comprises a change in, or an occurrence of, a luminescence signal. Suitable methods and luminophores for luminescently labeling assay components are known in the art and described for example in Haugland, Richard P. (1996) Handbook of Fluorescent Probes and Research Chemicals (6th ed.). Examples of luminescent probes include, but are not limited to, aequorin and luciferases.

As used herein, the term "immunoconjugate" comprises an antibody or an antibody derivative associated with or linked to a second agent, such as a cytotoxic agent, a detectable agent, a

radioactive agent, a targeting agent, a human antibody, a humanized antibody, a chimeric antibody, a synthetic antibody, a semisynthetic antibody, or a multispecific antibody.

Examples of suitable fluorescent labels include, but are not limited to, fluorescein, rhodamine, tetramethylrhodamine, eosin, erythrosin, coumarin, methyl-coumarins, pyrene, Malacite green, stilbene, Lucifer Yellow, Cascade BlueTM, and Texas Red. Other suitable optical dyes are described in the Haugland, Richard P. (1996) Handbook of Fluorescent Probes and Research Chemicals (6th ed.).

In another aspect, the fluorescent label is functionalized to facilitate covalent attachment to a cellular component present in or on the surface of the cell or tissue such as a cell surface marker. Suitable functional groups, including, but not are limited to, isothiocyanate groups, amino groups, haloacetyl groups, maleimides, succinimidyl esters, and sulfonyl halides, all of which may be used to attach the fluorescent label to a second molecule. The choice of the functional group of the fluorescent label will depend on the site of attachment to either a linker, the agent, the marker, or the second labeling agent.

“Eukaryotic cells” comprise all of the life kingdoms except monera. They can be easily distinguished through a membrane-bound nucleus. Animals, plants, fungi, and protists are eukaryotes or organisms whose cells are organized into complex structures by internal membranes and a cytoskeleton. The most characteristic membrane-bound structure is the nucleus. Unless specifically recited, the term “host” includes a eukaryotic host, including, for example, yeast, higher plant, insect and mammalian cells. Non-limiting examples of eukaryotic cells or hosts include simian, bovine, porcine, murine, rat, avian, reptilian and human.

“Prokaryotic cells” that usually lack a nucleus or any other membrane-bound organelles and are divided into two domains, bacteria and archaea. In addition to chromosomal DNA, these cells can also contain genetic information in a circular loop called an episome. Bacterial cells are very small, roughly the size of an animal mitochondrion (about 1-2 μ m in diameter and 10 μ m long). Prokaryotic cells feature three major shapes: rod shaped, spherical, and spiral. Instead of going through elaborate replication processes like eukaryotes, bacterial cells divide by binary fission. Examples include but are not limited to *Bacillus* bacteria, *E. coli* bacterium, and *Salmonella* bacterium.

A "native" or "natural" antigen is a polypeptide, protein or a fragment which contains an epitope, which has been isolated from a natural biological source, and which can specifically bind to an antigen receptor, in particular a T cell antigen receptor (TCR), in a subject.

The terms "antigen" and "antigenic" refer to molecules with the capacity to be recognized by an antibody or otherwise act as a member of an antibody-ligand pair. "Specific binding" refers to the interaction of an antigen with the variable regions of immunoglobulin heavy and light chains. Antibody-antigen binding may occur *in vivo* or *in vitro*. The skilled artisan will understand that macromolecules, including proteins, nucleic acids, fatty acids, lipids, lipopolysaccharides and polysaccharides have the potential to act as an antigen. The skilled artisan will further understand that nucleic acids encoding a protein with the potential to act as an antibody ligand necessarily encode an antigen. The artisan will further understand that antigens are not limited to full-length molecules, but can also include partial molecules. The term "antigenic" is an adjectival reference to molecules having the properties of an antigen. The term encompasses substances which are immunogenic, i.e., immunogens, as well as substances which induce immunological unresponsiveness, or anergy, i.e., anergens.

An "altered antigen" is one having a primary sequence that is different from that of the corresponding wild-type antigen. Altered antigens can be made by synthetic or recombinant methods and include, but are not limited to, antigenic peptides that are differentially modified during or after translation, e.g., by phosphorylation, glycosylation, cross-linking, acylation, proteolytic cleavage, linkage to an antibody molecule, membrane molecule or other ligand. (Ferguson et al. (1988) Ann. Rev. Biochem. 57:285-320). A synthetic or altered antigen of the invention is intended to bind to the same TCR as the natural epitope.

A "self-antigen" also referred to herein as a native or wild-type antigen is an antigenic peptide that induces little or no immune response in the subject due to self-tolerance to the antigen. An example of a self-antigen is the melanoma specific antigen gp100.

The terms "major histocompatibility complex" or "MHC" refers to a complex of genes encoding cell-surface molecules that are required for antigen presentation to T cells and for rapid graft rejection. In humans, the MHC is also known as the "human leukocyte antigen" or "HLA" complex. The proteins encoded by the MHC are known as "MHC molecules" and are classified into class I and class II MHC molecules. Class I MHC includes membrane heterodimeric proteins made up of an α chain encoded in the MHC noncovalently linked with the β 2-microglobulin. Class I MHC molecules are expressed by nearly all nucleated cells

and have been shown to function in antigen presentation to CD8⁺ T cells. Class I molecules include HLA-A, B, and C in humans. Class II MHC molecules also include membrane heterodimeric proteins consisting of noncovalently associated α and β chains. Class II MHC molecules are known to function in CD4⁺ T cells and, in humans, include HLA-DP, -DQ, and DR. In a preferred embodiment, invention compositions and ligands can complex with MHC molecules of any HLA type. Those of skill in the art are familiar with the serotypes and genotypes of the HLA. See: bimas.dcrt.nih.gov/cgi-bin/molbio/hla coefficient viewing page. Rammensee H. G., Bachmann J., and Stevanovic S. MHC Ligands and Peptide Motifs (1997) Chapman & Hall Publishers; Schreuder G. M. Th. et al. The HLA dictionary (1999) Tissue Antigens 54:409-437.

The term "antigen-presenting matrix", as used herein, intends a molecule or molecules which can present antigen in such a way that the antigen can be bound by a T-cell antigen receptor on the surface of a T cell. An antigen-presenting matrix can be on the surface of an antigen-presenting cell (APC), on a vesicle preparation of an APC, or can be in the form of a synthetic matrix on a solid support such as a bead or a plate. An example of a synthetic antigen-presenting matrix is purified MHC class I molecules complexed to P2-microglobulin, multimers of such purified MHC class I molecules, purified MHC Class II molecules, or functional portions thereof, attached to a solid support.

The term "antigen presenting cells (APC)" refers to a class of cells capable of presenting one or more antigens in the form of antigen-MHC complex recognizable by specific effector cells of the immune system, and thereby inducing an effective cellular immune response against the antigen or antigens being presented. While many types of cells may be capable of presenting antigens on their cell surface for T-cell recognition, only professional APCs have the capacity to present antigens in an efficient amount and further to activate T-cells for cytotoxic T-lymphocyte (CTL) responses. APCs can be intact whole cells such as macrophages, B-cells and dendritic cells; or other molecules, naturally occurring or synthetic, such as purified MHC class I molecules complexed to β 2-microglobulin.

The term "dendritic cells (DCs)" refers to a diverse population of morphologically similar cell types found in a variety of lymphoid and non-lymphoid tissues (Steinman (1991) Ann. Rev. Immunol. 9:271-296). Dendritic cells constitute the most potent and preferred of mammalian APCs. A subset, if not all, of DCs are derived from bone marrow progenitor cells, circulate in small numbers in the peripheral blood and appear either as immature Langerhans' cells or

terminally differentiated mature cells. While DCs can be derived from monocytes, they possess distinct phenotypes. For example, a particular differentiating marker, CD14 antigen, is not found in dendritic cells but is expressed at very high levels in monocytes by monocytes. See for example Jersmann et al., (2005) *Immunol. Cell Biol.* 83:462.

Also, mature dendritic cells are not phagocytic, whereas the monocytes are strongly phagocytosing cells. Mature monocytes and DCs endocytose material through different mechanisms. Monocytes engulf by means of phagocytosis, whereas DCs utilize macropinocytosis. Thus, DCs generally engulf cargo of a smaller size than monocytes (See for example Conner and Schmid, (2003) *Nature* 433:37-44. It has been shown that DCs are as endocytically active as other antigen presenting cells, and provide all the signals necessary for T cell activation and proliferation (See, e.g. Levine and Chain, (1992) *ONAS* 89(17):8342.

The term "antigen presenting cell recruitment factors" or "APC recruitment factors" include both intact, whole cells as well as other molecules that are capable of recruiting antigen presenting cells. Examples of suitable APC recruitment factors include molecules such as interleukin 4 (IL4), granulocyte macrophage colony stimulating factor (GM-CSF), Sepragel and macrophage inflammatory protein 3 alpha (MIP3 α). These are available from Immunex, Schering-Plough and R&D Systems (Minneapolis, Minn.). They also can be recombinantly produced using the methods disclosed in *Current Protocols In Molecular Biology* (F. M. Ausubel et al., eds. (1987)). Peptides, proteins and compounds having the same biological activity as the above-noted factors are included within the scope of this invention.

The term "immune effector cells" refers to cells capable of binding an antigen and which mediate an immune response. These cells include, but are not limited to, T cells, B cells, monocytes, macrophages, NK cells and cytotoxic T lymphocytes (CTLs), for example CTL lines, CTL clones, and CTLs from tumor, inflammatory, or other infiltrates. Certain diseased tissue expresses specific antigens and CTLs specific for these antigens have been identified.

The term "immune effector molecule" as used herein, refers to molecules capable of antigen-specific binding, and includes antibodies, T cell antigen receptors, B cell antigen receptors, and MHC Class I and Class II molecules.

A "naive" immune effector cell is an immune effector cell that has never been exposed to an antigen capable of activating that cell. Activation of naive immune effector T cells requires

both recognition of the antigen:MHC complex and the simultaneous delivery of a costimulatory signal by a professional APC in order to proliferate and differentiate into antigen-specific armed effector T cells. Activated T cells can then activate specific B cells through immunological synapses by providing a co-stimulation signal. Activated B cells subsequently produce antibodies directed to a specific antigen. Naïve B cells can also be activated by T cell-independent mechanisms. This occurs when antigens are capable of binding to the B cell receptor and producing a co-stimulation signal.

"Immune response" broadly refers to the antigen-specific responses of lymphocytes to foreign substances. The terms "immunogen" and "immunogenic" refer to molecules with the capacity to elicit an immune response. All immunogens are antigens, however, not all antigens are immunogenic. An immune response of this invention can be humoral (via antibody activity) or cell-mediated (via T cell activation). The response may occur *in vivo* or *in vitro*. The skilled artisan will understand that a variety of macromolecules, including proteins, nucleic acids, fatty acids, lipids, lipopolysaccharides and polysaccharides have the potential to be immunogenic. The skilled artisan will further understand that nucleic acids encoding a molecule capable of eliciting an immune response necessarily encode an immunogen. The artisan will further understand that immunogens are not limited to full-length molecules, but may include partial molecules.

The term "passive immunity" refers to the transfer of immunity from one subject to another through the transfer of antibodies. Passive immunity may occur naturally, as when maternal antibodies are transferred to a fetus. Passive immunity may also occur artificially as when antibody compositions are administered to non-immune subjects. Antibody donors and recipients may be human or non-human subjects. Antibodies may be polyclonal or monoclonal, may be generated *in vitro* or *in vivo*, and may be purified, partially purified, or unpurified depending on the embodiment. In some embodiments described herein, passive immunity is conferred on a subject in need thereof through the administration of antibodies or antigen binding fragments that specifically recognize or bind to a particular antigen. In some embodiments, passive immunity is conferred through the administration of an isolated or recombinant polynucleotide encoding an antibody or antigen binding fragment that specifically recognizes or binds to a particular antigen.

In the context of this invention, a "ligand" is a polypeptide. In one aspect, the term "ligand" as used herein refers to any molecule that binds to a specific site on another molecule. In

other words, the ligand confers the specificity of the protein in a reaction with an immune effector cell or an antibody to a protein or DNA to a protein. In one aspect it is the ligand site within the protein that combines directly with the complementary binding site on the immune effector cell.

In one aspect, a peptide or ligand of the invention binds to an antigenic determinant or epitope on an immune effector cell, such as an antibody or a T cell receptor (TCR). A ligand may be an antigen, peptide, protein or epitope of the invention.

In another aspect, ligands may bind to a receptor on an antibody. In one embodiment, the ligand of the invention is about 4 to about 8 amino acids in length.

In a further aspect, ligands may bind to a receptor on an MHC class I molecule. In one embodiment, the ligand of the invention is about 7 to about 11 amino acids in length.

In a yet further aspect, ligands may bind to a receptor on an MHC class II molecule. In one embodiment, the ligand of the invention is about 10 to about 20 amino acids long.

As used herein, the term "educated, antigen-specific immune effector cell", is an immune effector cell as defined above, which has previously encountered an antigen. In contrast with its naive counterpart, activation of an educated, antigen-specific immune effector cell does not require a costimulatory signal. Recognition of the peptide:MHC complex is sufficient.

"Activated", when used in reference to a T cell, implies that the cell is no longer in G₀ phase, and begins to produce one or more of cytotoxins, cytokines, and other related membrane-associated proteins characteristic of the cell type (e.g., CD8⁺ or CD4⁺), is capable of recognizing and binding any target cell that displays the particular antigen on its surface, and releasing its effector molecules.

The term "cross-reactive" is used to describe compounds of the invention which are functionally overlapping. More particularly, the immunogenic properties of a native ligand and/or immune effector cells activated thereby are shared to a certain extent by the altered ligand such that the altered ligand is "cross-reactive" with the native ligand and/or the immune effector cells activated thereby. For purposes of this invention, cross-reactivity is manifested at multiple levels: (i) at the ligand level, e.g., the altered ligands can bind the TCR of and activate native ligand CTLs; (ii) at the T cell level, i.e., altered ligands of the invention bind the TCR of and activate a population of T cells (distinct from the population of native

ligand CTLs) which can effectively target and lyse cells displaying the native ligand; and (iii) at the antibody level, e.g., "anti"-altered ligand antibodies can detect, recognize and bind the native ligand and initiate effector mechanisms in an immune response which ultimately result in elimination of the native ligand from the host.

As used herein, the term "inducing an immune response in a subject" is a term well understood in the art and intends that an increase of at least about 2-fold, more preferably at least about 5-fold, more preferably at least about 10-fold, more preferably at least about 100-fold, even more preferably at least about 500-fold, even more preferably at least about 1000-fold or more in an immune response to an antigen (or epitope) can be detected or measured, after introducing the antigen (or epitope) into the subject, relative to the immune response (if any) before introduction of the antigen (or epitope) into the subject. An immune response to an antigen (or epitope), includes, but is not limited to, production of an antigen-specific (or epitope-specific) antibody, and production of an immune cell expressing on its surface a molecule which specifically binds to an antigen (or epitope). Methods of determining whether an immune response to a given antigen (or epitope) has been induced are well known in the art. For example, antigen-specific antibody can be detected using any of a variety of immunoassays known in the art, including, but not limited to, ELISA, wherein, for example, binding of an antibody in a sample to an immobilized antigen (or epitope) is detected with a detectably-labeled second antibody (e.g., enzyme-labeled mouse anti-human Ig antibody).

"Co-stimulatory molecules" are involved in the interaction between receptor-ligand pairs expressed on the surface of antigen presenting cells and T cells. Research accumulated over the past several years has demonstrated convincingly that resting T cells require at least two signals for induction of cytokine gene expression and proliferation (Schwartz (1990) *Science* 248:1349-1356 and Jenkins (1992) *Immunol. Today* 13:69-73). One signal, the one that confers specificity, can be produced by interaction of the TCR/CD3 complex with an appropriate MHC/peptide complex. The second signal is not antigen specific and is termed the "co-stimulatory" signal. This signal was originally defined as an activity provided by bone-marrow-derived accessory cells such as macrophages and dendritic cells, the so called "professional" APCs. Several molecules have been shown to enhance co-stimulatory activity. These are heat stable antigen (HSA) (Liu et al. (1992) *J. Exp. Med.* 175:437-445), chondroitin sulfate-modified MHC invariant chain (Ii-CS) (Naujokas et al. (1993) *Cell* 74:257-268), intracellular adhesion molecule 1 (ICAM-1) (Van (1992) *Cell* 71:1065-1068).

These molecules each appear to assist co-stimulation by interacting with their cognate ligands on the T cells. Co-stimulatory molecules mediate co-stimulatory signal(s), which are necessary, under normal physiological conditions, to achieve full activation of naive T cells. One exemplary receptor-ligand pair is the B7 co-stimulatory molecule on the surface of APCs and its counter-receptor CD28 or CTLA-4 on T cells (Freeman et al. (1993) *Science* 262:909-911; Young et al. (1992) *J. Clin. Invest.* 90:229 and Nabavi et al. (1992) *Nature* 360:266-268). Other important co-stimulatory molecules are CD40, CD54, CD80, and CD86. The term "co-stimulatory molecule" encompasses any single molecule or combination of molecules which, when acting together with a peptide/MHC complex bound by a TCR on the surface of a T cell, provides a co-stimulatory effect which achieves activation of the T cell that binds the peptide. The term thus encompasses B7, or other co-stimulatory molecule(s) on an antigen-presenting matrix such as an APC, fragments thereof (alone, complexed with another molecule(s), or as part of a fusion protein) which, together with peptide/MHC complex, binds to a cognate ligand and results in activation of the T cell when the TCR on the surface of the T cell specifically binds the peptide. Co-stimulatory molecules are commercially available from a variety of sources, including, for example, Beckman Coulter, Inc. (Fullerton, Calif.). It is intended, although not always explicitly stated, that molecules having similar biological activity as wild-type or purified co-stimulatory molecules (e.g., recombinantly produced or muteins thereof) are intended to be used within the spirit and scope of the invention.

As used herein, "solid phase support" or "solid support", used interchangeably, is not limited to a specific type of support. Rather a large number of supports are available and are known to one of ordinary skill in the art. Solid phase supports include silica gels, resins, derivatized plastic films, glass beads, cotton, plastic beads, alumina gels. As used herein, "solid support" also includes synthetic antigen-presenting matrices, cells, and liposomes. A suitable solid phase support may be selected on the basis of desired end use and suitability for various protocols. For example, for peptide synthesis, solid phase support may refer to resins such as polystyrene (e.g., PAM-resin obtained from Bachem Inc., Peninsula Laboratories, etc.), POLYHIPE.RTM. resin (obtained from Aminotech, Canada), polyamide resin (obtained from Peninsula Laboratories), polystyrene resin grafted with polyethylene glycol (TentaGel.RTM., Rapp Polymere, Tübingen, Germany) or polydimethylacrylamide resin (obtained from Milligen/Biosearch, Calif.).

An example of a solid phase support include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite. The nature of the carrier can be either soluble to some extent or insoluble. The support material may have virtually any possible structural configuration so long as the coupled molecule is capable of binding to a polynucleotide, polypeptide or antibody. Thus, the support configuration may be spherical, as in a bead, or cylindrical, as in the inside surface of a test tube, or the external surface of a rod. Alternatively, the surface may be flat such as a sheet, test strip, etc. or alternatively polystyrene beads. Those skilled in the art will know many other suitable carriers for binding antibody or antigen, or will be able to ascertain the same by use of routine experimentation.

The term "immunomodulatory agent", as used herein, is a molecule, a macromolecular complex, or a cell that modulates an immune response and encompasses a synthetic antigenic peptide of the invention alone or in any of a variety of formulations described herein; a polypeptide comprising a synthetic antigenic peptide of the invention; a polynucleotide encoding a peptide or polypeptide of the invention; a synthetic antigenic peptide of the invention bound to a Class I or a Class II MHC molecule on an antigen-presenting matrix, including an APC and a synthetic antigen-presenting matrix (in the presence or absence of co-stimulatory molecule(s)); a synthetic antigenic peptide of the invention covalently or non-covalently complexed to another molecule(s) or macromolecular structure; and an educated, antigen-specific immune effector cell which is specific for a peptide of the invention.

The term "modulate an immune response" includes inducing (increasing, eliciting) an immune response; and reducing (suppressing) an immune response. An immunomodulatory method (or protocol) is one that modulates an immune response in a subject.

As used herein, the term "cytokine" refers to any one of the numerous factors that exert a variety of effects on cells, for example, inducing growth or proliferation. Non-limiting examples of cytokines which may be used alone or in combination in the practice of the present invention include, interleukin-2 (IL-2), stem cell factor (SCF), interleukin 3 (IL-3), interleukin 6 (IL-6), interleukin 12 (IL-12), G-CSF, granulocyte macrophage-colony stimulating factor (GM-CSF), interleukin-1 alpha (IL-1 α), interleukin-11 (IL-11), MIP-11, leukemia inhibitory factor (LIF), c-kit ligand, thrombopoietin (TPO) and flt3 ligand. The present invention also includes culture conditions in which one or more cytokine is specifically excluded from the medium. Cytokines are commercially available from several

vendors such as, for example, Genzyme (Framingham, Mass.), Genentech (South San Francisco, Calif.), Amgen (Thousand Oaks, Calif.), R&D Systems (Minneapolis, Minn.) and Immunex (Seattle, Wash.). It is intended, although not always explicitly stated, that molecules having similar biological activity as wild-type or purified cytokines (e.g., recombinantly produced or muteins thereof) are intended to be used within the spirit and scope of the invention.

Diagnostic and Therapeutic Methods

A method is provided for inhibiting, competing or titrating the binding of a DNABII polypeptide or protein to a microbial DNA, by contacting the DNABII polypeptide or protein or the microbial DNA with an interfering agent, thereby inhibiting, competing or titrating the binding of the DNABII protein or polypeptide to the microbial DNA. In a further aspect, the DNABII polypeptide and the microbial DNA are detectably labeled, for example with luminescent molecules that will emit a signal when brought into close contact with each other. The contacting can be performed in vitro or in vivo.

In another aspect, a method for inhibiting, preventing or breaking down a microbial biofilm is provided by contacting the biofilm with an interfering agent, thereby inhibiting, preventing or breaking down the microbial biofilm. In a further aspect, the DNABII polypeptide and the microbial DNA are detectably labeled, for example with luminescent molecules that will emit a signal when brought into close contact with each other. The contacting can be performed in vitro or in vivo.

When practiced in vitro, the methods are useful to screen for or confirm interfering agents having the same, similar or opposite ability as the polypeptides, polynucleotides, antibodies, host cells, small molecules and compositions of this invention. Alternatively, they can be used to identify which interfering agent is best suited to treat a microbial infection. For example, one can screen for new agents or combination therapies by having two samples containing for example, the DNABII polypeptide and microbial DNA and the agent to be tested. The second sample contains the DNABII polypeptide and microbial DNA and an agent known to active, e.g., an anti-IHF antibody or a small molecule to serve as a positive control. In a further aspect, several samples are provided and the interfering agents are added to the system in increasing dilutions to determine the optimal dose that would likely be effective in treating a subject in the clinical setting. As is apparent to those of skill in the art, a negative control containing the DNABII polypeptide and the microbial DNA can be

provided. In a further aspect, the DNABII polypeptide and the microbial DNA are detectably labeled, for example with luminescent molecules that will emit a signal when brought into close contact with each other. The samples are contained under similar conditions for an effective amount of time for the agent to inhibit, compete or titrate the interaction between the DNABII polypeptide and microbial DNA and then the sample is assayed for emission of signal from the luminescent molecules. If the sample emits a signal, then the agent is not effective to inhibit binding.

In another aspect, the in vitro method is practiced in a miniaturized chamber slide system wherein the microbial (such as a bacterial) isolate causing an infection could be isolated from the human/animal then cultured to allow it to grow as a biofilm in vitro, see for example Experiment No. 1 below. The interfering agent (such as anti-IHF antibody) or potential interfering agent biofilm is added alone or in combination with another agent to the culture with or without increasing dilutions of the potential interfering agent or interfering agent such as an anti-IHF (or other antibody, small molecule, agent etc.) to find the optimal dose that would likely be effective at treating that patient when delivered to the subject where the infection existed. As apparent to those of skill in the art, a positive and negative control can be performed simultaneously.

In a further aspect, the method is practiced in a high throughput platform with the interfering agent (such as anti-IHF antibody) and/or potential interfering agent (alone or in combination with another agent) in a flow cell. The interfering agent (such as anti-IHF antibody) or potential interfering agent biofilm is added alone or in combination with another agent to the culture with or without increasing dilutions of the potential interfering agent or interfering agent such as an anti-IHF (or other antibody, small molecule, agent etc.) to find the optimal dose that would likely be effective at treating that patient when delivered to the subject where the infection existed. Biofilm isolates are sonicated to separate biofilm bacteria from DNABII polypeptide such as IHF bound to microbial DNA. The DNABII polypeptide – DNA complexes are isolated by virtue of the anti-IHF antibody on the platform. The microbial DNA is then be released with e.g. a salt wash, and used to identify the biofilm bacteria added. The freed DNA is then identified, e.g., by PCR sequenced. If DNA is not freed, then the interfering agent(s) successfully performed or bound the microbial DNA. If DNA is found in the sample, then the agent did not interfere with DNABII polypeptide-microbial DNA binding. As is apparent to those of skill in the art, a positive and/or negative control can be simultaneously performed.

The above methods also can be used as a diagnostic test since it is possible that a given bacterial species will respond better to reversal of its biofilm by one agent more than another, this rapid high throughput assay system could allow one skilled in the art to assay a panel of possible anti-IHF-like agents to identify the most efficacious of the group.

The advantage of these methods is that most clinical microbiology labs in hospitals are already equipped to perform these sorts of assays (i.e. determination of MIC, MBC values) using bacteria that are growing in liquid culture (or planktonically). As is apparent to those of skill in the art, bacteria generally do not grow planktonically when they are causing diseases. Instead they are growing as a stable biofilm and these biofilms are significantly more resistant to treatment by antibiotics, antibodies or other therapeutics. This resistance is why most MIC/MBC values fail to accurately predict efficacy in vivo. Thus, by determining what “dose” of agent could reverse a bacterial biofilm in vitro (as described above)

Applicants’ pre-clinical assay would be a more reliable predictor of clinical efficacy, even as an application of personalized medicine.

In addition to the clinical setting, the methods can be used to identify the microbe causing the infection and/or confirm effective interfering agents in an industrial setting.

In a further aspect of the above methods, an antibiotic or antimicrobial known to inhibit growth of the underlying infection is added sequentially or concurrently, to determine if the infection can be inhibited. It is also possible to add the interfering agent to the microbial DNA or DNABII polypeptide before adding the missing complex to assay for biofilm inhibition.

When practiced in vivo in non-human animal such as a chinchilla, the method provides a pre-clinical screen to identify interfering agents that can be used alone or in combination with other agents to break down biofilms. Examples of this method are shown in Experiment Nos. 2 through 7, below.

In another aspect, provided herein is a method of inhibiting, preventing or breaking down a biofilm in a subject by administering to the subject an effective amount of an interfering agent, thereby inhibiting, preventing or breaking down the microbial biofilm. Examples of this method are shown in Experiment Nos. 2 through 7, below.

For the purpose of the above noted in vitro and in vivo methods, the interfering agent is of the group of:

- (a) an isolated or recombinant integration host factor (IHF) polypeptide or a fragment or an equivalent of each thereof;
- (b) an isolated or recombinant histone-like protein from *E.coli* strain U93 (HU) polypeptide or a fragment or an equivalent of each thereof;
- (c) an isolated or recombinant protein polypeptide identified in Table 8, Table 9A, Table 9B, Table 10 or a DNA binding peptide identified in Figure 6, or a fragment or an equivalent of each thereof;
- (d) an isolated or recombinant polypeptide of SEQ ID NO. 1 through 340, or a fragment or an equivalent of each thereof;
- (e) an isolated or recombinant C-terminal polypeptide of SEQ ID NO. 6 through 11, 28, 29, 42 through 100, Table 8 or those C-terminal polypeptides identified in Table 10 or a fragment or an equivalent of each thereof;
- (f) a polypeptide that competes with an integration host factor on binding to a microbial DNA;
- (g) a four-way junction polynucleotide resembling a Holliday junction, a 3 way junction polynucleotide resembling a replication fork, a polynucleotide that has inherent flexibility or bent polynucleotide;
- (h) an isolated or recombinant polynucleotide encoding any one of (a) through (f) or an isolated or recombinant polynucleotide of SEQ ID NO. 36 or an equivalent of each thereof, or a polynucleotide that hybridizes under stringent conditions to the polynucleotide its equivalent or its complement;
- (i) an antibody or antigen binding fragment that specifically recognizes or binds any one of (a) through (f), or an equivalent or fragment of each antibody or antigen binding fragment thereof;
- (j) isolated or recombinant polynucleotide encoding the antibody or antigen binding fragment of (i) or its complement; or
- (k) a small molecule that competes with the binding of a DNABII protein or polypeptide to a microbial DNA.

Also provided herein is a method for inducing an immune response in or conferring passive immunity on subject in need thereof, comprising, or alternatively consisting essentially of, or

yet further consisting of, administering to the subject an effective amount of one or more of the group:

- (a) an isolated or recombinant integration host factor (IHF) polypeptide, or a fragment or an equivalent of each thereof;
- (b) an isolated or recombinant histone-like protein from *E.coli* strain U93 (HU) polypeptide or a fragment or an equivalent of each thereof;
- (c) an isolated or recombinant protein polypeptide identified in Table 8, Table 9A, Table 9B, Table 10 or an DNA binding peptide identified in Figure 6, or a fragment or an equivalent of each thereof;
- (d) an isolated or recombinant polypeptide of SEQ ID NO. 1 through 340, or a fragment or an equivalent thereof;
- (e) an isolated or recombinant C-terminal polypeptide of SEQ ID NO. 6 through 11, 28, 29, 42 through 100, Table 8 or those C-terminal polypeptides identified in Table 10 or a fragment or an equivalent of each thereof;
- (f) an isolated or recombinant polynucleotide encoding any one of (a) through (e) or an isolated or recombinant polynucleotide of SEQ ID NO. 36 or an equivalent of each thereof, or a polynucleotide that hybridizes under stringent conditions to the polynucleotide, its equivalent or its complement;
- (g) an antibody or antigen binding fragment that specifically recognizes or binds any one of (a) through (e), or an equivalent or fragment of each thereof;
- (h) an isolated or recombinant polynucleotide encoding the antibody or antigen binding fragment of (g);
- (i) and antigen presenting cell pulsed with any one of (a) through (e); and
- (j) and antigen presenting cell transfected with one or more polynucleotides encoding any one of (a) through (e).

In one particular aspect, the interfering agent is an isolated or recombinant integration host factor (IHF) polypeptide or a fragment thereof, a C-terminal fragment of an IHF polypeptide or an equivalent of each thereof. In another particular aspect, the interfering agent is an isolated or recombinant HU polypeptide or a fragment thereof, a C-terminal fragment of HU polypeptide, or an equivalent of each thereof. Non-limiting examples of such are an IHF or

HU alpha or beta polypeptide; an IHF polypeptide; *Moraxella acatarrhalis* HU; *E. coli* HupA, HupB, himA, himD; *E. faecalis* HU (such as V583), HMGB1 (High Mobility Group Box 1, a protein with similar DNA binding and DNA substrate specificities but not in primary amino acid sequence to the DNABII family of proteins; a functional orthologue) and those identified in Table 8 or Table 10.

In a further aspect, the methods further comprise, or alternatively consist essentially of, or yet further consist of administering to the subject an effective amount of one or more of an antimicrobial, an antigenic peptide or an adjuvant.

A non-limiting example of an antimicrobial agent is another vaccine component such as a surface antigen, e.g. an OMP P5, rsPilA, OMP 26, OMP P2, or Type IV Pilin protein (see Jurcisek and Bakaletz (2007) J. of Bacteriology 189(10):3868-3875 and Murphy, TF, Bakaletz, LO and Smeesters, PR (2009) The Pediatric Infectious Disease Journal, 28:S121-S126).

The agents and compositions of this invention can be concurrently or sequentially administered with other antimicrobial agents and/or surface antigens. In one particular aspect, administration is locally to the site of the infection by direct injection or by inhalation for example. Other non-limiting examples of administration include by one or more method comprising transdermally, urethrally, sublingually, rectally, vaginally, ocularly, subcutaneous, intramuscularly, intraperitoneally, intranasally, by inhalation or orally.

Microbial infections and disease that can be treated by the methods of this invention include infection by the organisms identified in Experiment No. 1 and Table 8, e.g., *Streptococcus agalactiae*, *Neisseria meningitidis*, *Treponemes, denticola, pallidum*), *Burkholderia cepacia*), or *Burkholderia pseudomallei*. In one aspect, the microbial infection is one or more of *Haemophilus influenzae* (nontypeable), *Moraxella catarrhalis*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis*. These microbial infections may be present in the upper, mid and lower airway (otitis, sinusitis, bronchitis but also exacerbations of chronic obstructive pulmonary disease (COPD), chronic cough, complications of and/or primary cause of cystic fibrosis (CF) and community acquired pneumonia (CAP). Thus, by practicing the in vivo methods of this invention, these diseases and complications from these infections can also be prevented or treated.

Infections might also occur in the oral cavity (*caries, periodontitis*) and caused by *Streptococcus mutans*, *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*.

Infections might also be localized to the skin (abscesses, 'staph' infections, impetigo, secondary infection of burns, Lyme disease) and caused by *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* and *Borrelia burgdorferi*. Infections of the urinary tract (UTI) can also be treated and are typically caused by *Escherichia coli*. Infections of the gastrointestinal tract (GI) (diarrhea, cholera, gall stones, gastric ulcers) are typically caused by *Salmonella enterica serovar*, *Vibrio cholerae* and *Helicobacter pylori*. Infections of the genital tract include and are typically caused by *Neisseria gonorrhoeae*. Infections can be of the bladder or of an indwelling device caused by *Enterococcus faecalis*. Infections associated with implanted prosthetic devices, such as artificial hip or knee replacements, or dental implants, or medical devices such as pumps, catheters, stents, or monitoring systems, typically caused by a variety of bacteria, can be treated by the methods of this invention. These devices can be coated or conjugated to an agent as described herein. Thus, by practicing the in vivo methods of this invention, these diseases and complications from these infections can also be prevented or treated.

Infections caused by *Streptococcus agalactiae* can also be treated by the methods of this invention and it is the major cause of bacterial septicemia in newborns. Infections caused by *Neisseria meningitidis* which can cause meningitis can also be treated.

Thus, routes of administration applicable to the methods of the invention include intranasal, intramuscular, urethrally, intratracheal, subcutaneous, intradermal, topical application, intravenous, rectal, nasal, oral, inhalation, and other enteral and parenteral routes of administration. Routes of administration may be combined, if desired, or adjusted depending upon the agent and/or the desired effect. An active agent can be administered in a single dose or in multiple doses. Embodiments of these methods and routes suitable for delivery, include systemic or localized routes. In general, routes of administration suitable for the methods of the invention include, but are not limited to, direct injection, enteral, parenteral, or inhalational routes.

Parenteral routes of administration other than inhalation administration include, but are not limited to, topical, transdermal, subcutaneous, intramuscular, intraorbital, intracapsular, intraspinal, intrasternal, and intravenous routes, i.e., any route of administration other than through the alimentary canal. Parenteral administration can be conducted to effect systemic or local delivery of the inhibiting agent. Where systemic delivery is desired, administration typically involves invasive or systemically absorbed topical or mucosal administration of pharmaceutical preparations.

The interfering agents of the invention can also be delivered to the subject by enteral administration. Enteral routes of administration include, but are not limited to, oral and rectal (e.g., using a suppository) delivery.

Methods of administration of the active through the skin or mucosa include, but are not limited to, topical application of a suitable pharmaceutical preparation, transcutaneous transmission, transdermal transmission, injection and epidermal administration. For transdermal transmission, absorption promoters or iontophoresis are suitable methods. Iontophoretic transmission may be accomplished using commercially available "patches" that deliver their product continuously via electric pulses through unbroken skin for periods of several days or more.

In various embodiments of the methods of the invention, the interfering agent will be administered by inhalation, injection or orally on a continuous, daily basis, at least once per day (QD), and in various embodiments two (BID), three (TID), or even four times a day. Typically, the therapeutically effective daily dose will be at least about 1 mg, or at least about 10 mg, or at least about 100 mg, or about 200 – about 500 mg, and sometimes, depending on the compound, up to as much as about 1 g to about 2.5 g.

Dosing of can be accomplished in accordance with the methods of the invention using capsules, tablets, oral suspension, suspension for intra-muscular injection, suspension for intravenous infusion, gel or cream for topical application, or suspension for intra-articular injection.

Dosage, toxicity and therapeutic efficacy of compositions described herein can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, for example, to determine the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀. Compositions which exhibit high therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The

dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the methods, the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

In some embodiments, an effective amount of a composition sufficient for achieving a therapeutic or prophylactic effect, ranges from about 0.000001 mg per kilogram body weight per administration to about 10,000 mg per kilogram body weight per administration. Suitably, the dosage ranges are from about 0.0001 mg per kilogram body weight per administration to about 100 mg per kilogram body weight per administration. Administration can be provided as an initial dose, followed by one or more “booster” doses. Booster doses can be provided a day, two days, three days, a week, two weeks, three weeks, one, two, three, six or twelve months after an initial dose. In some embodiments, a booster dose is administered after an evaluation of the subject’s response to prior administrations.

The skilled artisan will appreciate that certain factors may influence the dosage and timing required to effectively treat a subject, including but not limited to, the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of the therapeutic compositions described herein can include a single treatment or a series of treatments.

Polypeptides

Also provided herein are interfering agents and compositions for use in the methods described herein, wherein the interfering agent is of the group:

- (a) an isolated or recombinant integration host factor (IHF) polypeptide or a fragment or an equivalent of each thereof;
- (b) an isolated or recombinant histone-like protein from *E.coli* strain U93 (HU) polypeptide or a fragment or an equivalent of each thereof;

(c) an isolated or recombinant protein polypeptide identified in Table 8, Table 9A, Table 9B, Table 10 or a DNA binding peptide identified in Figure 6, or a fragment or an equivalent of each thereof;

(d) an isolated or recombinant polypeptide of SEQ ID NO. 1 through 340, or a fragment or an equivalent thereof;

(e) an isolated or recombinant C-terminal polypeptide of SEQ ID NO. 6 through 11, 28, 29, 42 through 100, Table 8 or those C-terminal polypeptides identified in Table 10 or a fragment or an equivalent of each thereof; or

(f) a polypeptide or polynucleotide that competes with an integration host factor on binding to a microbial DNA.

In one particular aspect, the interfering agent is an isolated or recombinant DNABII polypeptide or a fragment or an equivalent of each thereof. Non-limiting examples of such are an IHF or HU alpha or beta polypeptide; an IHF α polypeptide; *Moraxell acatarrhalis* HU; *E. coli* HupA, HupB, himA, himD; *E. faecalis* HU (such as V583), HMGB1 and those identified in Table 8.

In another aspect, the interfering agent is an isolated or recombinant polypeptide consisting essentially of an amino acid sequence selected from SEQ ID NO. 1 to 5 or 12 to 27, 30 to 35, 101-340 or a DNA binding peptide identified in Figure 6.

In another aspect, the isolated or recombinant polypeptide comprises, or alternatively consists essentially of, or yet further consists of SEQ ID NO. 1 or 2, with the proviso that the polypeptide is none of SEQ ID NO. 6 to 11, 28, 29, or 42 through 100.

In another aspect, the isolated or recombinant polypeptide comprises, or alternatively consists essentially of, or yet further consists of SEQ ID NO. 3, 4 or 5, with the proviso that the polypeptide is none of SEQ ID NO. 6 to 11, 28, 29, or 42 through 100.

In another aspect, the isolated or recombinant polypeptide comprises, or alternatively consists essentially of, or yet further consists of SEQ ID NO. 12, 14, 16, 18, 20, 22, 24, 26, 30 or 32, with the proviso that the polypeptide is none of SEQ ID NO. 6 to 11, 28, 29, or 42 through 100.

In another aspect, the isolated or recombinant polypeptide comprises, or alternatively consists essentially of, or yet further consists of SEQ ID NO. 13, 15, 17, 19, 21, 23, 25, 27, 31 33, 34,

or 35 with the proviso that the polypeptide is none of SEQ ID NO. 6 to 11, 28, 29, or 42 through 100.

In another aspect, the isolated or recombinant polypeptide comprises, or alternatively consists essentially of, or yet further consists of an isolated or recombinant polypeptide of the group of:

- a polypeptide comprising SEQ ID NO. 12 and 13;
- a polypeptide comprising SEQ ID NO. 14 and 15;
- a polypeptide comprising SEQ ID NO. 16 and 17;
- a polypeptide comprising SEQ ID NO. 18 and 19;
- a polypeptide comprising SEQ ID NO. 20 and 21;
- a polypeptide comprising SEQ ID NO. 23 and 24;
- a polypeptide comprising SEQ ID NO. 25 and 26;
- a polypeptide comprising SEQ ID NO. 30 and 31;
- a polypeptide comprising SEQ ID NO. 32 and 33;
- a polypeptide comprising SEQ ID NO. 34 and 35;
- a polypeptide comprising SEQ ID NO. 337 and 338; or
- a polypeptide comprising SEQ ID NO. 339 and 340;

with the proviso that the polypeptide is none of wild-type of any one of IHF alpha, IHF beta or SEQ ID NO. 6 to 11, 28, 29, or 42 through 100.

In another aspect, the isolated or recombinant polypeptide is of the group:

- a polypeptide consisting essentially of SEQ ID NO. 12 and 13;
- a polypeptide consisting essentially of SEQ ID NO. 14 and 15;
- a polypeptide consisting essentially of SEQ ID NO. 16 and 17;
- a polypeptide consisting essentially of SEQ ID NO. 18 and 19;
- a polypeptide consisting essentially of SEQ ID NO. 20 and 21;
- a polypeptide consisting essentially of SEQ ID NO. 23 and 24;
- a polypeptide consisting essentially of SEQ ID NO. 25 and 26;

- a polypeptide consisting essentially of SEQ ID NO. 30 and 31;
- a polypeptide consisting essentially of SEQ ID NO. 32 and 33;
- a polypeptide consisting essentially of SEQ ID NO. 34 and 35;
- a polypeptide consisting essentially of SEQ ID NO. 337 and 338; or
- a polypeptide consisting essentially of SEQ ID NO. 339 and 340;

with the proviso that the polypeptide is none of wild-type of any one of IHF alpha, IHF beta or SEQ ID NO. 6 to 11, 28, 29, or 42 through 100.

Further provided as agents for use in the methods of this invention are fragments or an equivalent of the isolated or recombinant polypeptides described above. An example of a fragment is a C-terminal polypeptide. In a further aspect, the isolated or recombinant polypeptide comprises, or alternatively consists essentially of, or yet further consists of two or more of the isolated or recombinant polypeptides described above.

For example, the isolated or recombinant polypeptide comprises, or alternatively consists essentially of, or yet further consists of any one of SEQ ID NO. 1 to 6, 12 to 27 or 30 to 33, or a fragment or an equivalent polypeptide, examples of which are identified in Table 8 or shown in Table 9A, Table 9B or Table 10. In one aspect, isolated wild-type polypeptides are excluded, i.e., that the polypeptide is none of SEQ ID NO. 6 through 11, 28, 29, or a wildtype sequence identified in Table 8 or shown in Table 9A.

In one aspect, this invention provides an isolated or recombinant polypeptide consisting essentially of an amino acid sequence of the group SEQ ID NO. 1 to 6, 12 to 27 or 30 to 35, 1 to 6 and 13 to 35, or a polypeptide comprising, or alternatively consisting essentially of, or yet further consisting of an amino acid corresponding to the β -3 and/or α -3 fragments of a *Haemophilus influenzae* IHFa or IHF β , non-limiting examples of which include SEQ ID NO. 12 through 27, or a fragment or equivalent thereof of each thereof. In another aspect, the invention provides an isolated or recombinant polypeptide comprising, or alternatively consisting essentially of, or yet further consisting of an amino acid sequence of the group SEQ ID NO. 1 to 4, or a fragment or an equivalent of each thereof, or a polypeptide comprising, or alternatively consisting essentially of, or yet further consisting of an amino acid corresponding to the β -3 and/or α -3 fragments of a *Haemophilus influenzae* IHFa or IHF β , non-limiting examples of which include SEQ ID NO. 12 through 27 or a fragment or a biological equivalent thereof which further comprises independently at least 2, or

alternatively at least 3, or alternatively at least 4, or alternatively at least 5, or at least 6, or alternatively at least 7, or alternatively at least 8, or alternatively at least 9 or alternatively at least 10 amino acids at the amino and/or carboxyl terminus of the polypeptide. In one aspect, isolated wildtype DNA binding polypeptides are excluded, i.e., that the polypeptide is none of SEQ ID NO. 6 through 11, 28, 29, or 42 through 100 or an isolated wildtype polypeptide sequence listed in Table 8 or shown in Table 9A.

In another aspect, this invention provides an isolated or recombinant polypeptide comprising, or alternatively consisting essentially of, or yet further consisting of, SEQ ID NO 1 or 2 alone or in combination with a polypeptide comprising, or alternatively consisting essentially of, or yet further consisting of an amino acid corresponding to the β -3 and/or α -3 fragments of a *Haemophilus influenzae* IHF α or IHF β , non-limiting examples of which include SEQ ID Nos. 12 through 27 or a fragment or a biological equivalent of each thereof. In one aspect, isolated wildtype DNA binding polypeptides are excluded, i.e., that the polypeptide is none of SEQ ID NO. 6 through 11, 28, 29, or 42 through 100 or an isolated wildtype polypeptide sequence listed in Table 8 or shown in Table 9A.

In a yet further aspect, this invention provides an isolated or recombinant polypeptide comprising or alternatively consisting essentially of, or yet further consisting of, SEQ ID NO. 3 or 4 or a fragment or an equivalent of each thereof, alone or in combination with a polypeptide comprising, or alternatively consisting essentially of, or yet further consisting of an amino acid corresponding to the β -3 and/or α -3 fragments of a *Haemophilus influenzae* IHF α or IHF β , non-limiting examples of which include SEQ ID NO. 12 through 27, and 34-35 or a biological equivalent of each thereof. In one aspect, isolated wildtype DNA binding polypeptides are excluded, i.e., that the polypeptide is none of SEQ ID NO. 6 through 11, 28, 29, or 42 through 100 or an isolated wildtype polypeptide sequence listed in Table 8 or shown in Table 9A.

This invention also provides isolated or recombinant polypeptides comprising or alternatively consisting essentially of, or yet further consisting of, two or more, or three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, ten or more, eleven or more, twelve or more, thirteen or more of all fourteen of the isolated polypeptides or a fragment or an equivalent of each thereof. Examples of such include isolated or recombinant polypeptides comprising SEQ ID NO. 1 through 4, e.g., SEQ ID NO. 1 and 2, or alternatively 1 and 3 or alternatively 1 and 4, or alternatively 2 and 3, or alternatively SEQ ID NO. 1, 2 and 3 or alternatively, 2, 3 and 4, or alternatively 1, 3 and 4. The polypeptides can

be in any orientation, e.g., SEQ ID NO. 1, 2, and 3 or SEQ ID NO. 3, 2 and 1 or alternatively 2, 1 and 3, or alternatively, 3, 1 and 2. Biological equivalents of these polypeptides are further included in this invention with the proviso that the sequences do not include isolated wildtype sequences such as those identified in Tables 8 and 9.

In another aspect, this invention provides an isolated or recombinant polypeptide comprising or alternatively consisting essentially of, or yet further consisting of, SEQ ID NO. 1 or 2 and 3 or 4, or a fragment or an equivalent of each thereof, with the proviso that the polypeptide is none of SEQ ID NO. 5 through 10, and they may further comprise any one or more of SEQ ID Nos. 11 through 26, e.g., 11 and 12, or alternatively 1 and 11, or alternatively 2 and 11, or alternatively, 1 and 12, or alternatively 2 and 12, or alternatively 11, 12 and 1, or alternatively 2, 11 and 12. In this embodiment, SEQ ID NO. 1 or 2 is located upstream or amino terminus from SEQ ID NO. 3 or 4, with the proviso that the amino acid sequence is not an isolated wildtype polypeptide, e.g., none of SEQ ID NO. 6 through 11, 28 and 29. In another aspect, the isolated polypeptide comprises SEQ ID NO. 3 or 4 located upstream or amino terminus to SEQ ID NO. 1 or 2. Fragments Biological equivalents of these polypeptides are further included in this invention with the proviso that the sequence do not include isolated wildtype polypeptides.

In one embodiment, any polypeptide or protein having sequence identity to the wildtype polypeptides or those disclosed in Pedulla et al. (1996) PNAS 93:15411-15416 is excluded from this invention.

In any of the above embodiments, a peptide linker can be added to the N-terminus or C-terminus of the polypeptide. A “linker” or “peptide linker” refers to a peptide sequence linked to either the N-terminus or the C-terminus of a polypeptide sequence. In one aspect, the linker is from about 1 to about 20 amino acid residues long or alternatively 2 to about 10, about 3 to about 5 amino acid residues long. An example of a peptide linker is Gly-Pro-Ser-Leu-Lys-Leu (SEQ ID NO: 37). Other examples include Gly-Gly-Gly; Gly-Pro-Ser-Leu (SEQ ID NO: 38); Gly-Pro-Ser; Pro-Ser-Leu-Lys (SEQ ID NO: 39); Gly-Pro-Ser-Leu-Lys (SEQ ID NO: 40) and Ser-Leu-Lys-Leu (SEQ ID NO: 41).

The isolated polypeptides of this invention are intended to include isolated wildtype and recombinantly produced polypeptides and proteins from prokaryotic and eukaryotic host cells, as well as muteins, analogs and fragments thereof, examples of such cells are described above. In some embodiments, the term also includes antibodies and anti-idiotypic antibodies

as described herein. Such polypeptides can be isolated or produced using the methods known in the art and briefly described herein.

It is understood that functional equivalents or variants of the wild type polypeptide or protein also are within the scope of this invention, for example, those having conservative amino acid substitutions of the amino acids, see for example, Table 9. Other analogs include fusion proteins comprising a protein or polypeptide of this invention which can include a polypeptide joined to an antigen presenting matrix.

In a further aspect, the polypeptides are conjugated or linked to a detectable label. Suitable labels are known in the art and described herein.

In a yet further aspect, the polypeptides with or without a detectable label can be contained or expressed on the surface of a host prokaryotic or eukaryotic host cell, such as a dendritic cell.

The proteins and polypeptides are obtainable by a number of processes known to those of skill in the art, which include purification, chemical synthesis and recombinant methods. Polypeptides can be isolated from preparations such as host cell systems by methods such as immunoprecipitation with antibody, and standard techniques such as gel filtration, ion-exchange, reversed-phase, and affinity chromatography. For such methodology, see for example Deutscher et al. (1999) *Guide To Protein Purification: Methods In Enzymology* (Vol. 182, Academic Press). Accordingly, this invention also provides the processes for obtaining these polypeptides as well as the products obtainable and obtained by these processes.

The polypeptides also can be obtained by chemical synthesis using a commercially available automated peptide synthesizer such as those manufactured by Perkin/ Elmer/Applied Biosystems, Inc., Model 430A or 431A, Foster City, CA, USA. The synthesized polypeptide can be precipitated and further purified, for example by high performance liquid chromatography (HPLC). Accordingly, this invention also provides a process for chemically synthesizing the proteins of this invention by providing the sequence of the protein and reagents, such as amino acids and enzymes and linking together the amino acids in the proper orientation and linear sequence.

Alternatively, the proteins and polypeptides can be obtained by well-known recombinant methods as described, for example, in Sambrook et al. (1989) *supra*, using a host cell and vector systems described herein.

Also provided by this application are the polypeptides described herein conjugated to a

detectable agent for use in the diagnostic methods. For example, detectably labeled polypeptides can be bound to a column and used for the detection and purification of antibodies. They also are useful as immunogens for the production of antibodies as described below. The polypeptides of this invention are useful in an in vitro assay system to screen for agents or drugs, which modulate cellular processes.

It is well known to those skilled in the art that modifications can be made to the peptides of the invention to provide them with altered properties. As used herein the term "amino acid" refers to either natural and/or unnatural or synthetic amino acids, including glycine and both the D or L optical isomers, and amino acid analogs and peptidomimetics. A peptide of three or more amino acids is commonly called an oligopeptide if the peptide chain is short. If the peptide chain is long, the peptide is commonly called a polypeptide or a protein.

Peptides of the invention can be modified to include unnatural amino acids. Thus, the peptides may comprise D-amino acids, a combination of D- and L-amino acids, and various "designer" amino acids (e.g., .beta.-methyl amino acids, C-.alpha.-methyl amino acids, and N-.alpha.-methyl amino acids, etc.) to convey special properties to peptides. Additionally, by assigning specific amino acids at specific coupling steps, peptides with .alpha.-helices .beta. turns, .beta. sheets, .gamma.-turns, and cyclic peptides can be generated. Generally, it is believed that .alpha.-helical secondary structure or random secondary structure is preferred.

The polypeptides of this invention also can be combined with various solid phase carriers, such as an implant, a stent, a paste, a gel, a dental implant, or a medical implant or liquid phase carriers, such as beads, sterile or aqueous solutions, pharmaceutically acceptable carriers, pharmaceutically acceptable polymers, liposomes, micelles, suspensions and emulsions. Examples of non-aqueous solvents include propyl ethylene glycol, polyethylene glycol and vegetable oils. When used to prepare antibodies or induce an immune response in vivo, the carriers also can include an adjuvant that is useful to non-specifically augment a specific immune response. A skilled artisan can easily determine whether an adjuvant is required and select one. However, for the purpose of illustration only, suitable adjuvants include, but are not limited to Freund's Complete and Incomplete, mineral salts and polynucleotides. Other suitable adjuvants include monophosphoryl lipid A (MPL), mutant derivatives of the heat labile enterotoxin of E. coli, mutant derivatives of cholera toxin, CPG oligonucleotides, and adjuvants derived from squalene.

This invention also provides a pharmaceutical composition comprising or alternatively

consisting essentially of, or yet further consisting of, any of a polypeptide, analog, mutein, or fragment of this invention, alone or in combination with each other or other agents, such as an antibiotic and an acceptable carrier or solid support. These compositions are useful for various diagnostic and therapeutic methods as described herein.

Polynucleotides

This invention also provides isolated or recombinant polynucleotides encoding one or more of the above-identified isolated or recombinant polypeptides and their respective complementary strands. Vectors comprising the isolated or recombinant polynucleotides are further provided examples of which are known in the art and briefly described herein. In one aspect where more than one isolated or recombinant polynucleotide is to be expressed as a single unit, the isolated or recombinant polynucleotides can be contained within a polycistronic vector. The polynucleotides can be DNA, RNA, mRNA or interfering RNA, such as siRNA, miRNA or dsRNA.

In another aspect, this invention provides an interfering agent that is a polynucleotide that interferes with the binding of the DNA to a polypeptide or protein in a microbial biofilm, or a four-way junction polynucleotide resembling a Holliday junction, a 3 way junction polynucleotide resembling a replication fork, a polynucleotide that has inherent flexibility or bent polynucleotide which can treat or inhibit DNABII polynucleotide from binding to microbial DNA as well treat, prevent or inhibit biofilm formation and associated infections and disorders. One of skill in the art can make such polynucleotides using the information provided herein and knowledge of those of skill in the art. See Goodman and Kay (1999) *J. Biological Chem.* 274(52):37004-37011 and Kamashev and Rouviere-Yaniv (2000) *EMBO J.* 19(23):6527-6535.

The invention further provides the isolated or recombinant polynucleotide operatively linked to a promoter of RNA transcription, as well as other regulatory sequences for replication and/or transient or stable expression of the DNA or RNA. As used herein, the term "operatively linked" means positioned in such a manner that the promoter will direct transcription of RNA off the DNA molecule. Examples of such promoters are SP6, T4 and T7. In certain embodiments, cell-specific promoters are used for cell-specific expression of the inserted polynucleotide. Vectors which contain a promoter or a promoter/enhancer, with termination codons and selectable marker sequences, as well as a cloning site into which an inserted piece of DNA can be operatively linked to that promoter are known in the art and

commercially available. For general methodology and cloning strategies, see Gene Expression Technology (Goeddel ed., Academic Press, Inc. (1991)) and references cited therein and Vectors: Essential Data Series (Gacsa and Ramji, eds., John Wiley & Sons, N.Y. (1994)) which contains maps, functional properties, commercial suppliers and a reference to GenEMBL accession numbers for various suitable vectors.

In one embodiment, polynucleotides derived from the polynucleotides of the invention encode polypeptides or proteins having diagnostic and therapeutic utilities as described herein as well as probes to identify transcripts of the protein that may or may not be present. These nucleic acid fragments can be prepared, for example, by restriction enzyme digestion of larger polynucleotides and then labeled with a detectable marker. Alternatively, random fragments can be generated using nick translation of the molecule. For methodology for the preparation and labeling of such fragments, see Sambrook, et al. (1989) *supra*.

Expression vectors containing these nucleic acids are useful to obtain host vector systems to produce proteins and polypeptides. It is implied that these expression vectors must be replicable in the host organisms either as episomes or as an integral part of the chromosomal DNA. Non-limiting examples of suitable expression vectors include plasmids, yeast vectors, viral vectors and liposomes. Adenoviral vectors are particularly useful for introducing genes into tissues *in vivo* because of their high levels of expression and efficient transformation of cells both *in vitro* and *in vivo*. When a nucleic acid is inserted into a suitable host cell, e.g., a prokaryotic or a eukaryotic cell and the host cell replicates, the protein can be recombinantly produced. Suitable host cells will depend on the vector and can include mammalian cells, animal cells, human cells, simian cells, insect cells, yeast cells, and bacterial cells constructed using known methods. See Sambrook, et al. (1989) *supra*. In addition to the use of viral vector for insertion of exogenous nucleic acid into cells, the nucleic acid can be inserted into the host cell by methods known in the art such as transformation for bacterial cells; transfection using calcium phosphate precipitation for mammalian cells; or DEAE-dextran; electroporation; or microinjection. See, Sambrook et al. (1989) *supra*, for methodology. Thus, this invention also provides a host cell, e.g. a mammalian cell, an animal cell (rat or mouse), a human cell, or a prokaryotic cell such as a bacterial cell, containing a polynucleotide encoding a protein or polypeptide or antibody.

A polynucleotide can comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure can be imparted before or after assembly of the polynucleotide. The sequence of nucleotides can be

interrupted by non-nucleotide components. A polynucleotide can be further modified after polymerization, such as by conjugation with a labeling component. The term also refers to both double- and single-stranded molecules. Unless otherwise specified or required, any embodiment of this invention that is a polynucleotide encompasses both the double-stranded form and each of two complementary single-stranded forms known or predicted to make up the double-stranded form.

When the vectors are used for gene therapy in vivo or ex vivo, a pharmaceutically acceptable vector is preferred, such as a replication-incompetent retroviral or adenoviral vector.

Pharmaceutically acceptable vectors containing the nucleic acids of this invention can be further modified for transient or stable expression of the inserted polynucleotide. As used herein, the term “pharmaceutically acceptable vector” includes, but is not limited to, a vector or delivery vehicle having the ability to selectively target and introduce the nucleic acid into dividing cells. An example of such a vector is a “replication-incompetent” vector defined by its inability to produce viral proteins, precluding spread of the vector in the infected host cell. An example of a replication-incompetent retroviral vector is LNL6 (Miller et al. (1989) *BioTechniques* 7:980-990). The methodology of using replication-incompetent retroviruses for retroviral-mediated gene transfer of gene markers has been established. (Bordignon (1989) *PNAS USA* 86:8912-8952; Culver (1991) *PNAS USA* 88:3155; and Rill (1991) *Blood* 79(10):2694-2700).

This invention also provides genetically modified cells that contain and/or express the polynucleotides of this invention. The genetically modified cells can be produced by insertion of upstream regulatory sequences such as promoters or gene activators (see, U.S. Patent No. 5,733,761).

The polynucleotides can be conjugated to a detectable marker, e.g., an enzymatic label or a radioisotope for detection of nucleic acid and/or expression of the gene in a cell. A wide variety of appropriate detectable markers are known in the art, including fluorescent, radioactive, enzymatic or other ligands, such as avidin/biotin, which are capable of giving a detectable signal. In one aspect, one will likely desire to employ a fluorescent label or an enzyme tag, such as urease, alkaline phosphatase or peroxidase, instead of radioactive or other environmentally undesirable reagents. In the case of enzyme tags, calorimetric indicator substrates can be employed to provide a means visible to the human eye or spectrophotometrically, to identify specific hybridization with complementary nucleic acid-containing samples. Thus, this invention further provides a method for detecting a

single-stranded polynucleotide or its complement, by contacting target single-stranded polynucleotide with a labeled, single-stranded polynucleotide (a probe) which is a portion of the polynucleotide of this invention under conditions permitting hybridization (preferably moderately stringent hybridization conditions) of complementary single-stranded polynucleotides, or more preferably, under highly stringent hybridization conditions. Hybridized polynucleotide pairs are separated from un-hybridized, single-stranded polynucleotides. The hybridized polynucleotide pairs are detected using methods known to those of skill in the art and set forth, for example, in Sambrook et al. (1989) *supra*.

The polynucleotide embodied in this invention can be obtained using chemical synthesis, recombinant cloning methods, PCR, or any combination thereof. Methods of chemical polynucleotide synthesis are known in the art and need not be described in detail herein. One of skill in the art can use the sequence data provided herein to obtain a desired polynucleotide by employing a DNA synthesizer or ordering from a commercial service.

The polynucleotides of this invention can be isolated or replicated using PCR. The PCR technology is the subject matter of U.S. Patent Nos. 4,683,195; 4,800,159; 4,754,065; and 4,683,202 and described in PCR: The Polymerase Chain Reaction (Mullis et al. eds., Birkhauser Press, Boston (1994)) or MacPherson et al. (1991) and (1995) *supra*, and references cited therein. Alternatively, one of skill in the art can use the sequences provided herein and a commercial DNA synthesizer to replicate the DNA. Accordingly, this invention also provides a process for obtaining the polynucleotides of this invention by providing the linear sequence of the polynucleotide, nucleotides, appropriate primer molecules, chemicals such as enzymes and instructions for their replication and chemically replicating or linking the nucleotides in the proper orientation to obtain the polynucleotides. In a separate embodiment, these polynucleotides are further isolated. Still further, one of skill in the art can insert the polynucleotide into a suitable replication vector and insert the vector into a suitable host cell (prokaryotic or eukaryotic) for replication and amplification. The DNA so amplified can be isolated from the cell by methods known to those of skill in the art. A process for obtaining polynucleotides by this method is further provided herein as well as the polynucleotides so obtained.

RNA can be obtained by first inserting a DNA polynucleotide into a suitable host cell. The DNA can be delivered by any appropriate method, e.g., by the use of an appropriate gene delivery vehicle (e.g., liposome, plasmid or vector) or by electroporation. When the cell replicates and the DNA is transcribed into RNA; the RNA can then be isolated using methods

known to those of skill in the art, for example, as set forth in Sambrook et al. (1989) *supra*. For instance, mRNA can be isolated using various lytic enzymes or chemical solutions according to the procedures set forth in Sambrook et al. (1989) *supra*, or extracted by nucleic-acid-binding resins following the accompanying instructions provided by manufactures.

Polynucleotides exhibiting sequence complementarity or homology to a polynucleotide of this invention are useful as hybridization probes or as an equivalent of the specific polynucleotides identified herein. Since the full coding sequence of the transcript is known, any portion of this sequence or homologous sequences, can be used in the methods of this invention.

It is known in the art that a “perfectly matched” probe is not needed for a specific hybridization. Minor changes in probe sequence achieved by substitution, deletion or insertion of a small number of bases do not affect the hybridization specificity. In general, as much as 20% base-pair mismatch (when optimally aligned) can be tolerated. Preferably, a probe useful for detecting the aforementioned mRNA is at least about 80% identical to the homologous region. More preferably, the probe is 85% identical to the corresponding gene sequence after alignment of the homologous region; even more preferably, it exhibits 90% identity.

These probes can be used in radioassays (e.g. Southern and Northern blot analysis) to detect, prognose, diagnose or monitor various cells or tissues containing these cells. The probes also can be attached to a solid support or an array such as a chip for use in high throughput screening assays for the detection of expression of the gene corresponding a polynucleotide of this invention. Accordingly, this invention also provides a probe comprising or corresponding to a polynucleotide of this invention, or its equivalent, or its complement, or a fragment thereof, attached to a solid support for use in high throughput screens.

The total size of fragment, as well as the size of the complementary stretches, will depend on the intended use or application of the particular nucleic acid segment. Smaller fragments will generally find use in hybridization embodiments, wherein the length of the complementary region may be varied, such as between at least 5 to 10 to about 100 nucleotides, or even full length according to the complementary sequences one wishes to detect.

Nucleotide probes having complementary sequences over stretches greater than 5 to 10 nucleotides in length are generally preferred, so as to increase stability and selectivity of the

hybrid, and thereby improving the specificity of particular hybrid molecules obtained. More preferably, one can design polynucleotides having gene-complementary stretches of 10 or more or more than 50 nucleotides in length, or even longer where desired. Such fragments may be readily prepared by, for example, directly synthesizing the fragment by chemical means, by application of nucleic acid reproduction technology, such as the PCR technology with two priming oligonucleotides as described in U.S. Patent No. 4,603,102 or by introducing selected sequences into recombinant vectors for recombinant production. In one aspect, a probe is about 50-75 or more alternatively, 50-100, nucleotides in length.

The polynucleotides of the present invention can serve as primers for the detection of genes or gene transcripts that are expressed in cells described herein. In this context, amplification means any method employing a primer-dependent polymerase capable of replicating a target sequence with reasonable fidelity. Amplification may be carried out by natural or recombinant DNA-polymerases such as T7 DNA polymerase, Klenow fragment of E. coli DNA polymerase, and reverse transcriptase. For illustration purposes only, a primer is the same length as that identified for probes.

One method to amplify polynucleotides is PCR and kits for PCR amplification are commercially available. After amplification, the resulting DNA fragments can be detected by any appropriate method known in the art, e.g., by agarose gel electrophoresis followed by visualization with ethidium bromide staining and ultraviolet illumination.

Methods for administering an effective amount of a gene delivery vector or vehicle to a cell have been developed and are known to those skilled in the art and described herein. Methods for detecting gene expression in a cell are known in the art and include techniques such as in hybridization to DNA microarrays, in situ hybridization, PCR, RNase protection assays and Northern blot analysis. Such methods are useful to detect and quantify expression of the gene in a cell. Alternatively expression of the encoded polypeptide can be detected by various methods. In particular it is useful to prepare polyclonal or monoclonal antibodies that are specifically reactive with the target polypeptide. Such antibodies are useful for visualizing cells that express the polypeptide using techniques such as immunohistology, ELISA, and Western blotting. These techniques can be used to determine expression level of the expressed polynucleotide.

Antibodies and Derivatives Thereof

This invention also provides an antibody that binds and/or specifically recognizes and binds an isolated polypeptide for use in the methods of the invention. The antibody can be any of the various antibodies described herein, non-limiting examples of such include a polyclonal antibody, a monoclonal antibody, a chimeric antibody, a human antibody, a veneered antibody, a diabody, a humanized antibody, an antibody derivative, a recombinant humanized antibody, or a derivative or fragment of each thereof. In one aspect, the fragment comprises, or alternatively consists essentially of, or yet further consists of the CDR of the antibody. In one aspect, the antibody is detectably labeled or further comprises a detectable label conjugated to it. Also provided is a hybridoma cell line that produces a monoclonal antibody of this invention. Compositions comprising or alternatively consisting essentially of or yet further, consisting of one or more of the above embodiments are further provided herein. Further provided are polynucleotides that encode the amino acid sequence of the antibodies and fragments as well as methods to produce recombinantly or chemically synthesize the antibody polypeptides and fragments thereof. The antibody polypeptides can be produced in a eukaryotic or prokaryotic cell, or by other methods known in the art and described herein.

Antibodies can be generated using conventional techniques known in the art and are well-described in the literature. Several methodologies exist for production of polyclonal antibodies. For example, polyclonal antibodies are typically produced by immunization of a suitable mammal such as, but not limited to, chickens, goats, guinea pigs, hamsters, horses, mice, rats, and rabbits. An antigen is injected into the mammal, which induces the B-lymphocytes to produce immunoglobulins specific for the antigen. Immunoglobulins may be purified from the mammal's serum. Antibodies specific to IHF α and IHF β can be generated by injection of polypeptides corresponding to different epitopes of IHF α and IHF β . For example, antibodies can be generated using the 20 amino acids of each subunit such as TFRPGQKLKSRVENASPKDE (SEQ ID NO.34) for IHF α and KYVPHFKPGKELRDRANIYG (SEQ ID No. 35) for IHF β . Variations of this methodology include modification of adjuvants, routes and site of administration, injection volumes per site and the number of sites per animal for optimal production and humane treatment of the animal. For example, adjuvants typically are used to improve or enhance an immune response to antigens. Most adjuvants provide for an injection site antigen depot, which allows for a slow release of antigen into draining lymph nodes. Other adjuvants include surfactants which promote concentration of protein antigen molecules over a large surface area and immunostimulatory molecules. Non-limiting examples of adjuvants for polyclonal

antibody generation include Freund's adjuvants, Ribi adjuvant system, and Titermax.

Polyclonal antibodies can be generated using methods known in the art some of which are described in U.S. Patent Nos. 7,279,559; 7,119,179; 7,060,800; 6,709,659; 6,656,746; 6,322,788; 5,686,073; and 5,670,153.

Monoclonal antibodies can be generated using conventional hybridoma techniques known in the art and well-described in the literature. For example, a hybridoma is produced by fusing a suitable immortal cell line (e.g., a myeloma cell line such as, but not limited to, Sp2/0, Sp2/0-AG14, NSO, NS1, NS2, AE-1, L.5, P3X63Ag8.653, Sp2 SA3, Sp2 MAI, Sp2 SS1, Sp2 SA5, U397, MLA 144, ACT IV, MOLT4, DA-1, JURKAT, WEHI, K-562, COS, RAJI, NIH 3T3, HL-60, MLA 144, NAMAIWA, NEURO 2A, CHO, PerC.6, YB2/O) or the like, or heteromyelomas, fusion products thereof, or any cell or fusion cell derived there from, or any other suitable cell line as known in the art (see, those at the following web addresses e.g., atcc.org, lifetech.com., last accessed on November 26, 2007), with antibody producing cells, such as, but not limited to, isolated or cloned spleen, peripheral blood, lymph, tonsil, or other immune or B cell containing cells, or any other cells expressing heavy or light chain constant or variable or framework or CDR sequences, either as endogenous or heterologous nucleic acid, as recombinant or endogenous, viral, bacterial, algal, prokaryotic, amphibian, insect, reptilian, fish, mammalian, rodent, equine, ovine, goat, sheep, primate, eukaryotic, genomic DNA, cDNA, rDNA, mitochondrial DNA or RNA, chloroplast DNA or RNA, hnRNA, mRNA, tRNA, single, double or triple stranded, hybridized, and the like or any combination thereof. Antibody producing cells can also be obtained from the peripheral blood or, preferably the spleen or lymph nodes, of humans or other suitable animals that have been immunized with the antigen of interest. Any other suitable host cell can also be used for expressing-heterologous or endogenous nucleic acid encoding an antibody, specified fragment or variant thereof, of the present invention. The fused cells (hybridomas) or recombinant cells can be isolated using selective culture conditions or other suitable known methods, and cloned by limiting dilution or cell sorting, or other known methods.

Other suitable methods of producing or isolating antibodies of the requisite specificity can be used, including, but not limited to, methods that select recombinant antibody from a peptide or protein library (e.g., but not limited to, a bacteriophage, ribosome, oligonucleotide, RNA, cDNA, or the like, display library; e.g., as available from various commercial vendors such as MorphoSys (Martinsreid/Planegg, Del.), BioInvent (Lund, Sweden), Affitech (Oslo, Norway) using methods known in the art. Art known methods are described in the patent

literature some of which include U.S. Patent Nos. 4,704,692; 5,723,323; 5,763,192; 5,814,476; 5,817,483; 5,824,514; 5,976,862. Alternative methods rely upon immunization of transgenic animals (e.g., SCID mice, Nguyen et al. (1977) *Microbiol. Immunol.* 41:901-907 (1997); Sandhu et al. (1996) *Crit. Rev. Biotechnol.* 16:95-118; Eren et al. (1998) *Immunol.* 93:154-161 that are capable of producing a repertoire of human antibodies, as known in the art and/or as described herein. Such techniques, include, but are not limited to, ribosome display (Hanes et al. (1997) *Proc. Natl. Acad. Sci. USA*, 94:4937-4942; Hanes et al. (1998) *Proc. Natl. Acad. Sci. USA* 95:14130-14135); single cell antibody producing technologies (e.g., selected lymphocyte antibody method ("SLAM") (U.S. Patent No. 5,627,052, Wen et al. (1987) *J. Immunol.* 17:887-892; Babcook et al. (1996) *Proc. Natl. Acad. Sci. USA* 93:7843-7848); gel microdroplet and flow cytometry (Powell et al. (1990) *Biotechnol.* 8:333-337; One Cell Systems, (Cambridge, Mass).; Gray et al. (1995) *J. Imm. Meth.* 182:155-163; and Kenny et al. (1995) *Bio. Technol.* 13:787-790); B-cell selection (Steenbakketers et al. (1994) *Molec. Biol. Reports* 19:125-134).

Antibody derivatives of the present invention can also be prepared by delivering a polynucleotide encoding an antibody of this invention to a suitable host such as to provide transgenic animals or mammals, such as goats, cows, horses, sheep, and the like, that produce such antibodies in their milk. These methods are known in the art and are described for example in U.S. Patent Nos. 5,827,690; 5,849,992; 4,873,316; 5,849,992; 5,994,616; 5,565,362; and 5,304,489.

The term "antibody derivative" includes post-translational modification to linear polypeptide sequence of the antibody or fragment. For example, U.S. Patent No. 6,602,684 B1 describes a method for the generation of modified glycol-forms of antibodies, including whole antibody molecules, antibody fragments, or fusion proteins that include a region equivalent to the Fc region of an immunoglobulin, having enhanced Fc-mediated cellular toxicity, and glycoproteins so generated.

The antibodies of the invention also include derivatives that are modified by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from generating an anti-idiotypic response. Antibody derivatives include, but are not limited to, antibodies that have been modified by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Additionally, the derivatives may contain one or more non-classical amino acids.

Antibody derivatives also can be prepared by delivering a polynucleotide of this invention to provide transgenic plants and cultured plant cells (e.g., but not limited to tobacco, maize, and duckweed) that produce such antibodies, specified portions or variants in the plant parts or in cells cultured therefrom. For example, Cramer et al. (1999) *Curr. Top. Microbol. Immunol.* 240:95-118 and references cited therein, describe the production of transgenic tobacco leaves expressing large amounts of recombinant proteins, e.g., using an inducible promoter.

Transgenic maize have been used to express mammalian proteins at commercial production levels, with biological activities equivalent to those produced in other recombinant systems or purified from natural sources. See, e.g., Hood et al. (1999) *Adv. Exp. Med. Biol.* 464:127-147 and references cited therein. Antibody derivatives have also been produced in large amounts from transgenic plant seeds including antibody fragments, such as single chain antibodies (scFv's), including tobacco seeds and potato tubers. See, e.g., Conrad et al. (1998) *Plant Mol. Biol.* 38:101-109 and references cited therein. Thus, antibodies can also be produced using transgenic plants, according to known methods.

Antibody derivatives also can be produced, for example, by adding exogenous sequences to modify immunogenicity or reduce, enhance or modify binding, affinity, on-rate, off-rate, avidity, specificity, half-life, or any other suitable characteristic. Generally part or all of the non-human or human CDR sequences are maintained while the non-human sequences of the variable and constant regions are replaced with human or other amino acids.

In general, the CDR residues are directly and most substantially involved in influencing antigen binding. Humanization or engineering of antibodies can be performed using any known method such as, but not limited to, those described in U.S. Patent Nos. 5,723,323; 5,976,862; 5,824,514; 5,817,483; 5,814,476; 5,763,192; 5,723,323; 5,766,886; 5,714,352; 6,204,023; 6,180,370; 5,693,762; 5,530,101; 5,585,089; 5,225,539; and 4,816,567.

Chimeric, humanized or primatized antibodies of the present invention can be prepared based on the sequence of a reference monoclonal antibody prepared using standard molecular biology techniques. DNA encoding the heavy and light chain immunoglobulins can be obtained from the hybridoma of interest and engineered to contain non-reference (e.g., human) immunoglobulin sequences using standard molecular biology techniques. For example, to create a chimeric antibody, the murine variable regions can be linked to human constant regions using methods known in the art (U.S. Patent No. 4,816,567). To create a humanized antibody, the murine CDR regions can be inserted into a human framework using methods known in the art (U.S. Patent No. 5,225,539 and U.S. Patents Nos. 5,530,101;

5,585,089; 5,693,762 and 6,180,370). Similarly, to create a primatized antibody the murine CDR regions can be inserted into a primate framework using methods known in the art (WO 93/02108 and WO 99/55369).

Techniques for making partially to fully human antibodies are known in the art and any such techniques can be used. According to one embodiment, fully human antibody sequences are made in a transgenic mouse which has been engineered to express human heavy and light chain antibody genes. Multiple strains of such transgenic mice have been made which can produce different classes of antibodies. B cells from transgenic mice which are producing a desirable antibody can be fused to make hybridoma cell lines for continuous production of the desired antibody. (See for example, Russel et al. (2000) *Infection and Immunity* April 2000:1820-1826; Gallo et al. (2000) *European J. of Immun.* 30:534-540; Green (1999) *J. of Immun. Methods* 231:11-23; Yang et al. (1999A) *J. of Leukocyte Biology* 66:401-410; Yang (1999B) *Cancer Research* 59(6):1236-1243; Jakobovits (1998) *Advanced Drug Delivery Reviews* 31:33-42; Green and Jakobovits (1998) *J. Exp. Med.* 188(3):483-495; Jakobovits (1998) *Exp. Opin. Invest. Drugs* 7(4):607-614; Tsuda et al. (1997) *Genomics* 42:413-421; Sherman-Gold (1997) *Genetic Engineering News* 17(14); Mendez et al. (1997) *Nature Genetics* 15:146-156; Jakobovits (1996) *Weir's Handbook of Experimental Immunology, The Integrated Immune System Vol. IV*, 194.1-194.7; Jakobovits (1995) *Current Opinion in Biotechnology* 6:561-566; Mendez et al. (1995) *Genomics* 26:294-307; Jakobovits (1994) *Current Biology* 4(8):761-763; Arbones et al. (1994) *Immunity* 1(4):247-260; Jakobovits (1993) *Nature* 362(6417):255-258; Jakobovits et al. (1993) *Proc. Natl. Acad. Sci. USA* 90(6):2551-2555; and U.S. Patent No. 6,075,181.)

The antibodies of this invention also can be modified to create chimeric antibodies. Chimeric antibodies are those in which the various domains of the antibodies' heavy and light chains are coded for by DNA from more than one species. See, e.g., U.S. Patent No. 4,816,567.

Alternatively, the antibodies of this invention can also be modified to create veneered antibodies. Veneered antibodies are those in which the exterior amino acid residues of the antibody of one species are judiciously replaced or "veneered" with those of a second species so that the antibodies of the first species will not be immunogenic in the second species thereby reducing the immunogenicity of the antibody. Since the antigenicity of a protein is primarily dependent on the nature of its surface, the immunogenicity of an antibody could be reduced by replacing the exposed residues which differ from those usually found in another mammalian species antibodies. This judicious replacement of exterior residues should have

little, or no, effect on the interior domains, or on the interdomain contacts. Thus, ligand binding properties should be unaffected as a consequence of alterations which are limited to the variable region framework residues. The process is referred to as "veneering" since only the outer surface or skin of the antibody is altered, the supporting residues remain undisturbed.

The procedure for "veneering" makes use of the available sequence data for human antibody variable domains compiled by Kabat et al. (1987) Sequences of Proteins of Immunological Interest, 4th ed., Bethesda, Md., National Institutes of Health, updates to this database, and other accessible U.S. and foreign databases (both nucleic acid and protein). Non-limiting examples of the methods used to generate veneered antibodies include EP 519596; U.S. Patent No. 6,797,492; and described in Padlan et al. (1991) Mol. Immunol. 28(4-5):489-498.

The term "antibody derivative" also includes "diabodies" which are small antibody fragments with two antigen-binding sites, wherein fragments comprise a heavy chain variable domain (VH) connected to a light chain variable domain (VL) in the same polypeptide chain. (See for example, EP 404,097; WO 93/11161; and Hollinger et al. (1993) Proc. Natl. Acad. Sci. USA 90:6444-6448.) By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. (See also, U.S. Patent No. 6,632,926 to Chen et al. which discloses antibody variants that have one or more amino acids inserted into a hypervariable region of the parent antibody and a binding affinity for a target antigen which is at least about two fold stronger than the binding affinity of the parent antibody for the antigen).

The term "antibody derivative" further includes engineered antibody molecules, fragments and single domains such as scFv, dAbs, nanobodies, minibodies, Unibodies, and Affibodies (Holliger & Hudson (2005) Nature Biotech 23(9):1126-36; U.S. Patent Publication US 2006/0211088; PCT Publication W02007/059782; US Patent No. 5,831,012).

The term "antibody derivative" further includes "linear antibodies". The procedure for making linear antibodies is known in the art and described in Zapata et al. (1995) Protein Eng. 8(10):1057-1062. Briefly, these antibodies comprise a pair of tandem Fd segments (V_H-C_H1-V_H-C_H1) which form a pair of antigen binding regions. Linear antibodies can be bispecific or monospecific.

The antibodies of this invention can be recovered and purified from recombinant cell cultures by known methods including, but not limited to, protein A purification, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. High performance liquid chromatography ("HPLC") can also be used for purification.

Antibodies of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a eukaryotic host, including, for example, yeast, higher plant, insect and mammalian cells, or alternatively from a prokaryotic host as described above. A number of antibody production systems are described in Birch & Radner (2006) *Adv. Drug Delivery Rev.* 58: 671-685.

If an antibody being tested binds with protein or polypeptide, then the antibody being tested and the antibodies provided by this invention are equivalent. It also is possible to determine without undue experimentation, whether an antibody has the same specificity as the antibody of this invention by determining whether the antibody being tested prevents an antibody of this invention from binding the protein or polypeptide with which the antibody is normally reactive. If the antibody being tested competes with the antibody of the invention as shown by a decrease in binding by the monoclonal antibody of this invention, then it is likely that the two antibodies bind to the same or a closely related epitope. Alternatively, one can pre-incubate the antibody of this invention with a protein with which it is normally reactive, and determine if the antibody being tested is inhibited in its ability to bind the antigen. If the antibody being tested is inhibited then, in all likelihood, it has the same, or a closely related, epitopic specificity as the antibody of this invention.

The term "antibody" also is intended to include antibodies of all immunoglobulin isotypes and subclasses. Particular isotypes of a monoclonal antibody can be prepared either directly by selecting from an initial fusion, or prepared secondarily, from a parental hybridoma secreting a monoclonal antibody of different isotype by using the sib selection technique to isolate class switch variants using the procedure described in Steplewski et al. (1985) *Proc. Natl. Acad. Sci. USA* 82:8653 or Spira et al. (1984) *J. Immunol. Methods* 74:307. Alternatively, recombinant DNA techniques may be used.

The isolation of other monoclonal antibodies with the specificity of the monoclonal antibodies described herein can also be accomplished by one of ordinary skill in the art by

producing anti-idiotypic antibodies. Herlyn et al. (1986) Science 232:100. An anti-idiotypic antibody is an antibody which recognizes unique determinants present on the monoclonal antibody of interest.

In some aspects of this invention, it will be useful to detectably or therapeutically label the antibody. Suitable labels are described supra. Methods for conjugating antibodies to these agents are known in the art. For the purpose of illustration only, antibodies can be labeled with a detectable moiety such as a radioactive atom, a chromophore, a fluorophore, or the like. Such labeled antibodies can be used for diagnostic techniques, either in vivo, or in an isolated test sample.

The coupling of antibodies to low molecular weight haptens can increase the sensitivity of the antibody in an assay. The haptens can then be specifically detected by means of a second reaction. For example, it is common to use haptens such as biotin, which reacts avidin, or dinitrophenol, pyridoxal, and fluorescein, which can react with specific anti-hapten antibodies. See, Harlow and Lane (1988) supra.

The variable region of the antibodies of the present invention can be modified by mutating amino acid residues within the VH and/or VL CDR 1, CDR 2 and/or CDR 3 regions to improve one or more binding properties (e.g., affinity) of the antibody. Mutations may be introduced by site-directed mutagenesis or PCR-mediated mutagenesis and the effect on antibody binding, or other functional property of interest, can be evaluated in appropriate in vitro or in vivo assays. Preferably conservative modifications are introduced and typically no more than one, two, three, four or five residues within a CDR region are altered. The mutations may be amino acid substitutions, additions or deletions.

Framework modifications can be made to the antibodies to decrease immunogenicity, for example, by "backmutating" one or more framework residues to the corresponding germline sequence.

In addition, the antibodies of the invention may be engineered to include modifications within the Fc region to alter one or more functional properties of the antibody, such as serum half-life, complement fixation, Fc receptor binding, and/or antigen-dependent cellular cytotoxicity. Such modifications include, but are not limited to, alterations of the number of cysteine residues in the hinge region to facilitate assembly of the light and heavy chains or to increase or decrease the stability of the antibody (U.S. Patent No. 5,677,425); and amino acid

mutations in the Fc hinge region to decrease the biological half life of the antibody (U.S. Patent No. 6,165,745).

Additionally, the antibodies of the invention may be chemically modified. Glycosylation of an antibody can be altered, for example, by modifying one or more sites of glycosylation within the antibody sequence to increase the affinity of the antibody for antigen (U.S. Patents Nos. 5,714,350 and 6,350,861). Alternatively, to increase antibody-dependent cell-mediated cytotoxicity, a hypofucosylated antibody having reduced amounts of fucosyl residues or an antibody having increased bisecting GlcNAc structures can be obtained by expressing the antibody in a host cell with altered glycosylation mechanism (Shields, R. L. et al., 2002 J. Biol. Chem. 277:26733-26740; Umana et al., 1999 Nat. Biotech. 17:176-180).

The antibodies of the invention can be pegylated to increase biological half-life by reacting the antibody or fragment thereof with polyethylene glycol (PEG) or a reactive ester or aldehyde derivative of PEG, under conditions in which one or more PEG groups become attached to the antibody or antibody fragment. Antibody pegylation may be carried out by an acylation reaction or an alkylation reaction with a reactive PEG molecule (or an analogous reactive water-soluble polymer). As used herein, the term "polyethylene glycol" is intended to encompass any of the forms of PEG that have been used to derivatize other proteins, such as mono (C1-C10) alkoxy- or aryloxy-polyethylene glycol or polyethylene glycol-maleimide. The antibody to be pegylated can be an aglycosylated antibody. Methods for pegylating proteins are known in the art and can be applied to the antibodies of the invention (EP 0 154 316 and EP 0 401 384).

Additionally, antibodies may be chemically modified by conjugating or fusing the antigen-binding region of the antibody to serum protein, such as human serum albumin, to increase half-life of the resulting molecule. Such approach is for example described in EP 0322094 and EP 0 486 525.

The antibodies or fragments thereof of the present invention may be conjugated to a diagnostic agent and used diagnostically, for example, to monitor the development or progression of a disease and determine the efficacy of a given treatment regimen. Examples of diagnostic agents include enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody or fragment

thereof, or indirectly, through a linker using techniques known in the art. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase. Examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin. Examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin. An example of a luminescent material includes luminol. Examples of bioluminescent materials include luciferase, luciferin, and aequorin. Examples of suitable radioactive material include ^{125}I , ^{131}I , Indium-111, Lutetium-171, Bismuth-212, Bismuth-213, Astatine-211, Copper-62, Copper-64, Copper-67, Yttrium-90, Iodine-125, Iodine-131, Phosphorus-32, Phosphorus-33, Scandium-47, Silver-111, Gallium-67, Praseodymium-142, Samarium-153, Terbium-161, Dysprosium-166, Holmium-166, Rhenium-186, Rhenium-188, Rhenium-189, Lead-212, Radium-223, Actinium-225, Iron-59, Selenium-75, Arsenic-77, Strontium-89, Molybdenum-99, Rhodium-105, Palladium-109, Praseodymium-143, Promethium-149, Erbium-169, Iridium-194, Gold-198, Gold-199, and Lead-211. Monoclonal antibodies may be indirectly conjugated with radiometal ions through the use of bifunctional chelating agents that are covalently linked to the antibodies. Chelating agents may be attached through amines (Meares et al., 1984 Anal. Biochem. 142: 68-78); sulfhydryl groups (Koyama 1994 Chem. Abstr. 120: 217262t) of amino acid residues and carbohydrate groups (Rodwell et al. 1986 PNAS USA 83: 2632-2636; Quadri et al. 1993 Nucl. Med. Biol. 20: 559-570).

Further, the antibodies or fragments thereof of the present invention may be conjugated to a therapeutic agent. Suitable therapeutic agents include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin, antimetabolites (such as methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, fludarabine, 5-fluorouracil, decarbazine, hydroxyurea, asparaginase, gemcitabine, cladribine), alkylating agents (such as mechlorethamine, thioepa, chlorambucil, melphalan, carmustine (BSNU), lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, dacarbazine (DTIC), procarbazine, mitomycin C, cisplatin and other platinum derivatives, such as carboplatin), antibiotics (such as dactinomycin (formerly actinomycin), bleomycin, daunorubicin (formerly daunomycin), doxorubicin, idarubicin, mithramycin, mitomycin, mitoxantrone, plicamycin, anthramycin (AMC)),

diphtheria toxin and related molecules (such as diphtheria A chain and active fragments thereof and hybrid molecules), ricin toxin (such as ricin A or a deglycosylated ricin A chain toxin), cholera toxin, a Shiga-like toxin (SLT-I, SLT-II, SLT-IIIV), LT toxin, C3 toxin, Shiga toxin, pertussis toxin, tetanus toxin, soybean Bowman-Birk protease inhibitor, *Pseudomonas* exotoxin, alorin, saporin, modeccin, gelanin, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, *Phytolacca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin toxins and mixed toxins.

Additional suitable conjugated molecules include ribonuclease (RNase), DNase I, an antisense nucleic acid, an inhibitory RNA molecule such as a siRNA molecule, an immunostimulatory nucleic acid, aptamers, ribozymes, triplex forming molecules, and external guide sequences. Aptamers are small nucleic acids ranging from 15-50 bases in length that fold into defined secondary and tertiary structures, such as stem-loops or G-quartets, and can bind small molecules, such as ATP (U.S. Patent No. 5,631,146) and theophiline (U.S. Patent No. 5,580,737), as well as large molecules, such as reverse transcriptase (U.S. Patent No. 5,786,462) and thrombin (U.S. Patent No. 5,543,293).

Ribozymes are nucleic acid molecules that are capable of catalyzing a chemical reaction, either intramolecularly or intermolecularly. Ribozymes typically cleave nucleic acid substrates through recognition and binding of the target substrate with subsequent cleavage. Triplex forming function nucleic acid molecules can interact with double-stranded or single-stranded nucleic acid by forming a triplex, in which three strands of DNA form a complex dependant on both Watson-Crick and Hoogsteen base-pairing. Triplex molecules can bind target regions with high affinity and specificity.

The functional nucleic acid molecules may act as effectors, inhibitors, modulators, and stimulators of a specific activity possessed by a target molecule, or the functional nucleic acid molecules may possess a de novo activity independent of any other molecules.

The therapeutic agents can be linked to the antibody directly or indirectly, using any of a large number of available methods. For example, an agent can be attached at the hinge region of the reduced antibody component via disulfide bond formation, using cross-linkers such as N-succinyl 3-(2-pyridyldithio)propionate (SPDP), or via a carbohydrate moiety in the Fc region of the antibody (Yu et al. 1994 Int. J. Cancer 56: 244; Upešlaciš et al., "Modification of Antibodies by Chemical Methods," in Monoclonal antibodies: principles and applications, Birch et al. (eds.), pages 187-230 (Wiley-Liss, Inc. 1995); Price, "Production and

Characterization of Synthetic Peptide-Derived Antibodies," in Monoclonal antibodies: Production, engineering and clinical application, Ritter et al. (eds.), pages 60-84 (Cambridge University Press 1995)).

Techniques for conjugating therapeutic agents to antibodies are well known (Amon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody in Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates" 1982 Immunol. Rev. 62:119-58).

The antibodies of the invention or antigen-binding regions thereof can be linked to another functional molecule such as another antibody or ligand for a receptor to generate a bi-specific or multi-specific molecule that binds to at least two or more different binding sites or target molecules. Linking of the antibody to one or more other binding molecules, such as another antibody, antibody fragment, peptide or binding mimetic, can be done, for example, by chemical coupling, genetic fusion, or noncovalent association. Multi-specific molecules can further include a third binding specificity, in addition to the first and second target epitope.

Bi-specific and multi-specific molecules can be prepared using methods known in the art. For example, each binding unit of the bi-specific molecule can be generated separately and then conjugated to one another. When the binding molecules are proteins or peptides, a variety of coupling or cross-linking agents can be used for covalent conjugation. Examples of cross-linking agents include protein A, carbodiimide, N-succinimidyl-S-acetyl-thioacetate (SATA), 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), o-phenylenedimaleimide (oPDM), N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), and sulfosuccinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (sulfo-SMCC) (Karpovsky et al., 1984 J. Exp. Med. 160:1686; Liu et al., 1985 Proc. Natl. Acad. Sci. USA 82:8648). When the binding molecules are antibodies, they can be conjugated by sulfhydryl bonding of the C-terminus hinge regions of the two heavy chains.

The antibodies or fragments thereof of the present invention may be linked to a moiety that is toxic to a cell to which the antibody is bound to form "depleting" antibodies. These antibodies are particularly useful in applications where it is desired to deplete an NK cell.

The antibodies of the invention may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

The antibodies also can be bound to many different carriers. Thus, this invention also provides compositions containing the antibodies and another substance, active or inert. Examples of well-known carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylase, natural and modified cellulose, polyacrylamide, agarose, and magnetite. The nature of the carrier can be either soluble or insoluble for purposes of the invention. Those skilled in the art will know of other suitable carriers for binding monoclonal antibodies, or will be able to ascertain such, using routine experimentation.

Isolation, Culturing and Expansion of APCs, Including Dendritic Cells

This invention also provides isolated host cells comprising one or more of an isolated polypeptides or isolated polynucleotides or the vectors of this invention. In one aspect the isolated host cell is a eukaryotic cell such as antigen presenting cell (APC), e.g. a dendritic cell. In another aspect, the isolated host cell is a prokaryotic cell. In one aspect, the invention is an isolated host cell that is cultured under conditions that promote expression of the polynucleotide. This invention also provides the host cell, expression system and polypeptide produced by the expression system.

The following is a brief description of two fundamental approaches for the isolation of APC. These approaches involve (1) isolating bone marrow precursor cells (CD34⁺) from blood and stimulating them to differentiate into APC; or (2) collecting the precommitted APCs from peripheral blood. In the first approach, the patient must be treated with cytokines such as GM-CSF to boost the number of circulating CD34⁺ stem cells in the peripheral blood.

The second approach for isolating APCs is to collect the relatively large numbers of precommitted APCs already circulating in the blood. Previous techniques for isolating committed APCs from human peripheral blood have involved combinations of physical procedures such as metrizamide gradients and adherence/nonadherence steps (Freudenthal et al. (1990) PNAS 87:7698-7702); Percoll gradient separations (Mehta-Damani et al. (1994) J.

Immunol. 153:996-1003); and fluorescence activated cell sorting techniques (Thomas et al. (1993) J. Immunol. 151:6840-52).

One technique for separating large numbers of cells from one another is known as countercurrent centrifugal elutriation (CCE). Cell samples are placed in a special elutriation rotor. The rotor is then spun at a constant speed of, for example, 3000 rpm. Once the rotor has reached the desired speed, pressurized air is used to control the flow rate of cells. Cells in the elutriator are subjected to simultaneous centrifugation and a washout stream of buffer which is constantly increasing in flow rate. This results in fractional cell separations based largely but not exclusively on differences in cell size.

In one aspect of the invention, the APC are precommitted or mature dendritic cells which can be isolated from the white blood cell fraction of a mammal, such as a murine, simian or a human (See, e.g., WO 96/23060). The white blood cell fraction can be from the peripheral blood of the mammal. This method includes the following steps: (a) providing a white blood cell fraction obtained from a mammalian source by methods known in the art such as leukapheresis; (b) separating the white blood cell fraction of step (a) into four or more subfractions by countercurrent centrifugal elutriation, (c) stimulating conversion of monocytes in one or more fractions from step (b) to dendritic cells by contacting the cells with calcium ionophore, GM-CSF and IL-13 or GM-CSF and IL-4, (d) identifying the dendritic cell-enriched fraction from step (c), and (e) collecting the enriched fraction of step (d), preferably at about 4 °C. One way to identify the dendritic cell-enriched fraction is by fluorescence-activated cell sorting. The white blood cell fraction can be treated with calcium ionophore in the presence of other cytokines, such as recombinant (rh) rhIL-12, rhGM-CSF, or rhIL-4. The cells of the white blood cell fraction can be washed in buffer and suspended in $\text{Ca}^{++}/\text{Mg}^{++}$ free media prior to the separating step. The white blood cell fraction can be obtained by leukapheresis. The dendritic cells can be identified by the presence of at least one of the following markers: HLA-DR, HLA-DQ, or B7.2, and the simultaneous absence of the following markers: CD3, CD16, CD56, CD57, and CD19, CD20. Monoclonal antibodies specific to these cell surface markers are commercially available.

More specifically, the method requires collecting an enriched collection of white cells and platelets from leukapheresis that is then further fractionated by countercurrent centrifugal elutriation (CCE) (Abrahamsen et al. (1991) J. Clin. Apheresis. 6:48-53). In this technique, cells are subject to simultaneous centrifugation and a washout stream of buffer which is

constantly increasing in flow rate. The constantly increasing countercurrent flow of buffer leads to fractional cell separations that are largely based on cell size.

Quality control of APC and more specifically DC collection and confirmation of their successful activation in culture is dependent upon a simultaneous multi-color FACS analysis technique which monitors both monocytes and the dendritic cell subpopulation as well as possible contaminant T lymphocytes. Cell sorting is based on differential expression of cell surface markers including CD3 (T cells), CD16/CD56/CD57 (NK/LAK cells), and CD19/CD20 (B cells). DCs are distinguishable from monocytes based in part on levels of CD14, which is expressed at very high levels in monocytes compared to DCs. DCs show high levels of expression of HLA-DR, significant HLA-DQ and B7.2 (but little or no B7.1) at the time they are circulating in the blood (in addition they express Leu M7 and M9, myeloid markers which are also expressed by monocytes and neutrophils).

When combined with a third color reagent for analysis of dead cells, propidium iodide (PI), it is possible to make positive identification of all cell subpopulations

The goal of FACS analysis at the time of collection is to confirm that the DCs are enriched in the expected fractions, to monitor neutrophil contamination, and to make sure that appropriate markers are expressed. This rapid bulk collection of enriched DCs from human peripheral blood, suitable for clinical applications, is absolutely dependent on the analytic FACS technique described above for quality control. If need be, mature DCs can be immediately separated from monocytes at this point by fluorescent sorting for "cocktail negative" cells. It may not be necessary to routinely separate DCs from monocytes because, the monocytes themselves are still capable of differentiating into DCs or functional DC-like cells in culture.

Once collected, the DC rich/monocyte APC fractions (usually 150 through 190) can be pooled and cryopreserved for future use, or immediately placed in short term culture.

Alternatively, others have reported that a method for upregulating (activating) dendritic cells and converting monocytes to an activated dendritic cell phenotype. This method involves the addition of calcium ionophore to the culture media convert monocytes into activated dendritic cells. Adding the calcium ionophore A23187, for example, at the beginning of a 24-48 hour culture period resulted in uniform activation and dendritic cell phenotypic conversion of the pooled "monocyte plus DC" fractions: characteristically, the activated population becomes uniformly CD14 (Leu M3) negative, and upregulates HLA-DR, HLA-DQ, ICAM-1, B7.1,

and B7.2. Furthermore this activated bulk population functions as well on a small numbers basis as a further purified.

Specific combination(s) of cytokines have been used successfully to amplify (or partially substitute) for the activation/conversion achieved with calcium ionophore: these cytokines include but are not limited to purified or recombinant ("rh") rhGM-CSF, rhIL-2, and rhIL-4. Each cytokine when given alone is inadequate for optimal upregulation.

Presentation of Antigen to the APC

For purposes of immunization, the polypeptides (e.g., SEQ ID NO. 1 through 33) can be delivered to antigen-presenting cells as protein/peptide or in the form of cDNA encoding the protein/peptide. Antigen-presenting cells (APCs) can consist of dendritic cells (DCs), monocytes/macrophages, B lymphocytes or other cell type(s) expressing the necessary MHC/co-stimulatory molecules. The methods described below focus primarily on DCs which are the most potent, preferred APCs.

Pulsing is accomplished in vitro/ex vivo by exposing APCs to the antigenic protein or polypeptide(s) of this invention. The protein or peptide(s) are added to APCs at a concentration of 1-10 μ m for approximately 3 hours. Transfection of APCs with polynucleotides encoding antigens or antigenic polypeptides is accomplished by exposing APCs to the nucleic acids in the presence of transfection agents known in the art, including but not limited to cationic lipids. Transfected or pulsed APCs can subsequently be administered to the host via an intravenous, subcutaneous, intranasal, intramuscular or intraperitoneal route of delivery.

Protein/peptide antigen can also be delivered in vivo with adjuvant via the intravenous, subcutaneous, intranasal, intramuscular or intraperitoneal route of delivery.

Foster Antigen Presenting Cells

Foster antigen presenting cells are particularly useful as a target cell. Foster APCs are derived from the human cell line 174X CEM.T2, referred to as T2, which contains a mutation in its antigen processing pathway that restricts the association of endogenous peptides with cell surface MHC class I molecules (Zweerink et al. (1993) J. Immunol. 150:1763-1771). This is due to a large homozygous deletion in the MHC class II region encompassing the genes TAP 1, TAP2, LMP 1, and LMP2, which are required for antigen presentation to MHC class I-restricted CD8⁺ CTLs. In effect, only "empty" MHC class I molecules are presented on the surface of these cells. Exogenous peptide added to the culture medium binds to these

MHC molecules provided that the peptide contains the allele-specific binding motif. These T2 cells are referred to herein as "foster" APCs. They can be used in conjunction with this invention to present antigen(s).

Transduction of T2 cells with specific recombinant MHC alleles allows for redirection of the MHC restriction profile. Libraries tailored to the recombinant allele will be preferentially presented by them because the anchor residues will prevent efficient binding to the endogenous allele.

High level expression of MHC molecules makes the APC more visible to the CTLs. Expressing the MHC allele of interest in T2 cells using a powerful transcriptional promoter (e.g., the CMV promoter) results in a more reactive APC (most likely due to a higher concentration of reactive MHC-peptide complexes on the cell surface).

Expansion of Immune Effector Cells

The present invention makes use of these APCs to stimulate production of an enriched population of antigen-specific immune effector cells. The antigen-specific immune effector cells are expanded at the expense of the APCs, which die in the culture. The process by which naive immune effector cells become educated by other cells is described essentially in Coulie (1997) *Molec. Med. Today* 3:261-268.

The APCs prepared as described above are mixed with naive immune effector cells. Preferably, the cells may be cultured in the presence of a cytokine, for example IL2. Because dendritic cells secrete potent immunostimulatory cytokines, such as IL12, it may not be necessary to add supplemental cytokines during the first and successive rounds of expansion. In any event, the culture conditions are such that the antigen-specific immune effector cells expand (i.e. proliferate) at a much higher rate than the APCs. Multiple infusions of APCs and optional cytokines can be performed to further expand the population of antigen-specific cells.

In one embodiment, the immune effector cells are T cells. In a separate embodiment, the immune effector cells can be genetically modified by transduction with a transgene coding for example, IL-2, IL-11 or IL-13. Methods for introducing transgenes in vitro, ex vivo and in vivo are known in the art.

Functional Analysis with Antibodies

Antibodies of this invention can be used to purify the polypeptides of this invention and to

identify biological equivalent polypeptide and/or polynucleotides. They also can be used to identify agents that modify the function of the polypeptides of this invention. These antibodies include polyclonal antisera, monoclonal antibodies, and various reagents derived from these preparations that are familiar to those practiced in the art and described above.

Antibodies that neutralize the activities of proteins encoded by identified genes can also be used in vivo and in vitro to demonstrate function by adding such neutralizing antibodies into in vivo and in vitro test systems. They also are useful as pharmaceutical agents to modulate the activity of polypeptides of the invention.

Various antibody preparations can also be used in analytical methods such as ELISA assays or Western blots to demonstrate the expression of proteins encoded by the identified genes by test cells in vitro or in vivo. Fragments of such proteins generated by protease degradation during metabolism can also be identified by using appropriate polyclonal antisera with samples derived from experimental samples.

The antibodies of the invention may be used for vaccination or to boost vaccination, alone or in combination with peptides or protein-based vaccines or dendritic-cell based vaccines.

Compositions

Compositions are further provided. The compositions comprise a carrier and one or more of an isolated polypeptide of the invention, an isolated polynucleotide of the invention, a vector of the invention, an isolated host cell of the invention, a small molecule or an antibody of the invention. The carriers can be one or more of a solid support or a pharmaceutically acceptable carrier. The compositions can further comprise an adjuvant or other components suitable for administrations as vaccines. In one aspect, the compositions are formulated with one or more pharmaceutically acceptable excipients, diluents, carriers and/or adjuvants. In addition, embodiments of the compositions of the present invention include one or more of an isolated polypeptide of the invention, an isolated polynucleotide of the invention, a vector of the invention, a small molecule, an isolated host cell of the invention, or an antibody of the invention, formulated with one or more pharmaceutically acceptable auxiliary substances.

For oral preparations, any one or more of an isolated or recombinant polypeptide as described herein, an isolated or recombinant polynucleotide as described herein, a vector as described herein, an isolated host cell as described herein, a small molecule or an antibody as described herein can be used alone or in pharmaceutical formulations of the invention comprising, or consisting essentially of, the compound in combination with appropriate additives to make

tablets, powders, granules or capsules, for example, with conventional additives, such as lactose, mannitol, corn starch or potato starch; with binders, such as crystalline cellulose, cellulose derivatives, acacia, corn starch or gelatins; with disintegrators, such as corn starch, potato starch or sodium carboxymethylcellulose; with lubricants, such as talc or magnesium stearate; and if desired, with diluents, buffering agents, moistening agents, preservatives and flavoring agents. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

Pharmaceutical formulations and unit dose forms suitable for oral administration are particularly useful in the treatment of chronic conditions, infections, and therapies in which the patient self-administers the drug. In one aspect, the formulation is specific for pediatric administration.

The invention provides pharmaceutical formulations in which the one or more of an isolated polypeptide of the invention, an isolated polynucleotide of the invention, a vector of the invention, an isolated host cell of the invention, or an antibody of the invention can be formulated into preparations for injection in accordance with the invention by dissolving, suspending or emulsifying them in an aqueous or nonaqueous solvent, such as vegetable or other similar oils, synthetic aliphatic acid glycerides, esters of higher aliphatic acids or propylene glycol; and if desired, with conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives or other antimicrobial agents. A non-limiting example of such is a antimicrobial agent such as other vaccine components such as surface antigens, e.g. an OMP P5, rsPilA, OMP 26, OMP P2, or Type IV Pilin protein (see Jurcisek and Bakaletz (2007) J. of Bacteriology 189(10):3868-3875 and Murphy, TF, Bakaletz, LO and Smeesters, PR (2009) The Pediatric Infectious Disease Journal, 28:S121-S126) and antibacterial agents. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.), or phosphate buffered saline (PBS). In all cases, a composition for

parenteral administration must be sterile and should be fluid to the extent that easy syringability exists.

Aerosol formulations provided by the invention can be administered via inhalation and can be propellant or non-propellant based. For example, embodiments of the pharmaceutical formulations of the invention comprise a compound of the invention formulated into pressurized acceptable propellants such as dichlorodifluoromethane, propane, nitrogen and the like. For administration by inhalation, the compounds can be delivered in the form of an aerosol spray from a pressurized container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer. A non-limiting example of a non-propellant is a pump spray that is ejected from a closed container by means of mechanical force (*i.e.*, pushing down a piston with one's finger or by compression of the container, such as by a compressive force applied to the container wall or an elastic force exerted by the wall itself (*e.g.* by an elastic bladder)).

Suppositories of the invention can be prepared by mixing a compound of the invention with any of a variety of bases such as emulsifying bases or water-soluble bases. Embodiments of this pharmaceutical formulation of a compound of the invention can be administered rectally via a suppository. The suppository can include vehicles such as cocoa butter, carbowaxes and polyethylene glycols, which melt at body temperature, yet are solidified at room temperature.

Unit dosage forms for oral or rectal administration, such as syrups, elixirs, and suspensions, may be provided wherein each dosage unit, for example, teaspoonful, tablespoonful, tablet or suppository, contains a predetermined amount of the composition containing one or more compounds of the invention. Similarly, unit dosage forms for injection or intravenous administration may comprise a compound of the invention in a composition as a solution in sterile water, normal saline or another pharmaceutically acceptable carrier.

Embodiments of the pharmaceutical formulations of the invention include those in which one or more of an isolated polypeptide of the invention, an isolated polynucleotide of the invention, a vector of the invention, a small molecule for use in the invention, an isolated host cell of the invention, or an antibody of the invention is formulated in an injectable composition. Injectable pharmaceutical formulations of the invention are prepared as liquid solutions or suspensions; or as solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection. The preparation may also be emulsified or the active ingredient

encapsulated in liposome vehicles in accordance with other embodiments of the pharmaceutical formulations of the invention.

In an embodiment, one or more of an isolated polypeptide of the invention, an isolated polynucleotide of the invention, a vector of the invention, an isolated host cell of the invention, or an antibody of the invention is formulated for delivery by a continuous delivery system. The term "continuous delivery system" is used interchangeably herein with "controlled delivery system" and encompasses continuous (e.g., controlled) delivery devices (e.g., pumps) in combination with catheters, injection devices, and the like, a wide variety of which are known in the art.

Mechanical or electromechanical infusion pumps can also be suitable for use with the present disclosure. Examples of such devices include those described in, for example, U.S. Pat. Nos. 4,692,147; 4,360,019; 4,487,603; 4,360,019; 4,725,852; 5,820,589; 5,643,207; 6,198,966; and the like. In general, delivery of a compound of the invention can be accomplished using any of a variety of refillable, pump systems. Pumps provide consistent, controlled release over time. In some embodiments, a compound of the invention is in a liquid formulation in a drug-impermeable reservoir, and is delivered in a continuous fashion to the individual.

In one embodiment, the drug delivery system is an at least partially implantable device. The implantable device can be implanted at any suitable implantation site using methods and devices well known in the art. An implantation site is a site within the body of a subject at which a drug delivery device is introduced and positioned. Implantation sites include, but are not necessarily limited to, a subdermal, subcutaneous, intramuscular, or other suitable site within a subject's body. Subcutaneous implantation sites are used in some embodiments because of convenience in implantation and removal of the drug delivery device.

Drug release devices suitable for use in the disclosure may be based on any of a variety of modes of operation. For example, the drug release device can be based upon a diffusive system, a convective system, or an erodible system (e.g., an erosion-based system). For example, the drug release device can be an electrochemical pump, osmotic pump, an electroosmotic pump, a vapor pressure pump, or osmotic bursting matrix, e.g., where the drug is incorporated into a polymer and the polymer provides for release of drug formulation concomitant with degradation of a drug-impregnated polymeric material (e.g., a biodegradable, drug-impregnated polymeric material). In other embodiments, the drug

release device is based upon an electrodiffusion system, an electrolytic pump, an effervescent pump, a piezoelectric pump, a hydrolytic system, etc.

Drug release devices based upon a mechanical or electromechanical infusion pump can also be suitable for use with the present disclosure. Examples of such devices include those described in, for example, U.S. Pat. Nos. 4,692,147; 4,360,019; 4,487,603; 4,360,019; 4,725,852, and the like. In general, a subject treatment method can be accomplished using any of a variety of refillable, non-exchangeable pump systems. Pumps and other convective systems are generally preferred due to their generally more consistent, controlled release over time. Osmotic pumps are used in some embodiments due to their combined advantages of more consistent controlled release and relatively small size (see, e.g., PCT published application no. WO 97/27840 and U.S. Pat. Nos. 5,985,305 and 5,728,396). Exemplary osmotically-driven devices suitable for use in the disclosure include, but are not necessarily limited to, those described in U.S. Pat. Nos. 3,760,984; 3,845,770; 3,916,899; 3,923,426; 3,987,790; 3,995,631; 3,916,899; 4,016,880; 4,036,228; 4,111,202; 4,111,203; 4,203,440; 4,203,442; 4,210,139; 4,327,725; 4,627,850; 4,865,845; 5,057,318; 5,059,423; 5,112,614; 5,137,727; 5,234,692; 5,234,693; 5,728,396; and the like. A further exemplary device that can be adapted for the present disclosure is the Synchromed infusion pump (Medtronic).

In some embodiments, the drug delivery device is an implantable device. The drug delivery device can be implanted at any suitable implantation site using methods and devices well known in the art. As noted herein, an implantation site is a site within the body of a subject at which a drug delivery device is introduced and positioned. Implantation sites include, but are not necessarily limited to a subdermal, subcutaneous, intramuscular, or other suitable site within a subject's body.

Suitable excipient vehicles for a compound of the invention are, for example, water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. In addition, if desired, the vehicle may contain minor amounts of auxiliary substances such as wetting or emulsifying agents or pH buffering agents. Methods of preparing such dosage forms are known, or will be apparent upon consideration of this disclosure, to those skilled in the art. See, e.g., Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pennsylvania, 17th edition, 1985. The composition or formulation to be administered will, in any event, contain a quantity of the compound adequate to achieve the desired state in the subject being treated.

Compositions of the present invention include those that comprise a sustained-release or controlled release matrix. In addition, embodiments of the present invention can be used in conjunction with other treatments that use sustained-release formulations. As used herein, a sustained-release matrix is a matrix made of materials, usually polymers, which are degradable by enzymatic or acid-based hydrolysis or by dissolution. Once inserted into the body, the matrix is acted upon by enzymes and body fluids. A sustained-release matrix desirably is chosen from biocompatible materials such as liposomes, polylactides (polylactic acid), polyglycolide (polymer of glycolic acid), polylactide co-glycolide (copolymers of lactic acid and glycolic acid), polyanhydrides, poly(ortho)esters, polypeptides, hyaluronic acid, collagen, chondroitin sulfate, carboxylic acids, fatty acids, phospholipids, polysaccharides, nucleic acids, polyamino acids, amino acids such as phenylalanine, tyrosine, isoleucine, polynucleotides, polyvinyl propylene, polyvinylpyrrolidone and silicone. Illustrative biodegradable matrices include a polylactide matrix, a polyglycolide matrix, and a polylactide co-glycolide (co-polymers of lactic acid and glycolic acid) matrix.

In another embodiment, the interfering agent (as well as combination compositions) is delivered in a controlled release system. For example, a compound of the invention may be administered using intravenous infusion, an implantable osmotic pump, a transdermal patch, liposomes, or other modes of administration. In one embodiment, a pump may be used (Sefton (1987) CRC Crit. Ref. Biomed. Eng. 14:201; Buchwald et al. (1980) Surgery 88:507; Saudek et al. (1989) N. Engl. J. Med. 321:574). In another embodiment, polymeric materials are used. In yet another embodiment a controlled release system is placed in proximity of the therapeutic target, i.e., the liver, thus requiring only a fraction of the systemic dose. In yet another embodiment, a controlled release system is placed in proximity of the therapeutic target, thus requiring only a fraction of the systemic. Other controlled release systems are discussed in the review by Langer (1990) Science 249:1527-1533.

In another embodiment, the compositions of the present invention (as well as combination compositions separately or together) include those formed by impregnation of an inhibiting agent described herein into absorptive materials, such as sutures, bandages, and gauze, or coated onto the surface of solid phase materials, such as surgical staples, zippers and catheters to deliver the compositions. Other delivery systems of this type will be readily apparent to those skilled in the art in view of the instant disclosure.

The present invention provides methods and compositions for the administration of a one or more of an interfering agent to a host (e.g., a human) for the treatment of a microbial

infection. In various embodiments, these methods of the invention span almost any available method and route suitable for drug delivery, including in vivo and ex vivo methods, as well as systemic and localized routes of administration.

Screening Assays

The present invention provides methods for screening for equivalent agents, such as equivalent monoclonal antibodies to a polyclonal antibody as described herein and various agents that modulate the activity of the active agents and pharmaceutical compositions of the invention or the function of a polypeptide or peptide product encoded by the polynucleotide of this invention. For the purposes of this invention, an “agent” is intended to include, but not be limited to a biological or chemical compound such as a simple or complex organic or inorganic molecule, a peptide, a protein (e.g. antibody), a polynucleotide (e.g. anti-sense) or a ribozyme. A vast array of compounds can be synthesized, for example polymers, such as polypeptides and polynucleotides, and synthetic organic compounds based on various core structures, and these are also included in the term “agent.” In addition, various natural sources can provide compounds for screening, such as plant or animal extracts, and the like. It should be understood, although not always explicitly stated that the agent is used alone or in combination with another agent, having the same or different biological activity as the agents identified by the inventive screen.

One embodiment is a method for screening agents capable of interacting with, binding to, or inhibiting the DNA-DNABII (e.g., IHF) interaction. The present invention provides in Figure 6B the three-dimensional structure of the microbial DNA and IHF. Accordingly, the disclosure permits the use of virtual design techniques, also known as computer-aided, in silico design or modeling, to design, select, and synthesize agents capable of interacting with, binding to, or inhibiting the DNA-DNABII (e.g., IHF) interaction. In turn, the candidate agents may be effective in the treatment of biofilms and associated diseases or conditions (medical, industrial or veterinary) as described herein. Thus, the present disclosure also provides agents identified or designed by the in silico methods.

Three-dimensional structure of a IHF-DNA complex is illustrated in Figure 6B and a representative structure, with X, Y and Z coordinates, are provided in Protein Data Bank Accession Number: 1IHF, with relevant details provided in Rice et al. Cell 87:1295-1306 (1996). The three-dimensional structure of the IHF protein in the IHF-DNA complex can be used for the screening method. A suitable agent is one that can be positioned relative to the

IHF protein structure in the IHF-DNA complex with interactions at least one, or alternatively two, or three, or four, or five, or six, or seven, or eight, or nine, or at least ten of the amino acid residues that are identified to be involved in interacting with DNA.

Figure 6A illustrates the amino acid residues involved in IHF-DNA interaction, using the E. coli IHF sequence (SEQ ID NO: 42) as an example. Such amino acid residues, indicated by the lower level of arrows in Figure 6A, are further described below, indicated with bold and underlined letters. Namely, with the E. coli IHF, the amino acids involved in DNA binding are T4, K5, A6, E28, Q43, K45, S47, G48, N51, R55, K57, R60, R63, N64, P65, K66, R76, T80, R82 or Q85.

MAL**TKA**EMSE YLFDKLGLSK RDAKELV**E**LF FEEIRRALEN GE**Q****V****K****L****S****G**FG **N****F****D****L****R****D****K****N****Q****R**
PG**R****N****P****K**TGED IPITAR**R****R****V****V****T** FRPG**Q****K****L****K****S**R VENASPKDE (SEQ ID NO: 42)

Thus, one embodiment of the present disclosure provides a computer-implemented method for identifying an agent that inhibits, competes or titrates the binding of a DNABII polypeptide or protein to a microbial DNA, that inhibits, prevents or breaks down a microbial biofilm, that inhibits, prevents or breaks down a biofilm in a subject, or that inhibits, prevents or treats a microbial infection that produces a biofilm in a subject, comprising positioning a three-dimensional structure of a candidate agent against a three-dimensional structure of an integration host factor (IHF) protein, wherein the three-dimensional structure of the IHF protein is based on X, Y and Z atomic structure coordinates determined from a crystalline form of an IHF and DNA complex, wherein interaction of the agent with the IHF at two or more IHF amino acids selected from T4, K5, A6, E28, Q43, K45, S47, G48, N51, R55, K57, R60, R63, N64, P65, K66, R76, T80, R82 or Q85 as represented in SEQ ID NO: 42, or the equivalent of each, identifies that the agent inhibits, competes or titrates the binding of a DNABII polypeptide or protein to a microbial DNA, inhibits, prevents or breaks down a microbial biofilm, inhibits, prevents or breaks down a biofilm, or inhibits, prevents or treats a microbial infection that produces a biofilm.

In one aspect, a candidate agent interacts with the IHF protein at least one, or two, or three of amino acids 63, 64, 65, or 66. In one aspect, a candidate agent interacts with the IHF protein at least one, or two, or three of R63, N64, P65, K66. In another aspect, a candidate agent interacts with the IHF protein at least at one of 63 or 66. In another aspect, a candidate agent interacts with the IHF protein at least at one of R63 or K66.

It would be appreciated in the art that the exact locations and amino acid residues vary depending on the IHF sequence. One of the skill in the art, however, can readily identify such locations and amino acid residues based on the sequences. For instance, an IHF sequence can be aligned with the IHF sequence of *E. coli* (SEQ ID NO: 42), as illustrated in Table 9, to reveal those that correspond to the amino acids in *E. coli* IHF that interact with DNA. Likewise, the three-dimensional structure of such an IHF sequence in an IHF-DNA complex can be used for the screening.

In addition to the computer-implemented methods as provided herein, the present disclosure also provides custom computer system that includes, e.g., processor, memory and/or program, for performing the methods, as well as a computer readable medium, such as a non-transitory computer readable medium that stores suitable computer program or code for carrying out the methods.

Accordingly, another embodiment provides a custom computing apparatus comprising:

- at least one processor;
- a memory coupled to the at least one processor;
- a storage medium in communication with the memory and the at least one processor, the storage medium containing a set of processor executable instructions that, when executed by the processor configure the custom computing apparatus to identify an agent that inhibits, competes or titrates the binding of a DNABII polypeptide or protein to a microbial DNA, that inhibits, prevents or breaks down a microbial biofilm, that inhibits, prevents or breaks down a biofilm in a subject, or that inhibits, prevents or treats a microbial infection that produces a biofilm in a subject, wherein the configuration comprises:

- positioning a three-dimensional structure of a candidate agent against a three-dimensional structure of an integration host factor (IHF) protein, wherein the three-dimensional structure of the IHF protein is based on X, Y and Z atomic structure coordinates determined from a crystalline form of an IHF and DNA complex, wherein interaction of the agent with the IHF at two or more IHF amino acids selected from T4, K5, A6, E28, Q43, K45, S47, G48, N51, R55, K57, R60, R63, N64, P65, K66, R76, T80, R82 or Q85 as represented in SEQ ID NO: 42, or the equivalent of each, identifies that the agent inhibits, competes or titrates the binding of a DNABII polypeptide or protein to a microbial DNA, inhibits, prevents or breaks down a microbial biofilm, inhibits, prevents or breaks down a biofilm, or inhibits, prevents or treats a microbial infection that produces a biofilm.

Yet another embodiment provides a non-transitory computer medium comprising a set of processor executable instructions that, when executed by a processor, identifying an agent that inhibits, competes or titrates the binding of a DNABII polypeptide or protein to a microbial DNA, that inhibits, prevents or breaks down a microbial biofilm, that inhibits, prevents or breaks down a biofilm in a subject, or that inhibits, prevents or treats a microbial infection that produces a biofilm in a subject, comprising positioning a three-dimensional structure of a candidate agent against a three-dimensional structure of an integration host factor (IHF) protein, wherein the three-dimensional structure of the IHF protein is based on X, Y and Z atomic structure coordinates determined from a crystalline form of an IHF and DNA complex, wherein interaction of the agent with the IHF at two or more IHF amino acids selected from T4, K5, A6, E28, Q43, K45, S47, G48, N51, R55, K57, R60, R63, N64, P65, K66, R76, T80, R82 or Q85 as represented in SEQ ID NO: 42, or the equivalent of each, identifies that the agent inhibits, competes or titrates the binding of a DNABII polypeptide or protein to a microbial DNA, inhibits, prevents or breaks down a microbial biofilm, inhibits, prevents or breaks down a biofilm, or inhibits, prevents or treats a microbial infection that produces a biofilm.

Methods of in silico molecule or drug designs are well known in the art, see generally Kapetanovic (2008) Chem Biol. Interact., 171(2):165-76. Briefly, the atomic coordinates of the three-dimensional structure are input into a computer so that images of the structure and various parameters are shown on the display. The design typically involves positioning a three-dimensional structure to the three-dimensional structure of the target molecule. The positioning can be controlled by the user with assistance from a computer's graphic interface, and can be further guided by a computer algorithm looking for potential good matches. Positioning also involves moving either or both of the three-dimensional structures around at any dimension.

Then, the resultant data are input into a virtual compound and/or agent library. Since a virtual library is contained in a virtual screening software such as DOCK-4 (Kuntz, UCSF), the above-described data may be input into such a software. Candidate agents may be searched for, using a three-dimensional structure database of virtual or non-virtual drug candidate compounds, such as MDDR (Prous Science, Spain).

A candidate agent is found to be able to bind to DNA and/or DNABII protein if a desired interaction between the candidate agent and either or both is found. The interaction can be quantitative, e.g, strength of interaction and/or number of interaction sites, or qualitative, e.g., interaction or lack of interaction. The output of the method, accordingly, can be quantitative or qualitative. In one aspect, therefore, the present disclosure also provides a method for identifying an agent that does not inhibit the interaction or alternatively, strengthens the interaction between the DNA and protein.

The potential inhibitory or binding effect (i.e., interaction or association) of an agent such as a small molecule compound may be analyzed prior to its actual synthesis and testing by the use of computer modeling techniques. If the theoretical structure of the given compound suggests insufficient interaction and association between it and microbial DNA in the biofilm and/or DNABII protein, synthesis and testing of the agent can be obviated. However, if computer modeling indicates a strong interaction, the agent can then be synthesized and tested for its ability to bind to or inhibit the interaction using various methods such as in vitro or in vivo experiments. Methods of testing an agent's ability to inhibit or titrate a biofilm, alone or in connection with another agent, are disclosed herein. In this manner, synthesis of inoperative agents and compounds can be avoided.

One skilled in the art may use any of several methods to screen chemical or biological entities or fragments for their ability to associate with DNABII or microbial DNA and more particularly with the specific binding sites. Selected fragments or chemical entities may then be positioned in a variety of orientations, or docked, within an individual binding site of DNA or DNABII polypeptide. Docking may be accomplished using software such as QUANTA, SYBYL, followed by energy minimization and molecular dynamics with standard molecular mechanics forcefields, such as CHARMM and AMBER.

Commercial computer programs are also available for in silico design. Examples include, without limitation, GRID (Oxford University, Oxford, UK), MCSS (Molecular Simulations, Burlington, Mass.), AUTODOCK (Scripps Research Institute, La Jolla, Calif.), DOCK (University of California, San Francisco, Calif.), GLIDE (Schrodinger Inc.), FlexX (Tripos Inc.) and GOLD (Cambridge Crystallographic Data Centre).

Once an agent or compound has been designed or selected by the above methods, the efficiency with which that agent or compound may bind to each other can be tested and

optimized by computational evaluation. For example, an effective DNABII fragment or may preferably demonstrate a relatively small difference in energy between its bound and free states (i.e., a small deformation energy of binding).

A compound designed or selected can be further computationally optimized so that in its bound state it would preferably lack repulsive electrostatic interaction with the target protein. Such non-complementary (e.g., electrostatic) interactions include repulsive charge-charge, dipole-dipole, and charge-dipole interactions. Specifically, the sum of all electrostatic interactions between the agent and DNABII and/or microbial DNA in the biofilm when the agent or compound is bound to either agent, preferably make a neutral or favorable contribution to the enthalpy of binding.

Computer softwares are also available in the art to evaluate compound deformation energy and electrostatic interaction. Examples include, without limitation, Gaussian 92 [Gaussian, Inc., Pittsburgh, Pa.]; AMBER [University of California at San Francisco]; QUANTA/CHARMM [Molecular Simulations, Inc., Burlington, Mass.]; and Insight II/Discover [Biosym Technologies Inc., San Diego, Calif.].

Once an binding agent has been optimally selected or designed, as described above, substitutions may then be made in some of its atoms or side groups in order to improve or modify its binding properties. Generally, initial substitutions are conservative, i.e., the replacement group will have approximately the same size, shape, hydrophobicity and charge as the original group. It should, of course, be understood that components known in the art to alter conformation should be avoided. Such substituted chemical compounds may then be analyzed for efficiency of fit to the DNABII protein and/or microbial DNA in the biofilm by the same computer methods described in detail, above.

One preferred embodiment is a method for screening small molecules capable of interacting with the protein or polynucleotide of the invention. For the purpose of this invention, "small molecules" are molecules having low molecular weights (MW) that are, in one embodiment, capable of binding to a protein of interest thereby altering the function of the protein. Preferably, the MW of a small molecule is no more than 1,000. Methods for screening small molecules capable of altering protein function are known in the art. For example, a miniaturized arrayed assay for detecting small molecule-protein interactions in cells is discussed by You et al. (1997) Chem. Biol. 4:961-968.

To practice the screening method in vitro, suitable cell culture or tissue infected with the microbial to be treated are first provided. The cells are cultured under conditions (temperature, growth or culture medium and gas (CO₂)) and for an appropriate amount of time to attain exponential proliferation without density dependent constraints. It also is desirable to maintain an additional separate cell culture that is not infected as a control.

As is apparent to one of skill in the art, suitable cells can be cultured in micro-titer plates and several agents can be assayed at the same time by noting genotypic changes, phenotypic changes or a reduction in microbial titer.

When the agent is a composition other than a DNA or RNA, such as a small molecule as described above, the agent can be directly added to the cell culture or added to culture medium for addition. As is apparent to those skilled in the art, an "effective" amount must be added which can be empirically determined.

When the agent is an antibody or antigen binding fragment, the agent can be contacted or incubated with the target antigen and polyclonal antibody as described herein under conditions to perform a competitive ELISA. Such methods are known to the skilled artisan.

The assays also can be performed in a subject. When the subject is an animal such as a rat, chinchilla, mouse or simian, the method provides a convenient animal model system that can be used prior to clinical testing of an agent in a human patient. In this system, a candidate agent is a potential drug if symptoms of the disease or microbial infection is reduced or eliminated, each as compared to untreated, animal having the same infection. It also can be useful to have a separate negative control group of cells or animals that are healthy and not treated, which provides a basis for comparison.

The agents and compositions can be used in the manufacture of medicaments and for the treatment of humans and other animals by administration in accordance with conventional procedures, such as an active ingredient in pharmaceutical compositions.

Combination Therapy

The compositions and related methods of the present invention may be used in combination with the administration of other therapies. These include, but are not limited to, the administration of DNase enzymes, antibiotics, antimicrobials, or other antibodies.

In some embodiments, the methods and compositions include a deoxyribonuclease (DNase) enzyme that acts synergistically with the anti-DNABII antibody. A DNase is any enzyme

that catalyzes the cleavage of phosphodiester linkages in the DNA backbone. Three non-limiting examples of DNase enzymes that are known to target not only cruciform structures, but also a variety of secondary structure of DNA include DNase I, T4 EndoVII and T7 Endo I. In certain embodiments, the effective amount of anti-DNABII antibody needed to destabilize the biofilm is reduced when combined with a DNase. When administered *in vitro*, the DNase can be added directly to the assay or in a suitable buffer known to stabilize the enzyme. The effective Unit dose of DNase and the assay conditions may vary, and can be optimized according to procedures known in the art.

In other embodiments, the methods and compositions can be combined with antibiotics and/or antimicrobials. Antimicrobials are substances that kill or inhibit the growth of microorganisms such as bacteria, fungi, or protozoans. Although biofilms are generally resistant to the actions of antibiotics, compositions and methods described herein can be used to sensitize the infection involving a biofilm to traditional therapeutic methods for treating infections. In other embodiments, the use of antibiotics or antimicrobials in combination with methods and compositions described herein allow for the reduction of the effective amount of the antimicrobial and/or biofilm reducing agent. Some non-limiting examples of antimicrobials and antibiotics useful in combination with methods of the current invention include amoxicillin, amoxicillin-clavulanate, cefdinir, azithromycin, and sulfamethoxazole-trimethoprim. The therapeutically effective dose of the antimicrobial and/or antibiotic in combination with the biofilm reducing agent can be readily determined by traditional methods. In some embodiments the dose of the antimicrobial agent in combination with the biofilm reducing agent is the average effective dose which has been shown to be effective in other bacterial infections, for example, bacterial infections wherein the etiology of the infection does not include a biofilm. In other embodiments, the dose is 0.1, 0.15, 0.2, 0.25, 0.30, 0.35, 0.40, 0.45, 0.50, 0.55, 0.60, 0.65, 0.70, 0.75, 0.8, 0.85, 0.9, 0.95, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.5, 3.0 or 5 times the the average effective dose. The antibiotic or antimicrobial can be added prior to, concurrent with, or subsequent to the addition of the anti-DNABII antibody.

In other embodiments, the methods and compositions can be combined with antibodies that treat the bacterial infection. One example of an antibody useful in combination with the methods and compositions described herein is an antibody directed against an unrelated outer membrane protein (i.e. OMP P5). Treatment with this antibody alone does not debulk a biofilm *in vitro*. Combined thereapy with this antibody and a biofilm reducing agent results

in a greater effect than that which could be achieved by either reagent used alone at the same concentration. Other antibodies that may produce a synergistic effect when combined with a biofilm reducing agent or methods to reduce a biofilm include anti-rsPilA, anti-OMP26, anti-OMP P2, and anti-whole OMP preparations.

The compositions and methods described herein can be used to sensitize the bacterial infection involving a biofilm to common therapeutic modalities effective in treating bacterial infections without a biofilm but are otherwise ineffective in treating bacterial infections involving a biofilm. In other embodiments, the compositions and methods described herein can be used in combination with therapeutic modalities that are effective in treating bacterial infections involving a biofilm, but the combination of such additional therapy and biofilm reducing agent or method produces a synergistic effect such that the effective dose of either the biofilm reducing agent or the additional therapeutic agent can be reduced. In other instances the combination of such additional therapy and biofilm reducing agent or method produces a synergistic effect such that the treatment is enhanced. An enhancement of treatment can be evidenced by a shorter amount of time required to treat the infection.

The additional therapeutic treatment can be added prior to, concurrent with, or subsequent to methods or compositions used to reduce the biofilm, and can be contained within the same formulation or as a separate formulation.

Kits

Kits containing the agents and instructions necessary to perform the in vitro and in vivo methods as described herein also are claimed. Accordingly, the invention provides kits for performing these methods which may include an interfering of this invention as well as instructions for carrying out the methods of this invention such as collecting tissue and/or performing the screen, and/or analyzing the results, and/or administration of an effective amount of an interfering agent as defined herein. These can be used alone or in combination with other suitable antimicrobial agents.

For example, a kit can comprise, or alternatively consist essentially of, or yet further consist of any one or more agent of the group of an isolated or recombinant integration host factor (IHF) polypeptide or a fragment or an equivalent of each thereof; an isolated or recombinant protein polypeptide identified in Table 8, Table 9, Table 10 a DNA binding peptide identified in Figure 6, or a fragment or an equivalent of each thereof; an isolated or recombinant polypeptide of SEQ ID NO. 1 through 33, or a fragment or an equivalent of each thereof; an

isolated or recombinant C-terminal polypeptide of SEQ ID NO. 5 through 11, 28, 29 or those identified in Table 8, Table 10 or a fragment or an equivalent of each thereof; a polypeptide that competes with an integration host factor on binding to a microbial DNA; a four-way junction polynucleotide resembling a Holliday junction, a 3 way junction polynucleotide resembling a replication fork, a polynucleotide that has inherent flexibility or bent polynucleotide; an isolated or recombinant polynucleotide encoding any one of the above noted polypeptides; an antibody that specifically recognizes or binds any one of the above noted polypeptides, or an equivalent or fragment thereof; or a small molecule that competes with the binding of a DNABII protein or polypeptide to a microbial DNA, and instructions for use. The kit can further comprising one or more of an adjuvant, an antigenic peptide or an antimicrobial. Examples of carriers include a liquid carrier, a pharmaceutically acceptable carrier, a solid phase carrier, a pharmaceutically acceptable carrier, a pharmaceutically acceptable polymer, a liposome, a micelle, an implant, a stent, a paste, a gel, a dental implant, or a medical implant.

The following examples are intended to illustrate, and not limit, the inventions disclosed herein.

Experimental

Experiment No. 1

IHF antibodies, proteins and polypeptides were a generous gift from Nash. The methods to produce them are well known to the skilled artisan, e.g., as described in Granston and Nash, (1993) J. Mol. Biol., 234:45-59; Nash et al., (1987) Journal of Bacteriology, 169(9):4124-4127; and Rice et al., (1996) Cell, 87:1295-1306.. Briefly, to overproduce IHF- α , the *himA* gene was inserted downstream from the P_L promoter in the bacterial plasmid pAD284. Transformants of strain K5607, a lambda lysogen of strain C600himA42 that had received the desired plasmid, were identified by screening ampicillin-resistant transformants for the ability to grow bacteriophage Mu (13). DNA was prepared from *himA*⁺ transformants according to standard DNA isolation techniques, and the orientation of the *himA* gene was determined by restriction enzyme cleavage. Plasmid pP_LhimA-1, which has the *himA* gene in the proper orientation for expression by the P^L promoter, was transformed into strain N5271, which contains a cryptic lambda prophage expressing the cI857 thermoinducible repressor, to yield strain K5770.

To overproduce IHF- β , plasmid pKT23-hip323, which contains a fusion of the IHF-, -coding sequence to the bacteriophage lambda P_L promoter was used. pKT23-hip323 was introduced into N5271 to give strain E443. To facilitate the selection of pKT23-hip323 in the presence of another plasmid, changed its selectable marker was changed from ampicillin resistance (*bla*⁺) to chloramphenicol resistance (*cat*⁺). A *cat*-containing fragment was isolated from plasmid pBR325 as described by Flamm and Weisberg and was inserted into the unique *ScaI* site in *bla*. The ligated DNA was introduced into strain E403, which carries a *hip* mutation and which synthesizes temperature-sensitive X repressor, and chloramphenicol-resistant transformants were selected at low temperature. One such transformant (E735) was *hip*⁺ and ampicillin sensitive; it therefore appears to carry a *bla cat*⁺ derivative of pKT23-hip323 (pE735).

To generate a strain that overproduces both subunits of IHF, E735 was transformed with plasmid pP_LhimA-1, selecting a transformant (E738) that had become ampicillin resistant and had retained chloramphenicol resistance. The generation of a second strain that overproduces both subunits of IHF depended on the construction of plasmid pP_Lhip himA-5, which was made by ligating blunted (*SstII* restriction enzyme site) containing the *pheT* and *himA* genes into the pKT23-hip323 plasmid. This is described in further detail in (Nash et al., (1987) *J. of Bacteriology*, 169(9): 4124-4127. *himA*⁺ transformants of strain K5607 were identified by screening for HimA⁺, and the plasmid DNA was analyzed by restriction digestion. In all cases where the plasmid structure was obvious, two copies of *himA* had been ligated as a tandem direct repeat into the vector. It is not known if the presence of two copies of the *himA* gene on this plasmid is demanded by the selection, but it should be recalled that a single copy of the *himA* gene in plasmid pP_LhimA-1 is sufficient to complement a *himA* mutant. Plasmid pP_LhiphimA-5 was used to transform strain N5271 to yield strain K5746.

Cells were grown in shaking water bath at 31°C in TBY medium (10 g of tryptone, 5 g of yeast extract, and 5 g of sodium chloride per liter). At mid-log phase (optical density at 650 nm, ca. 0.6), the cells were shifted to a 42°C water bath and shaking was continued.

Typically, 300 ml of culture was centrifuged and suspended in 0.6 to 0.9 ml of TEG (20 mM Tris hydrochloride (pH 7.4), 1 mM sodium EDTA, 10% glycerol) containing 20 mM NaCl. The cells were disrupted with six 20-s bursts of sonication, with 40 s between each burst. A portion of the sonic extract was centrifuged in a Sorvall SS34 rotor for 20 min at 15,000 rpm.

Samples of the sonic extract were analyzed by sodium dodecyl sulfate (SDS) gel electrophoresis according to standard molecular biology techniques.

Purification of IHF was done according to the following: A 3.6-liter batch of cells was induced for 3 h. All subsequent steps were carried out at 0 to 4°C. The cell pellet from 3.3-liters was suspended in 10 ml of TEG containing 20 mM NaCl to give a total volume of 29 ml; this suspension was disrupted in two batches, each receiving six bursts of 3 min of sonication separated by 90-s intervals. The sonic extract was centrifuged for 20 min at 15,000 rpm, yielding 16.9 ml of clarified extract. A 10% (vol/vol) solution (1.1 ml) of polymin P (BDH Chemicals Ltd.) was added slowly to the clarified extract; after being stirred for 20 min, the mixture was centrifuged for 30 min at 10,000 rpm. The resulting pellet was suspended in 10 ml of TEG containing 500 mM NaCl; after being stirred for 15 min, the mixture was centrifuged for 20 min at 12,000 rpm. The supernatant (10.3 ml) was adjusted to 50% saturation by the addition of 3.2 g of ammonium sulfate, stirred for 20 min, and centrifuged for 15 min at 15,000 rpm. The resulting supernatant was adjusted to 70% saturation by the addition of 1.64 g of ammonium sulfate, stirred for 20 min, and centrifuged for 15 min at 15,000 rpm. The resulting pellet was suspended in 1 ml of TG (50 mM Tris hydrochloride (pH 7.4) containing 10% glycerol) and dialyzed against two changes of TG. The dialyzed material (2.0 ml) was loaded onto a 1-ml column (0.5 by 5.8 cm) of phosphocellulose (P11; Whatman, Inc.) that had been equilibrated with TG. The column was washed with 3 ml of TG and developed with 20 ml of a linear gradient (0 to 1.2 M) of KCl in TG. Fractions of 0.5 ml were collected, stored at -20°C, and assayed for IHF activity.

Polyclonal anti-IHF was prepared as follows. Rabbits were injected with 250 µg of purified IHF with Freund's complete adjuvant. Booster immunizations of 250 µg of IHF with Freund's incomplete adjuvant were given 1, 7, and 12 weeks later. As determined by immunoblotting of IHF, sera collected 13 weeks after the initial injection had a high titer of IHF-reactive material. The animals were maintained for several years and, when necessary, given further booster immunization in order to maintain a high titer of anti-IHF in their sera. The antibody was not purified further. Crude sera was stored at -70°C.

Antibodies specific to each subunit were generated by using synthetic polypeptides corresponding to the most C-terminal 20 amino acid residues of each subunit (SEQ ID Nos. 34 and 35), to immunize rabbits according to Ditto et al., (1994) J. of Bacteriology,

176(12):3738-3748.

Experiment 2

This experiment describes an in vitro model for reversal of an established biofilm in 8-well chamber slide. The materials used in this experiment were: Chocolate Agar; sBHI (BHI with 2 mg heme /mL and 2 mg b-NAD /mL); 8-well Chamber slides (Nunc* Lab-Tek* Fisher catalog #12-565-18); Sterile 0.9% saline; LIVE/DEAD BacLight Bacterial Viability Kit (Fisher catalog #NC9439023) and Formalin.

On day 1, NTHI was struck for isolation on chocolate agar. It was then incubated for 20 hrs at 37°C and 5% CO₂. The next day, bacteria were suspended in 5 mL equilibrated (37°C, 5% CO₂) sBHI and optical density was adjusted to OD₄₉₀ = 0.65 in sBHI. Bacteria was diluted 1:6 in equilibrated sBHI (1 mL bacterial suspension + 5 mL sBHI). Bacteria was then incubated for 3 hours at 37°C in 5% CO₂, static (OD₄₉₀ should be approx 0.65). Next, the bacteria was again diluted 1:2500 in equilibrated sBHI and 200 µL of the bacterial suspension was added to each well of the chamber slide. For dilution, 10 µL bacteria was added to 990 µL sBHI in an eppendorf tube and 8 µL dilution was added to 192 µL sBHI in each chamber and incubated at 37°C, 5% CO₂, static.

On the third day (after 16 hours of incubation medium was aspirated from chamber by aspirating medium from the corner of the well so as not to disturb biofilm. Then 200 µL of equilibrated sBHI was added to each chamber and incubated for 37°C, 5% CO₂, static for 8 hours. After 8 hours, the medium was aspirated and 200 µL equilibrated sBHI was added to each untreated chamber; and 200 µL of interfering agent such as Rabbit anti-rsPilA; diluted 1:50 in sBHI and 200 µL Naive rabbit serum (or other appropriate serum control) diluted 1:50 in sBHI was added. They were then incubate at 37°C and 5% CO₂, static.

On day 4, after approximately 16 hours of incubation, aspirate sBHI was aspirated and the biofilm was washed twice with 200 µL sterile saline. The saline was aspirated and 200 µL Live/Dead stain was added. Next, 3 mL component A plus 3 mL component B in 1 mL sterile 10 mM phosphate buffered saline was added. It was then incubated for 15 minutes at room temperature, static, protected from light. Stain was aspirated and biofilm was washed twice with 200 µL sterile saline. Saline was aspirated and 200 µL formalin was added to fix biofilm. It was then incubate 15 minutes at room temperature, static, protected from light. Formalin was aspirated and biofilm was washed twice with 200 µL sterile saline. Gasket was removed and coverslip were placed on slide; coverslip were sealed with nail polish and

dried prior to viewing by confocal microscopy.

Using this method and anti-IHF antibody, Applicants reduced a biofilm produced by *Haemophilus influenzae* which is prevalent in sinusitis, bronchitis, otitis media and exacerbations of chronic obstructive pulmonary disease (COPD). Untreated biofilm mass was measured to be $4.24 \mu\text{m}^3 / \mu\text{m}^2$ with a mean thickness of $11.68 \mu\text{m}$. After treatment, the biofilm mass was reduced to $0.53 \mu\text{m}^3 / \mu\text{m}^2$ with a mean thickness of $1.31 \mu\text{m}$. Thus, this shows a 88.8 % reduction in mean thickness and an 87.5 % reduction in biomass.

Polyclonal antisera directed against the *E. coli* IHF was prepared in rabbits according to standard techniques using purified Integration Host Factor (IHF). Experiment 1 describes the expression and purification of IHF from *E. coli*.

Using this method and anti-IHF antibody, Applicants reduced a biofilm produced by *Streptococcus mutans* which is prevalent in initiation and progression of dental caries. Untreated biofilm mass was measured to be $1.17 \mu\text{m}^3 / \mu\text{m}^2$ with a mean thickness of $5.43 \mu\text{m}$. After treatment, the biofilm mass was reduced to $0.1 \mu\text{m}^3 / \mu\text{m}^2$ with a mean thickness of $0.47 \mu\text{m}$. Thus, this shows a 91.3 % reduction in mean thickness and an 91.6% % reduction in biomass. *In vitro* biofilm assays were repeated 3 times, on separate days. The percent reduction in the max height, average thickness, and biomass is depicted in Table 1 below.

Table 1

		Assay 1	Assay 2	Assay 3
<i>S. mutans</i>	Max height (μm)	56	24	41
	Ave thickness (μm)	65	65	67
	biomass ($\mu\text{m}^3 / \mu\text{m}^2$)	37	58	66

Using this method and anti-IHF antibody, Applicants reduced a biofilm produced by *Staphylococcus aureus* which is prevalent in localized and diffuse skin infections, chronic rhinosinusitis and nosocomial infections. Untreated biofilm mass was measured to be $0.3 \mu\text{m}^3 / \mu\text{m}^2$ with a mean thickness of $2.2 \mu\text{m}$ and a biofilm height of $17.5 \mu\text{m}$. After treatment, the biofilm mass was reduced to $0.3 \mu\text{m}^3 / \mu\text{m}^2$ with a mean thickness of $1.1 \mu\text{m}$ and a biofilm height of $8 \mu\text{m}$. Thus, this shows a 48.8 % reduction in mean thickness and an 2.7 % reduction in biomass (Figure 7).

Using this method and anti-IHF antibody, Applicants reduced a biofilm produced by *Moraxella catarrhalis* which is prevalent in exacerbation of COPD and otitis media.

Untreated biofilm mass was measured to be $0.72 \mu\text{m}^3/\mu\text{m}^2$ with a mean thickness of $1.48 \mu\text{m}$. After treatment, the biofilm mass was reduced to $0.13 \mu\text{m}^3/\mu\text{m}^2$ with a mean thickness of $0.65 \mu\text{m}$. Thus, this shows a 55.8 % reduction in mean thickness and an 82.1 % reduction in biomass. *In vitro* biofilm assays were repeated 3 times, on separate days. The percent reduction in the max height, average thickness, and biomass is depicted in Table 2 below.

Table 2

		Assay 1	Assay 2	Assay 3
<i>M. catarrhailis</i>	Max height (μm)	61	33	36
	Ave thickness (μm)	92	34	84
	biomass ($\mu\text{m}^3/\mu\text{m}^2$)	92	35	92

Using this method and anti-IHF antibody, Applicants reduced a biofilm produced by *Streptococcus pneumoniae* which is prevalent in sinusitis, pneumonia and otitis media. Untreated biofilm mass was measured to be $0.64 \mu\text{m}^3/\mu\text{m}^2$ with a mean thickness of $3.99 \mu\text{m}$. After treatment, the biofilm mass was reduced to $0.14 \mu\text{m}^3/\mu\text{m}^2$ with a mean thickness of $0.82 \mu\text{m}$. Thus, this shows a 79.5 % reduction in mean thickness and an 78.6 % reduction in biomass. *In vitro* biofilm assays were repeated 3 times, on separate days. The percent reduction in the max height, average thickness, and biomass is depicted in Table 3 below.

Table 3

		Assay 1	Assay 2	Assay 3
<i>S. pneumoniae</i>	Max height (μm)	49	51	44
	Ave thickness (μm)	25	64	79
	biomass ($\mu\text{m}^3/\mu\text{m}^2$)	51	61	79

Using this method and anti-IHF antibody, Applicants reduced a biofilm produced by *Pseudomonas aeruginosa* which is prevalent in cystic fibrosis, pneumonia, skin and soft tissue infections and on medical devices. Untreated biofilm mass was measured to be $7.0 \mu\text{m}^3/\mu\text{m}^2$ with a mean thickness of $25.70 \mu\text{m}$ and a biofilm height of $40 \mu\text{m}$. After treatment, the biofilm mass was reduced to $3.4 \mu\text{m}^3/\mu\text{m}^2$ with a mean thickness of $10.3 \mu\text{m}$ and a biofilm height of $27.5 \mu\text{m}$. Thus, this shows a 60.1 % reduction in mean thickness and an 50.8 % reduction in biomass (Figure 7).

Using this method and anti-IHF antibody, Applicants reduced a biofilm produced by

Neisseria gonorrhoeae which is in gonorrhea. Untreated biofilm mass was measured to be $9.5 \mu\text{m}^3/\mu\text{m}^2$ with a mean thickness of $22.02 \mu\text{m}$ and a biofilm height of $40 \mu\text{m}$. After treatment, the biofilm mass was reduced to $0.8 \mu\text{m}^3/\mu\text{m}^2$ with a mean thickness of $3.4 \mu\text{m}$ and a biofilm height of $27.5 \mu\text{m}$. Thus, this shows a 84.5% reduction in mean thickness and an 92.1 % reduction in biomass (Figure 7).

Using this method and anti-IHF antibody, Applicants reduced a biofilm produced by *Uropathogenic E. coli* which is prevalent in urinary tract infections. Untreated biofilm mass was measured to be $1.75 \mu\text{m}^3/\mu\text{m}^2$ with a mean thickness of $31.73 \mu\text{m}$. After treatment, the biofilm mass was reduced to $0.75 \mu\text{m}^3/\mu\text{m}^2$ with a mean thickness of $1.62 \mu\text{m}$. Thus, this shows a 94.9% reduction in mean thickness and an 56.9 % reduction in biomass. *In vitro* biofilm assays were repeated 3 times, on separate days. The percent reduction in the max height, average thickness, and biomass is depicted in Table 4 below.

Table 4

		Assay 1	Assay 2	Assay 3
<i>UPEC</i>	Max height (μm)	69	65	76
	Ave thickness (μm)	97	33	95
	biomass ($\mu\text{m}^3/\mu\text{m}^2$)	98	96	57

Using this method and anti-IHF antibody, Applicants reduced a biofilm produced by *Staphylococcus epidermidis* which is prevalent in infections of the skin. *In vitro* biofilm assays were repeated 3 times, on separate days. The percent reduction in the max height, average thickness, and biomass is depicted in Table 5 below.

Table 5

		Assay 1	Assay 2	Assay 3
<i>S. epidermidis</i>	Max height (μm)	42	38	56
	Ave thickness (μm)	62	71	92
	biomass ($\mu\text{m}^3/\mu\text{m}^2$)	62	76	88

Experiment No. 3

Middle ear infection (or otitis media, OM) is a highly prevalent disease worldwide, afflicting 50 – 330 million children globally each year. The socioeconomic burden of OM is also great,

with cost estimates between \$5-6 billion in the United States alone annually. All three of the predominant bacterial pathogens of OM are known to form biofilms both in vitro and in vivo and recently, clinicians have come to appreciate that the chronicity and recurrence of OM is due, at least in part, to the formation of bacterial biofilms within the middle ear cavity. In one chinchilla model of OM, juvenile chinchillas are first given a viral ‘cold’, followed a week later by their being challenged intranasally with an inoculum of viable bacteria. Similar to the human condition wherein “my child has a cold and a week later gets an ear infection” chinchillas will also develop a bacterial OM approximately one week after a challenge, and while experiencing the viral upper respiratory tract infection. Once bacteria gain access to the middle ear (either via ascension of the Eustachian tube or following direct challenge to the middle ear space), they will form a robust biofilm. Applicants thus contemplate and indeed have already used chinchilla models as reported herein to demonstrate the protective efficacy of IHF immunization which results in rapid resolution of existing biofilms. This model is also useful for therapeutic approaches via either passive delivery of anti-DNABII antibody or via delivery of a small molecule or other agent known to bind to IHF or other DNABII family members.

Because the chinchilla model is used for development and pre-clinical testing of human vaccines, it is important to establish meaningful immunological parallels with the human host, particularly the child. Applicants have shown that effusions recovered from children with AOM due to NTHI, and middle ear fluids from chinchillas with experimental NTHI-induced OM, recognized immunodominant regions of OMP P5 in a similar hierarchical manner (see for e.g. Novotny LA, et al., (2000) *Infect Immun.* 68(4):2119-28; Novotny LA, et al., (2007) 9th International Symposium on Recent Advances in Otitis Media; St. Pete Beach, FL; Novotny LA, et al., (2002) *Vaccine* 20(29-30):3590-97). Applicants have also shown that chinchillas with experimental OM, children with natural OM, and adults with exacerbations of COPD, all recognized peptides representing PilA in a highly analogous manner (see for e.g. Adams LD, et al., (2007) 107th General Meeting, American Society for Microbiology; 2007; Toronto, ON; Adams LD, et al., (2007) 9th International Symposium on Recent Advances in Otitis Media; St. Pete Beach, FL.). Thus, chinchillas with experimental OM and children with natural disease respond similarly immunologically to at least two unrelated NTHI protein adhesins. This parallel was put to the ultimate test recently, when the chinchilla AV-NTHI superinfection model was used to conduct pre-clinical efficacy testing of a novel 11-valent Protein D-pneumococcal polysaccharide conjugate vaccine. Data obtained in the chinchilla predicted an efficacy of 34% whereas, when tested in children, the

efficacy obtained against *H. influenzae*-induced OM was 35.6% (see for e.g. Novotny LA, et al., (2006) Vaccine 24(22):4804-11 and Prymula R, et al., (2006) Lancet. 367(9512):740-8), thus lending strong support to the relevancy of this model for the development and testing of OM vaccine candidates.

Applicants have shown a dramatic reduction in the pre-formed biofilm remaining within the middle ears of chinchillas after receipt of 2 weekly TC immunizations with IHF delivered with a mucosal / systemic adjuvant.

48 adults were ordered from Rauscher's Chinchilla Ranch (LaRue, Ohio) and were acclimated to the vivarium for 7-10 days prior to the beginning of the study. Prior to TB [transbullar or direct challenge into the middle ear cavity] challenge, baseline otoscopy and tympanometry were performed as well as a limited volume prebleed to collect serum. Chinchillas were anesthetized and 300 µl of NTHI (strain #86-028NP) suspension containing approximately 1000 cfu were introduced into the middle ear space via transbullar challenge. Animals were allowed to recover, then monitored daily for adverse reactions as per IACUC accepted animal use protocols. Routine otoscopy and tympanometry were performed every 2-3 days throughout the study.

Four days after challenge (day +4), chinchillas were anesthetized and a CT scan was performed to visualize biofilms present within the middle ear and to obtain pre-immunization images. The surface of the pinnae were cleaned and hydrated by wiping with a sterile gauze pad moistened with sterile pyrogen free saline. After ~2 minutes (to allow the pinnae to dry), 50 µl of either the dmLT, IHF (purified *E. coli* IHF according to Experiment 1) + dmLT, or IHF + rsPilA + dmLT solution will be added to the pinna and gently massaged in. Three chinchillas per cohort were sacrificed and tissues / samples collected (on days +4, and +11) to begin to determine mechanism of action.

Eleven days after challenge (day +11), the secondary immunization occurred as described above wherein the pinnae are cleaned / hydrated and immunogens were topically administered. Three chinchillas per cohort were sacrificed and tissues / samples collected to begin to determine mechanism of action.

Eighteen days after challenge (day +18), chinchillas were anesthetized and a CT scan were performed to identify any biofilms present, as well as to compare to the pre-immunization CT scans. The animals were then bled to collect serum and euthanized. The bullae are dissected and any fluids present were aseptically collected, the bullae were then be opened to visualize the inferior bulla and any biofilm present. Images were collected and the bulla were washed

with 1 ml of sterile saline and re-imaged. The mucosa, along with any biofilm present, were collected from the right bulla and placed in a preweighed tube. These tissues were homogenized, serially diluted, and plated to determine cfu NTHI / mg wet weight tissue. The left bullae were filled with OCT compound and snap frozen for histological analysis.

Images of the left and right middle ear cavities, with resident biofilms, were scrambled and two images per animal were compiled into a single file for ranking by blinded evaluators. The relative amount of biomass remaining within the middle ear of each animal was ranked on a 0 to 4+ scale by blinded reviewers using the scale shown in Table 6 below. Titer ELISAs, cytokine arrays, and Biacore were run on the serum as well as on the collected middle ear effusions. The OCT embedded middle ears were sectioned and stained either for basic morphology and architecture (H&E) or for the presence of IHF using immunohistochemistry and/or immunofluorescence.

Table 6

Score	Criteria
0	No evidence of biomass.
1+	Biomass fills $\leq 25\%$ of middle ear space. Junction of the bony septa to inferior bulla is visible.
2+	Biomass fills $> 25\%$ to $\leq 50\%$ of middle ear space. Unable to visualize where the bony septa meet the inferior bulla.
3+	Biomass fills $> 50\%$ to $\leq 75\%$ of middle ear space. Biomass covers $> 50\%$ of the length of bony septa.
4+	Biomass fills $> 75\%$ to $\leq 100\%$ of middle ear space. Bony septa not visible; obscured by biomass.

Immunofluorescence imaging of frozen sections of biofilms formed *in vivo* was preformed according to the following. After dissection, the middle ear of each chinchilla was filled with OCT embedding compound (Fisher Scientific, Pittsburgh, PA) and flash frozen over liquid nitrogen. The bone which forms the inferior bulla was removed to leave the middle ear mucosa and existing biofilm intact. The resulting block was then bisected to reveal a cross section of the biofilm and re-embedded in OCT. Ten micron serial sections were cut using a Microm rotary cryotome, adhered to glass slides (Mercedes Medical, Sarasota, FL) and stored at -80°C . Sections were later stained to determine the relative incorporation of IHF within biofilms that had formed *in vivo*. Briefly, slides were air-dried, fixed in cold acetone, then equilibrated in buffer (0.05M Tris-HCl, 0.15M NaCl and 0.05% Tween 20, pH 7.4).

Sections were blocked with image-iT FX signal enhancer (Molecular Probes, Eugene, OR) and with Background Sniper (BioCare Medical, Concord, CA) per manufacturer's instructions. Sections were then incubated with a 1:200 dilution of polyclonal rabbit anti-IHF overnight at 4° C, in a humidified chamber. Slides were further rinsed and incubated with goat anti-rabbit IgG conjugated to AlexaFluor 594 (Invitrogen) for 30 minutes at room temp. As a counterstain, sections were incubated with DAPI and cover-slipped using ProLong Gold antifade reagent (Molecular Probes, Eugene, OR). Naive rabbit serum served as the negative control. Sections were viewed with a Zeiss LSM 510 Meta confocal system attached to a Zeiss Axiovert 200 inverted microscope (Carl Zeiss Inc., Thornwood, NY).

Significant differences in mean CFU/ mg tissue and mean CFU NTHI/ ml supernatant were determined by paired t-test. A p -value ≤ 0.05 was considered significant. Significance in relative biomass among cohorts was assessed by unpaired t-test. A p -value ≤ 0.05 was considered significant.

As shown in Figure 9, Panel A, the mean score for remaining biomass within the middle ears of chinchillas immunized with adjuvant only was 2.8 which indicated significant remaining disease and a lack of resolution of pre-formed biomass in most animals by day 18 after bacterial challenge of the middle ear. In contrast, the mean score for an *E. coli* IHF + adjuvant immunized animal was 1.5. Representative images of a residual biomass that received a mean score of +2.8 and one that received a mean score of +1.5 are shown in Fig. 9, Panel B. Also, as shown in Figure 10, the disease resolution was additionally measured by both histological evidence of altered biomass architecture within the middle ear (see Panel A) as well as a statistically significant reduction of bacterial load present within remaining biomass as measured by homogenization of the biomass and culture on chocolate agar (see Panel B). Furthermore, all animals immunized with isolated *E. coli* IHF mounted a strong local and systemic immune response and no animal presented with obvious secondary sequelae as the result of immunization as noted upon necropsy (data not shown).

The notable observed efficacy when anti-IHF used *in vitro* to debulk biofilms and also of purified *E. coli* IHF, when used as an immunogen *in vivo* to induce the formation of polyclonal antibodies that could resolve an ongoing biofilm disease, created a conundrum as to why mammalian hosts do not naturally mount an effective immune response to DNABII proteins that are associated with eDNA within the bacterial biofilms of recurrent and/or chronic disease states. In review of the solved structure of IHF when it is bound to DNA (Rice et al., (1996) Cell **87**(7): 1295-1306), it is clear that a significant portion of the protein

structure is occluded by bound DNA, which suggested the potential for occlusion of protective epitopes or domains of IHF and/or HU when bound to eDNA within a bacterial biofilm. It was hypothesized that use of native IHF, to which no DNA was bound, as the immunogen might provide a mechanism to overcome such occlusion and thereby foster production of protective antibodies.

To determine if eDNA could indeed prevent the development of protective antibodies directed against a DNABII family member upon immunization, whereas use of native protein was effective, a second larger cohort study was performed wherein the chinchilla study as detailed previously was essentially repeated.

For the second animal immunization study, twenty adult chinchillas (body mass between 500 - 700 g), shown to have no evidence of middle ear disease by tympanometry and video otoscopy, were enrolled and divided into four cohorts of 5 animals each. All animals were again challenged transbullarily with approximately 1000 CFU NTHI strain 86-028NP per bulla. Four days later, after a biofilm formed in the middle ear cavities of these animals, they were immunized by a transcutaneous route (TCI) as described above. Formulations consisted of either: 10 µg IHF admixed with 10 µg dmLT, 10 µg IHF that had been pre-bound to DNA + dmLT, DNA + dmLT, or 10 µg dmLT alone.

To determine if TCI with IHF pre-bound to DNA resulted in a similar immune response to that induced when the same nucleoprotein complex was delivered subcutaneously (SQ), and compared to that induced by IHF alone, two adult chinchillas were immunized to generate antisera. Each animal received a priming dose followed by two identical boosts delivered at 30-day intervals. Immunogens were admixed with the adjuvant monophosphoryl lipid A (MPL) (10 µg/dose) (Sigma-Aldrich, St. Louis, MO) due to its demonstrated strong adjuvant properties in the chinchilla host (See for example Bakaletz et al., (1999) *Infect Immun.* 67(6):2746-2762 and Kennedy et al., (2000) *Infect. Immun.* 68(5):2756-2765. One chinchilla was immunized with 10 µg of IHF + MPL and the other received 10 µg IHF bound to DNA + MPL. All doses were delivered SQ in a total volume of 200 µl. Fifteen days after receiving the final boost, animals were bled to procure serum and sera were assayed via Western blot and ELISA to determine both reciprocal titer and specificity of antibody reactivity to IHF.

Middle ears were again subsequently scored from 0 to 4+ for disease severity upon completion of the study. To better assure that the IHF and DNA remained in complex for immunization, synthetic oligonucleotides identical to those used in the published co-

crystalization study (see for e.g. Rice et al., (1996) Cell **87**(7): 1295-1306) of a high affinity IHF binding site from the bacteriophage lambda recombination site *attP* at a molar ratio of 2:1 [DNA (10 μ M) to IHF (5 μ M)] was used. This amount was at least 3 orders of magnitude over the K_d of IHF bound to this DNA target. As shown in Figure 11, IHF indeed bound dsDNA as demonstrated by its reduced mobility in an electrophoretic mobility shift assay.

As shown in Figure 12, animals immunized with isolated *E. coli* IHF showed a dramatic reduction in disease state with a mean residual middle ear biomass score of 0.9 as compared to the controls that had been immunized with adjuvant alone (mean biomass score = 2.2) or to those that had been immunized with DNA that had been admixed with adjuvant (mean biomass score = 2.8). Interestingly, those animals that had been immunized with the IHF-DNA complex also demonstrated middle ears with significant remaining bacterial biomass, yielding a mean biomass score of 2.5 which was not statistically significantly different from cohorts that received either the adjuvant alone or DNA that had been admixed with adjuvant. This outcome strongly suggested that if sufficient eDNA fragments were present, as one could hypothesize would be the case during natural disease, this situation could result in occlusion of critical IHF epitopes necessary for the generation of neutralizing antibodies.

To be assured that the observed DNA occlusion result was not specific to the use of a transcutaneous immunization route, these immunizations using a subcutaneous (SQ) immunization route to ensure delivery of the antigens to antigen presenting cells within the chinchilla host were repeated. As shown in Figure 13, whereas SQ immunization with isolated *E. coli* IHF yielded the generation of a strong immune response to isolated IHF, immunization with *E. coli* IHF that had been pre-bound to an excess of DNA failed to induce detectable antibodies that recognized IHF when assayed by Western blot. When assayed by ELISA, reciprocal titer *versus* isolated IHF was 1000 for the animal immunized with IHF that had been pre-bound to oligonucleotides, whereas that for the animal immunized with native IHF was 8000 (both animal's pre-immune reciprocal titers against IHF were 100). Collectively, these results are consistent with our hypothesis that the binding of IHF to eDNA, as would occur during a natural disease state, has the potential to block epitopes or domains of IHF necessary for generation of a protective acquired immune response. Further, immunization with native IHF (to which no DNA is bound) appeared to allow for the effective direction of the immune response toward the generation of protective or neutralizing antibodies, as demonstrated in both pre-clinical chinchilla studies described here.

Experiment No. 4

A number of oral bacteria (e.g., *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*) have been implicated in the pathogenesis of inflammatory diseases such as periodontitis and peri-implantitis, which destroy alveolar bone and gingiva. Investigations of the pathogenesis of these bacteria are hampered by lack of effective animal models. One of the challenges of investigating the pathogenicity of specific bacteria is the difficulty of establishing a biofilm when exogenous bacteria are introduced into the oral cavity of animals. Though animal models of periodontitis have been developed, cultivable bacteria are rarely recovered from the oral cavity of inoculated animals. Developing an effective animal model which can assess the pathogenicity of specific bacteria will greatly aid in elucidating their pathogenic mechanisms.

The surface of machined titanium dental implants (1.2 x 4.5mm) was modified by grit blasting with AlO₃ (100 μ m) and HCl etching (pH 7.8 for 20 min at 80°C). Machined and nano-textured implants were incubated in TSB medium inoculated with D7S clinical strain of *Aggregatibacter actinomycetemcomitans* (Aa) for 1 to 3 days at 37°C. The bacterial biofilm on the implants were analyzed by SEM, as well as by confocal laser scanning microscopy following staining with LIVE/DEAD® BacLight™. Implants with and without established

Aa biofilm were transmucosally placed into the alveolar bone of female rats between premolar and incisor region of the maxillae. To detect the presence of *Aa* biofilm on the implants placed in vivo, bacterial samples were collected from saliva and the oral surfaces of implants after 2 days. *Aa* was detected by culture, as well as by PCR analysis. Micro-CT and histological analysis of peri-implant bone and mucosal tissues was performed six weeks after implantation.

After one day of cultivation, agglomerates of coccoid-shaped *Aa* cells were found scattered throughout the implant. After two days, the number and size of the agglomerates decreased and more cells of varying lengths were observed ranging between bacteria with coccoid morphology. After three days of incubation, the agglomerates had almost disappeared, while large areas of the implant surface were covered with bacteria with rod-shaped morphology, forming a densely packed biofilm. LIVE/DEAD® staining of such three days *Aa* biofilm on the implants showed green signal for 75-80% of all biofilm bacteria, indicating living cells with uncompromised membrane integrity. Microbiological and PCR detection of *Aa* biofilm on implants placed in vivo were positive for samples from the implant surfaces and negative for the saliva samples as well as control implants. Clinical examination demonstrated significant peri-implant mucosal inflammation around implants with *Aa* biofilm, compared with control untreated implants. Micro-CT and histological analysis of peri-implant bone and mucosal tissues is pending. Nano-textured implant surfaces favor the establishment of *Aa* biofilm and increase risk of peri-implantitis.

Experiment No. 5

This experiment provides a mouse model for pre-clinical testing of interfering agents to treat Lyme disease. See Dresser et al. Pathogens 5(12)e1000680, Epub 2009 Dec. 4. Lyme disease is the most common tick-borne disease in the United States. Reported cases have more than doubled between 1992 and 2006, with approximately 29,000 new cases confirmed in 2008. Estimates are that the actual number of cases of Lyme disease may exceed that reported by a factor of 6 – 12 in endemic areas. By definition, these endemic areas are expanding as populations continue to move from cities to suburban and rural areas and whitetail deer (which carry the tick species *Ixodes*) increasingly roam these areas. Lyme disease is caused by the microorganism *Borrelia burgdorferi*, a spirochete. *B. burgdorferi* is transmitted via the bite of the *Ixodes* tick and subsequently disseminates, via the bloodstream, to other tissues and organs.

In this animal model, C3H/HeN mice are injected with spirochetes via dorsal subcutaneous and intraperitoneal injection, or via intravenous injection. Blood and biopsy specimens are recovered at approximately 7 days post infection for evaluation of microbial burden and assessment of pathology in tissues and organs. The methods and compositions of this invention are contemplated to develop both therapeutic as well as preventative strategies for reduction and/or elimination of the resulting *B. burgdorferi* biofilms which form subsequent to challenge and are believed to contribute to both the pathogenesis and chronic nature of the disease.

Experiment No. 6

This experiment provides a porcine model for pre-clinical testing of interfering agents to treat cystic fibrosis. See Stoltz et al. (2010) Science Translational Medicine 2(29): 29ra31.

Cystic fibrosis is an autosomal recessive disease due to mutations in a gene that encodes the CF transmembrane conductance regulator (called CFTR) anion channel. In this model, pigs which have been specifically bred to carry a defect in the genes called "CFTR" and called CF pigs spontaneously develop hallmark features of CF lung disease that includes infection of the lower airway by multiple bacterial species. The pigs can be immunized with the interfering agents to either 1) immunize these CF pigs with a polypeptide or other immunogenic agent thereby inducing the formation of antibodies which will eradicate bacterial biofilms in the lungs (similarly to how antibodies to IHF eradicated biofilms resident within the middle ears of chinchillas following active immunization as shown in Experiment No. 1, to deliver anti-IHF (or other interfering agent) to the lungs of these animals by nebulization to assess the amelioration of the signs of disease and associated pathologies.

Experiment No. 7

Applicants also provide a pre-clinical model for tuberculosis (TB). See Ordway et al. (2010) Anti. Agents and Chemotherapy 54:1820. The microorganism *Mycobacterium tuberculosis* is responsible for a growing global epidemic. Current figures suggest that there are approximately 8 million new cases of TB and about 2.7 million deaths due to TB annually. In addition to the role of this microbe as a co-infection of individuals with HIV (of the ~45 million infected with HIV, estimates are that ~1/3 are also co-infected with *M. tuberculosis*), its particularly troublesome that isolates have become highly resistant to multiple drugs and no new drug for TB has been introduced in over a quarter of a century. In this animal model,

SPF guinea pigs are maintained in a barrier colony and infected via aerosolized spray to deliver ~ 20 cfu of *M. tuberculosis* strain Erdman K01 bacilli into their lungs. Animals are sacrificed with determination of bacterial load and recovery of tissues for histopathological assessment on days 25, 50, 75, 100, 125 and 150 days post-challenge. Unlike mice which do not develop classic signs of TB, guinea pigs challenged in this manner develop well-organized granulomas with central necrosis, a hallmark of human disease. Further, like humans, guinea pigs develop severe pyogranulomatous and necrotizing lymphadenitis of the draining lymph nodes as part of the primary lesion complex. Use of this model will provide a pre-clinical screen to confirm and identify therapeutic as well as preventative strategies for reduction and/or elimination of the resulting *M. tuberculosis* biofilms which have been observed to form in the lungs of these animals subsequent to challenge and are believed to contribute to both the pathogenesis and chronicity of the disease.

Experiment No. 8

Multiple animal models of catheter/indwelling device biofilm infections are known. See Otto (2009) Nature Reviews Microbiology, 7:555. While typically considered normal skin flora, the microbe *Staphylococcus epidermidis* has become what many regard as a key opportunistic pathogen, ranking first among causative agents of nosocomial infections. Primarily, this bacterium is responsible for the majority of infections that develop on indwelling medical devices which are contaminated by this common skin colonizer during device insertion. While not typically life-threatening, the difficulty associated with treatment of these biofilm infections, combined with their frequency, makes them a serious public health burden. Current costs associated with treatment of vascular catheter associated bloodstream infections alone that are due to *S. epidermidis* amount to \$2 billion annually in the United States. In addition to *S. epidermidis*, *E. faecalis* and *S. aureus* are also contaminations found on indwelling medical devices. There are several animal models of catheter-associated *S. epidermidis* infections including rabbits, mice, guinea pigs and rats all of which are used to study the molecular mechanisms of pathogenesis and which lend themselves to studies of prevention and/or therapeutics. Rat jugular vein catheters have been used to evaluate therapies that interfere with *E. Faecalis*, *S. aureus* and *S.epidermidis* biofilm formation. Biofilm reduction is often measured three ways - (i) sonicate catheter and calculate CFUs, (ii) cut slices of catheter or simply lay on a plate and score, or (iii) the biofilm can be stained with crystal violet or another dye, eluted, and OD measured as a proxy for CFUs.

Experiment No. 9

Methods described herein may be used to elicit immune responses in humans and animals. Immunogenic compositions may be administered to a human and animal subjects in the presence of adjuvants such as but not limited to aluminum salts and liposomes. Those skilled in the art will understand that any number of pharmaceutically acceptable adjuvants can also be used. Immunogenic compositions may be administered to a human or animal subjects intramuscularly, subdermally, intranasally, or through any other suitable route. Immunogenic compositions may be prepared in a manner consistent with the selected mode of administration. Immunogenic compositions may take the form of polypeptides, nucleic acids, or a combination thereof, and may comprise full-length or partial antigens. Additionally or alternatively, immunogenic compositions may take the form of APCs pulsed with a particular antigen, or APCs transfected with one or more polynucleotides encoding a particular antigen. Administration may comprise a single dose of an immunogenic composition, or an initial administration, followed by one or more booster doses. Booster doses may be provided a day, two days, three days, a week, two weeks, three weeks, one, two, three, six or twelve months, or at any other time point after an initial dose. A booster dose may be administered after an evaluation of the subject's antibody titer.

Experiment No. 10

Methods described herein may be used to confer passive immunity on a non-immune subject. Passive immunity against a given antigen may be conferred through the transfer of antibodies or antigen binding fragments that specifically recognize or bind to a particular antigen. Antibody donors and recipients may be human or non-human subjects. Additionally or alternatively, the antibody composition may comprise an isolated or recombinant polynucleotide encoding an antibody or antigen binding fragment that specifically recognizes or binds to a particular antigen.

Passive immunity may be conferred in cases where the administration of immunogenic compositions poses a risk for the recipient subject, the recipient subject is immuno-compromised, or the recipient subject requires immediate immunity. Immunogenic compositions may be prepared in a manner consistent with the selected mode of administration. Compositions may comprise whole antibodies, antigen binding fragments, polyclonal antibodies, monoclonal antibodies, antibodies generated *in vivo*, antibodies generated *in vitro*, purified or partially purified antibodies, or whole serum. Administration may comprise a single dose of an antibody composition, or an initial administration followed by one or more booster doses. Booster doses may be provided a day, two days, three days, a

week, two weeks, three weeks, one, two, three, six or twelve months , or at any other time point after an initial dose. A booster dose may be administered after an evaluation of the subject's antibody titer.

Experiment 11

Members of the DNABII family are highly pleiotropic for multiple nucleoprotein systems including gene transcription, and moreover, are thus often essential. In contrast, both HU and IHF mutants have been generated in laboratory strains of *E. coli* and studied extensively. To determine what role IHF and HU have in eDNA formation, biofilms formed by *E. coli* strain MG1655 or its HU and IHF deficient derivatives were incubated with anti-IHF (prepared as described in Granston and Nash (1993) *J. Mol. Biol.*, 234:45-59.. As shown in Figure 8, whereas the parental isolate, strain MG1655 produced a robust biofilm *in vitro* (Panel A), both HU and IHF mutant strains were less robust (Panels C and E, respectively). An HU deficient mutant yielded a biofilm that was approximately $\frac{1}{2}$ the height of the wild type biofilm, whereas IHF deficient strains produced a biofilm that was approximately $\frac{2}{3}$ the height of the parental isolate. This result suggested that both proteins were involved in either the production and/or integrity of the *E. coli* biofilm EPS. After treatment with anti-IHF, the biofilm formed by the wild type isolate showed notable debulking and a mean percent reduction of 58.1% in height, 73.1% percent reduction in biomass and 87% reduction in mean thickness (Panel B), whereas that formed by the HU mutant showed no reduction in height and only a 30.9 % reduction on biomass and 37.2% reduction on mean thickness (Panel D). In contrast, no effect on the biofilm formed by the IHF mutant was observed in terms of loss of height (-3.4% reduction), biomass (-20.5% reduction) or mean thickness (-19.8% reduction) (Panel F). Collectively, these data indicated that for this strain of *E. coli*, IHF was likely the only structural element that could be targeted by use of the anti-IHF antibody. Since to date only the highly structured interwoven eDNA in biofilms that have been formed *in vivo* have been seen, confirmation of the expression of remaining DNABII family member by each respective mutant and any change in the resultant DNA structure awaits an *in vivo* immunofluorescent analysis.

Experiment 12

Whereas it was demonstrated in this application *in vitro* and *in vivo* that both anti-IHF and the use of IHF as an immunogen show utility in debulking biofilms and/or resolving biofilm disease respectively, it was unknown if debulking of NTHI biofilms with anti-IHF might also

allow synergism when used in conjunction with other therapeutic modalities. To this end the ability to induce augmented structural destabilization of pre-formed NTHI biofilms was assessed when a sub-optimal concentration of anti-IHF was used along with one of each of three unique reagents. These three reagents include 1) a DNA degrading enzyme (DNaseI) known to be able to degrade an NTHI biofilm *in vitro* (but used here at a suboptimal concentration), 2) antisera to an outer membrane protein of NTHI that did not destabilize a pre-formed NTHI biofilm when used alone (anti-OMP P5) (data not shown), and 3) an antibiotic typically used as a first line choice in children with chronic and/or recurrent OM (amoxicillin) but which has limited efficacy against bacteria resident within a biofilm community.

Figure 14A shows that treatment of an NTHI biofilm with a concentration of DNase shown to be sub-optimal has a marginal effect (see 14A II). Likewise, a 1:200 dilution of anti-IHF had little effect on the pre-formed NTHI biofilm (see 14A III). In contrast however, when these two reagents were used in concert, the biofilm was notably diminished (see 14A IV). Upon repeat of this experiment three times, we found that the most marked synergistic effect of debulking of the biofilm was measured as a diminution in height. Table 7 below depicts these results.

Table 7

	DNase	Anti-IHF	DNase + anti-IHF	
Max height (μm)	3.9	37.3	51.0	Assay 1
Max height (μm)	-11.5	16.4	37.7	Assay 2
Max height (μm)	-1.6	19.4	38.7	Assay 3

One simple explanation for this outcome was that as the DNABII protein was being titrated away from the periphery of the biofilm, the eDNA became more accessible to the action of the DNase.

Figure 14B shows the results of treatment with anti-OMP P5 on pre-formed NTHI biofilms. Although this antisera is strongly reactive with isolated NTHI OMP P5 (data not shown) and further, active immunization with isolated OMP P5 is effective in mediating significant protection against experimental OM in chinchilla models (see for e.g. 25. Bakaletz et al., (1997) *Vaccine* **15**(9): 955-961; Bakaletz et al., (1999) *Infect Immun* **67**(6): 2746-2762; Kennedy et al., (2000) *Infect Immun* **68**(5): 2756-2765; Kyd JM, et al., (2003) *Infect Immun* **71**(8): 4691-4699; Novotny LA, et al., (2000) *Infect Immun* **68**(4): 2119-2128; Novotny LA,

et al., (2002) *Vaccine* **20**(29-30): 3590-3597 and Novotny LA, et al., (2003) *J Immunol* **171**(4): 1978-1983) this antiserum does not induce a change in the structural integrity of an NTHI biofilm that has been formed *in vitro* when used at a dilution of 1:50 (see 14B II). Likewise a marginal effect when these biofilms were incubated with a sub-optimal dilution of anti-IHF (used at a 1:100 dilution here) was observed (see 14B I). When combined, however, the use of anti-P5 plus anti-IHF resulted in a reduction in the height of the biofilm that exceeded the sum of the two antisera when used singly (see 14B III), thus indicating a synergistic outcome. Hence, it was concluded that use of anti-IHF to destabilize the NTHI EPS matrix resulted in the exposure of the targeted bacterial cell surface protein (e.g. OMP P5) that would otherwise be obscured by eDNA as well as perhaps other components of the EPS, thus allowing immune mediated bacterial clearance by as yet unknown mechanisms.

Lastly, Figure 14C shows the results of treating pre-formed NTHI biofilms with amoxicillin. When used at a concentration of 64 µg/ml, amoxicillin had no measurable effect on the architecture of pre-formed NTHI biofilms (see 14C II) despite evidence of limited bacterial cell death. Treatment with IHF antisera at a 1:50 dilution substantially reduced the height of the biofilm as shown previously (see 14C III). Interestingly however, when the two reagents were used simultaneously, not only was there a dramatic reduction in the height of the biofilm (see 14C IV), but use of a vital dye indicated that the majority of the bacteria remaining in the biofilm were now dead as noted by the predominant fluorescence in the red channel within the imaged biofilm. This result showed that debulking of the biofilm with anti-IHF likely exposed the bacteria sufficiently so as to create conditions more akin to susceptibility to amoxicillin concentrations known to be effective when assayed against planktonic NTHI. This outcome may have been mediated by either increased physical exposure of bacteria within the remaining biofilm matrix to the action of amoxicillin and/or via increased release of bacteria into the planktonic phase as we showed earlier can occur during biofilm debulking by exposure to anti-IHF antibodies (see Fig. 4).

Experiment 13

Applicants have shown that the induction of anti-IHF antibodies by transcutaneous immunization is effective in resolving NTHI induced biofilms within the chinchilla middle ear. It was also shown that use of native IHF is essential to induction of a protective immune response, as when it is bound to eDNA, protective epitopes appear to have been masked as shown following both TCI and SQ immunization. Nonetheless, the rational design of vaccine candidates that will likely have broad protective efficacy requires a detailed understanding of

the immunodominant and/or protective epitopes of that protein, which may in fact be neither obvious nor necessarily continuous. Current vaccine candidate design strategies are more reductionist in their approach, and often aimed at identifying and using only that portion of the targeted protein that is absolutely necessary for induction of high avidity and protective antibodies. To this end, epitope mapping is done to determine the most effective immunogen.

Epitope mapping can be performed as described in Novotny, et al., (2009) *Vaccine* 28(1):279-289 and according to the following procedure. To generate immune serum Chinchillas can be parenterally immunized by subcutaneous (SQ) injection of 10 µg of the native IHF protein, a polypeptide of the C-terminal 20 amino acids of IHF α , a polypeptide of the C-terminal 20 amino acids of IHF β , or a polypeptide of amino acids 60-76 of TBP-DNABII administered co-mixed with the adjuvant monophosphoryl lipid A (MPL; Corixa) or with MPL alone (adjuvant-only control cohort). Two identical boosts can be delivered at 21-day intervals. Blood for serum can be collected 10 days after receipt of the final immunizing dose. Enzyme-linked immunosorbent assay (ELISA) and Western blotting of chinchilla antisera can be performed. For ELISA, sera can be assayed in a 96-well plate format against the immunogen delivered (0.2 µg protein/well). Titer can be defined as the reciprocal of the serum dilution that yielded an OD450 value of 0.1 greater than control wells which contain all components except serum. ELISA assays can be performed a minimum of three times and results are reported as the geometric mean. Western blotting can be performed against 1 µg of the respective immunogen using immune serum diluted 1:100 and detected with HRP-protein A (Zymed, South San Francisco, CA). Color was developed with CN/DAB substrate (Pierce, Rockford, IL).

A series of 20-mer synthetic peptides with a 5-residue overlap can be synthesized in order to epitope map the IHF protein of NTHI. Synthesis, purification and sequence confirmation of all synthetic peptides can be performed according to standard techniques (see for e.g. Bakaletz LO. et al., (1997) *Infect. Immun.* 71(8):4691-9 and Bakaletz LO., et al., (2001) *Vaccine* 68(4):2119-28). Amino acid analysis and mass spectral analysis can be used to confirm the purity, composition and amino acid sequence of each peptide.

Analysis of the interaction between synthetic peptides and antibodies present in sera, can be examined using a Biacore 3000 instrument as described and all reagents can be purchased from Biacore (Uppsala, Sweden). Briefly, peptides can be suspended in sodium acetate buffer, prior to immobilization to the surface of an activated reagent grade CM5 sensor chip

using amine coupling chemistry. For interaction analysis, 15 µl of serum (diluted 1:2 in HBS-EP buffer plus 1 mg carboxymethyl dextran/ml) can be injected across the sensor chip surface at a flow rate of 5 µl/min. The relative amount of antigen- or immunogen-specific antibody in each sample can be calculated as the difference in resonance units (RU) obtained 5 seconds before and 45 seconds after each sample can be injected. The sensor chip surface can be regenerated between samples with 25 mM NaOH. The synthetic peptides with the strongest interaction with the antibodies present in the sera can be the most promising candidates of protective epitopes capable of inducing high avidity protective antibodies *in vivo*.

Rabbit polyclonal antiserum can be generated against the most immunogenic peptides from the epitope mapping. This antiserum can be used to affinity purify neutralizing antibodies from non-neutralizing antibodies by column chromatography. To do so, both rabbit and chinchilla polyclonal antisera (the latter has been shown to be protective) can be used. Sera can be assayed both prior to as well as after running each over a column to which either native IHF was bound or to which IHF pre-admixed with DNA has been bound. Only the antibodies that do not bind to the DNA-IHF complex should be neutralizing. All flow-through and eluted antisera will then be assayed against the original antiserum pools via our *in vitro* biofilm assay for relative ability to debulk and NTHI biofilm as well as via ELISA, Western blot and biosensor assays to determine specificity and titer. As an additional test, epitope-targeted vaccine candidates can be bound to the column to determine if they can remove antibodies now proven to be neutralizing.

It is to be understood that while the invention has been described in conjunction with the above embodiments, that the foregoing description and examples are intended to illustrate and not limit the scope of the invention. Other aspects, advantages and modifications within the scope of the invention will be apparent to those skilled in the art to which the invention pertains.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. All nucleotide sequences provided herein are presented in the 5' to 3' direction.

The inventions illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms "comprising", "including," "containing", etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been

used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed.

Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification, improvement and variation of the inventions embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications, improvements and variations are considered to be within the scope of this invention. The materials, methods, and examples provided here are representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention.

The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

All publications, patent applications, patents, and other references mentioned herein are expressly incorporated by reference in their entirety, to the same extent as if each were incorporated by reference individually. In case of conflict, the present specification, including definitions, will control.

TABLE 8

Gram (+) - only HU, Gram (-) - all have HU some also IHF

Bacteria strain	Abbreviation	Protein name(s)	
<i>S. sobrinus</i> 6715	<i>Ss</i>	1310	(HU) (not fully sequenced)
<i>S. pyogenes</i> MGAS10270	<i>Spyog</i>	Spy1239	(HU)
<i>S. gordonii</i> Challis NCTC7868	<i>Sg</i>	SGO_0701	(HlpA)
<i>S. agalactiae</i> (Group B Strep) 2603V/R	GBS	SAG_0505	(Hup)
<i>S. mutans</i> UA159	<i>Sm</i>	Smu_589	(HU)
<i>S. pneumoniae</i> R6	<i>Spneu</i>	spr1020	(HU)
<i>S. gallolyticus</i> UCN34 (<i>S. bovis</i>)	<i>Sgall</i>	YP_003430069	(HlpA)
<i>S. aureus</i> MW2	<i>Sa</i>	MW1362	(HU)
<i>S. epidermidis</i> RP62A	<i>Se</i>	SERP1041	(Hup)
<i>E. coli</i> K12-MG1655	<i>Ec</i>	b1712	(HimA)
		b0912	(HimD)
			(HupA)
			(HupB)
<i>H. influenza</i> KW20 Rd	<i>Hi</i>	HI1221	(HimA)
		HI1313	(HimD)
		HI0430	(HupA)

<i>Salmonella enteric serovar typhi</i> Ctl8	Salm	Sty1771	(Hima)
		Sty0982	(Himd)
<i>Aggregatibacter actinomycetemcomitans</i> D11S-1	Aa	YP_0032555965	(IHFalpha)
		YP_003256209	(Ihfb)
<i>P. gingivalis</i> W83	Pg	YP_003255304	(HU)
		PG_0121	(Hup-1)
		PG_1258	(Hup-2)
<i>N. gonorrhoeae</i> FA1090 (Oklahoma)	Ng	NGO603	(IHFβ)
		NGO030	(IHFα)
<i>N. meningitides</i> MC58	Nm	NMB_0729	(Hima)
<i>P. aeruginosa</i>	Pa	NMB_1302	(Hima)
		PA3161	(Himd)
		PA1804	(HupB)
		PA2758	(Hima)
<i>H. pylori</i> 26695	Hp	Hp0835	(Hup)
<i>B. burgdorferi</i> B31	Bb	BB_0232	(Hbb)
<i>Moraxella catarrhalis</i> RH4	Mc	YP_003626307	(Hima)
		YP_003627027	(Himd)
		YP_003626775	(HupB)

TABLE 8 (CONTINUED)

<i>V. cholera</i> El Tor N16961	<i>Vc</i>	VC_0273	(HupA)
		VC_1914	(HipB)
		VC_1919	(HupB)
		VC_1222	(Hima)
<i>Burkholderia cenocepacia</i> HI2424	<i>Bc</i>	Bcen2424_1048	(IHFB)
		Bcen2424_1481	(IHFA)
<i>Burkholderia pseudomallei</i> 668	<i>Bp</i>	BURPS668_2881	(IHFB)
		BURPS668_1718	(IHFA)
<i>Mycobacterium tuberculosis</i> CDC1551	<i>Mtb</i>	MT_3064	(HU)
		MSMEG_2389	(Hup)
<i>Mycobacterium smegmatis</i> MC2	<i>Ms</i>		
<i>Treponema denticola</i> ATCC 35405	<i>Td</i>	TDE_1709	(HU)
<i>Treponema palladium</i> Nichols	<i>Tp</i>	TP_0251	(DNA binding protein II)
<i>Prevotella melaninogenica</i> ATCC 25845	<i>Pm</i>	PREME0022_2103	(HupB)
		PREME0022_0268	(HupA)
<i>Prevotella intermedia</i> 17	<i>Pi</i>	PREME0022_0341	(Hup)
		PREME0022_0340	(Hima)
		PIN_A0704	(Hup)
		PIN_A1504	(Hup-2)
<i>Bordetella pertusis</i> Tohama 1		PIN_0345	(Hima)
		PIN_0343	(Hypothetical protein)
		BP2572	(Ihfa)
	<i>Bpert</i>		

BP3530	(HupB)
BP0951	(InhFB)
Ef1550	(hup)

Enterococcus faecalis V583

Ef

9A	9A (cont.)
----	---------------

SEQ ID NOS 42-72, respectively, in order of appearance

Ec_HimA	-----MALTKAEMSEYLFDKLG-LS-----KDAKELVELFFEEIRRALEN
Salm_HimA	-----MALTKAEMSEYLFDKLG-LS-----KDAKELVELFFEEIRRALEN
Vc_HimA	-----MALTKAELAEALFEQLG-MS-----KDAKDTVEVFFEEIRKALES
Pa_HimA	-----MGALTKAEIAERLYEELG-LN-----KREAKELVELFFEEIRQALEH
Hi_HimA	-----MATITKLDIIEYLSDKYH-LS-----KQDTKNVVENFLEEIRLSLES
Aa_IHFalpha	-----MTLTKEVELAENLIEKFH-LS-----KREAKDLVESFFEEIRVALET
Mc_HimA	-----MGALTKADMVDELTIRLR-LT-----RQARKLVDGFFEEISQSLAQ
Ng_IHFalpha	-----MTLTKAELADILVDKVSNT-----KNDAKEIVELFFEEIRSTLAS
Nm_HimA	-----MTLTKAELADILVDKVSNT-----KNDAKEIVELFFEEIRSTLAS
Bc_IHFA	+30aaASTE-TPTLTKAELAEELLFDSVG-LN-----KREAKDMVEAFFEVIRDALEN
Bp_IHFA	+27aaTSAGDTPTLTKAELAEELLFDSVG-LN-----KREAKDMVEAFFEVIRDALEN
Bpert_IhfA	MGTTMLAEPRTLTKAELAEELLFERVG-LN-----KREAKDIVDTFFEEIRDALAR
Pm_HimA	-----MNNKEFIAALAARTGYT-----QDESQKMVKTVVDMMGKSFET
Pi_HimA	-----MNNKEFITALANRVGRS-----QDETQKLVKLTALQAMGDNFES
Tp_Dbp II	-----MKRVRRTSRFVVDALCDEV DLS-----RHVARVVD SFVSVVTAALER
Pm_Hup	-----MAKSAIQ LITSALAKQHNLS-----ADDAAAFVDAFFDIISSELKN-
Pi_hypo	-----MAKTALQLIADAVAKKHKIT-----VKEAEKFVSAIFDVVNEGLKT
Sa_HU	-----MNNKTDLINAVAEQADLT-----KKEAGSAVDAVFESTIONSLAK
Ec_hupA	-----MNNKTQLIDVIAEKAELS-----KTQAKAALESTLAAITESLKE
Se_Hup	-----MNNKTDLINAVAEQADLT-----KKEAGSAVDAVFESTIONSLAK
Ss_Hu	-----MANKQDLIAKVAEATELT-----KKDSAAAVDVFSSIEGFLSK
Spyog_HU	-----MANKQDLIAKVAEATELT-----KKDSAAAVDAVFSTIEAFLAE
Sgall_HlpA	-----MANKQDLIAKVAEATELT-----KKDSAAAVDAVFSAIESFLSE
GBS_Hup	-----MANKQDLIAKVAEATELT-----KKDSAAAVDAVFAAVADYLAE
Spneu_HU	-----MANKQDLIAKVAEATELT-----KKDSAAAVEAVFAAVADYLAA
Sg_HlpA	-----MANKQDLIAKVAEATELT-----KKDSAAAVDAVFAAVTEYLSK
Sm_HU	-----MANKQDLIAKVAEATELT-----KKDSAAAVDAVFSVAVSSYLAK
Ef_Hup	-----MANKAELIENVASS TGLT-----KKDATAAVDAVFSTIQETLAK
Hi_HupA	-----MNNKTDLIDAIANAELN-----KKQAKAALEATLDAITASLKE
Vc_HupA	-----MNNKTQLIDFIAEKADLT-----KVQAKAALEATLGAVEGALKD
Pa_HupB	-----MNNKSELIDAIASADIP-----KAVAGRAIDAVIESVTGALKA

α-1

Turn

α-2

T

Table 9A1

Table 9A1 (continued)

9A2	9A2 (cont.)
-----	----------------

SEQ ID NOS 73-76, respectively, in order of appearance

Aa HU	-----M N K T D L I D A I A S S A E L N-----K N Q A K A A L E A T L D A I T G S L K K
Vc_HupB	-----M N K T Q L V E Q I A A N A D I S-----K A S A G R A L D A F I E A V S G T L Q S
Ec_hupB	-----M N K S Q L I D K I A A G A D I S-----K A A A G R A L D A I I A S V T E S L K E
Mc_HupB	-----M N K S E L V D S I A Q S A G L T-----K E Q A A K A V N A F T E S V Q G A L Q R
	α-1 Turn α-2 T

Table 9A2

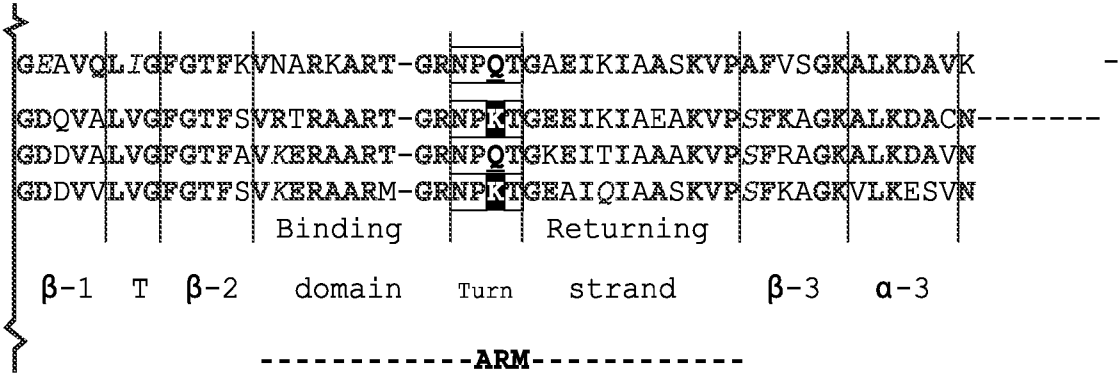


Table 9A2 (continued)

9A3	9A3 (cont.)
-----	----------------

SEQ ID NOS 77-100, respectively, in order of appearance

Bpert HupB	-----MNKTELIDHIAASKADIS-----KAAAGRSLDALIGAVKTTLKK
Mc HimD	----MQAVINKSNLIANLASVCEEL----EEDVVDEAVRLMIAMMVNELVY
Pm HupB	-----MNKTELIEKIAANAEVS-----KAAAKKALDATTEAIKEALAA
Pi Hup	-----MNKTELIEKIAAGAGLS-----KADSKKALDAMTAAIKEALVA
Td HU	-----MKQKRISKIDIIDSVYRNNPQYQLKQINAIANLFLDELSVLLQOG
Pg_Hup-1	-----MNKTDFTIAAVAERKANLT-----KADAQRAVNFAFAEVVTEQMNA
Hp_Hup	-----MNKAEFIDLVEAGKYNS-----KREAEAAISAFITLAVETALSK
Pm HupA	-----MTKADIINEIATSTGIA-----KKDVSAVVESFMETIKDSLLE
Pi Hup-2	-----MTKADIINEIASSTGIS-----KKDVSAVVESFMDAIKDSLLE
Pg_Hup-2	-----MTKADVNAIAKSTGID-----KETTLKVVESFMDTIKDSLSE
Mt HU	-----MNKAELIDVLTQKLGSD-----RQATAAVENVVDITIVRAVHK
Ms Hup	-----MNKAELIDVLTTKMGTD-----RQATAAVENVVDITIVRAVHK
Ec_HimD	-----MTKSELIERLATQQS-----HIPAKTVEDAVKEMLEHMASTLAC
Salm_HimD	-----MTKSELIERLATQQS-----HIPAKAVEDAVKEMLEHMASTLAC
Vc_HipB	-----MTKSELIERLCAEQT-----HLSAKEIEDAVKNILEHMASTLEA
Pa_HimD	-----MTKSELIERIVTHQG-----QLSAKDVELAIKTMLEQMSQALAT
Hi_HimD	-----MTKSELMEKLSAKQP-----TLPAKEIENMVKGILEFISQSLEN
Aa_IHFB	-----MTKSELIELLVQKNS-----NIPVKHVEEAVKAILEQMSYVLEH
Ng_IHFB	-----MVRLEAEVFAAKNGTHLLAKDVEYSVKVLVDITMTRSLAR
Nm HimD	-----MTKSELMVRLAEVFAAKNGTHLLAKDVEYSVKVLVDITMTRSLAR
Bc IHFB	-----MTKSELVAQLASRFP-----QLVLKDADFAVKTMLDAMSDALAK
Bp IHFB	-----MTKSELVAQLASRFP-----QLVLKDADFAVKTMLDAMSDALSK
Bpert IhfB	-----MTKSELIAALAARYP-----QLAARDTDYAVKTMLDAMTQALAS
Bb_Hbb	MSFSRRPKVTKSDIVDQISLNIKNNNL-KLEKKYIRLVIDAFFEELKSNLCS

α-1

Turn

α-2

T

Table 9A3

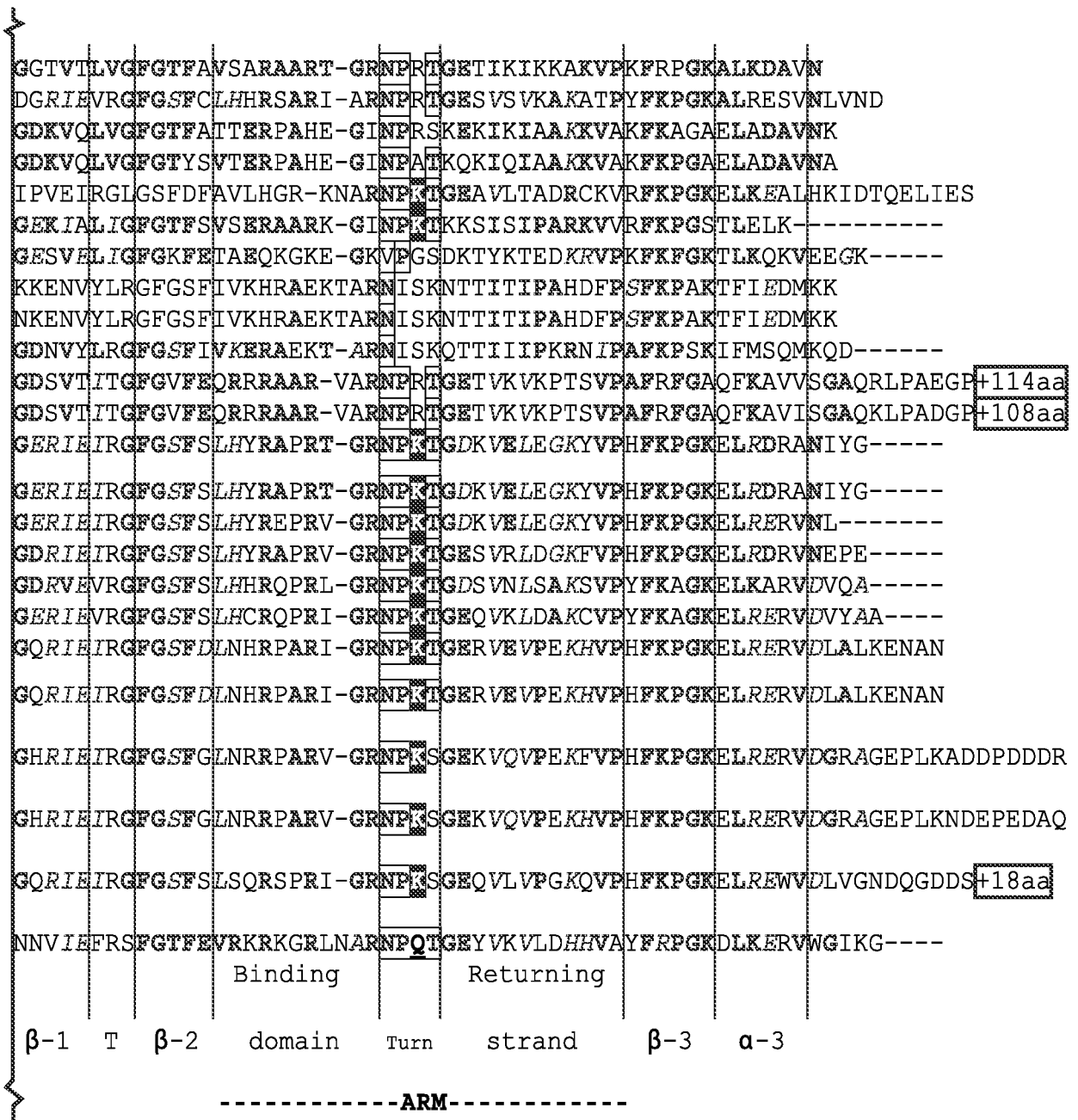


Table 9A3 (continued)

SEQ ID NOS 101-128, respectively, in order of appearance

Comparison to Liu et al 16 aa peptide motif

<i>Strep inter HU</i>	EVREERAARK-GRNPQTG
<i>Ec_HimA</i>	DLRDKNQRP-GRNPQTG
<i>Salm_HimA</i>	DLRDKNQRP-GRNPQTG
<i>Vc_HimA</i>	DLRDKNERP-GRNPQTG
<i>Pa_HimA</i>	DLRDKRQRP-GRNPQTG
<i>Hi_HimA</i>	ELRDKSSRP-GRNPQTG
<i>Aa_IHFalpha</i>	ELRDKASRP-GRNPQTG
<i>Mc_HimA</i>	ELKDKKPRP-GRNPQTG
<i>Ng_IHFalpha</i>	QLRDKPQRP-GRNPQTG
<i>Nm_HimA</i>	QLRDKPQRP-GRNPQTG
<i>Bc_IHFA</i>	QLRDKPQRP-GRNPQTG
<i>Bp_IHFA</i>	QLRDKPQRP-GRNPQTG
<i>Bpert_Ihfa</i>	QVRDKPPRP-GRNPQTG
<i>Pm_HimA</i>	EVKKRLERV-MVNPQTG
<i>Pi_HimA</i>	EVKKRLERI-MTNPQTG
<i>Tp_Dbp II</i>	ESRVRKASV-GKSIQTG
<i>Pm_Hup</i>	KVQAVKPR-ESVNVMTG
<i>Pi_hypo</i>	KVQAVKPR-ESVNVMTG
<i>Sa_HU</i>	EVREERAARK-GRNPQTG
<i>Ec_hupA</i>	KVNHRAERT-GRNPQTG
<i>Se_Hup</i>	EVREERAARK-GRNPQTG
<i>Ss_Hu</i>	EVREERAARK-GRNPQTG
<i>Spyog_HU</i>	EVREERAARK-GRNPQTG
<i>Sgall_HlpA</i>	EVREERAARK-GRNPQTG
<i>GBS_Hup</i>	EVREERAARK-GRNPQTG
<i>Spneu_HU</i>	EVREERAARK-GRNPQTG
<i>Sg_HlpA</i>	EVREERAARK-GRNPQTG
<i>Sm_HU</i>	EVREERAARK-GRNPQTG

Table 9B

SEQ ID NOS 129-139, respectively, in order of appearance

<i>Ef</i> Hup	EVREAAARK-GKNPQTG
<i>Hi</i> _HupA	KVNERAART-GKNPQTG
<i>Vc</i> _HupA	KVNHRSAART-GKNPQTG
<i>Bpert</i> HupB	AVSARAART-GKNPQTG
<i>Pa</i> _HupB	AVKERAART-GKNPQTG
<i>Aa</i> HU	SVRIRAART-GKNEKTG
<i>Pm</i> HupB	ATTERPAHE-GINPRSK
<i>Pi</i> Hup	SVTERPAHE-GINEATK
<i>Td</i> HU	FAVLHGR-KNARNEKTG
<i>Pg</i> _Hup-1	SVSERAARK-GINPKTK
<i>Hp</i> _Hup	ETAEQKGKE-GKVEGSD

Table 9B (continued)

SEQ ID NOS 140-159, respectively, in order of appearance
 Comparison to Liu et al 16 aa peptide motif

<i>Pm</i> HupA	FIVKHRAEKTARNISKN
<i>Pi</i> Hup-2	FIVKHRAEKTARNISKN
<i>Pg</i> Hup-2	IVKERAECT-ARNISKQ
<i>Mt</i> HU	EQRRRAAR-VARNPRTG
<i>Ms</i> Hup	EQRRRAAR-VARNPRTG
<i>Ec</i> HimD	SLHYRAPRT-GRNEKIG
<i>Salm</i> HimD	SLHYRAPRT-GRNEKIG
<i>Vc</i> HipB	SLHYREPRV-GRNEKIG
<i>Ec</i> hupB	AVKERAART-GRNPQTG
<i>Mc</i> HupB	SVKERAARM-GRNEKIG
<i>Pa</i> HimD	SLHYRAPRV-GRNEKIG
<i>Hi</i> HimD	SLHHRQPRL-GRNEKIG
<i>Aa</i> IHFB	SLHCRQPRI-GRNEKIG
<i>Nq</i> IHFB	DLNHRPARI-GRNEKIG
<i>Nm</i> HimD	DLNHRPARI-GRNEKIG
<i>Ec</i> IHFB	GLNRRPARV-GRNEKSG
<i>Bp</i> IHFB	GLNRRPARV-GRNEKSG
<i>Bpert</i> IhfB	SLSQRSPRI-GRNEKSG
<i>Mc</i> HimD	CLHHRSAIR-ARNERTG
<i>Bb</i> Hbb	EVRKRKGRLNARNPQTG

Table 9B (continued)

TABLE 10 (SEQ ID NOS 160-336, respectively, in order of appearance)

Bacteria strain, protein name	β3 sequence	α3 sequence	C-terminal 20 aa
<i>S. pyogenes</i> MGAS10270, HU	AFKAGK	ALKDAVK	IAASKVPAFKAGKALKDAVK
<i>S. gallolyticus</i> UCN34 (<i>S. bovis</i>), HlpA	AFKAGK	ALKDAVK	IAASKVPAFKAGKALKDAVK
<i>S. sobrinus</i> 6715 HU	AFKAGK	ALKDAVK	IAASKVPAFKAGKALKDAVK
<i>S. agalactiae</i> (Group B Strep) 2603V/R Hup	AFKAGK	ALKDAVK	IAASKVPAFKAGKALKDAVK
<i>S. pneumoniae</i> R6 HU	AFKAGK	ALKDAVK	IAASKVPAFKAGKALKDAVK
<i>S. gordonii</i> Challis NCTC7868, HlpA	AFKAGK	ALKDAVK	IAASKVPAFKAGKALKDAVK
<i>S. mutans</i> UA159, HU	AFKAGK	ALKDAVK	IKASKVPAFKAGKALKDAVK
<i>Enterococcus faecalis</i> V583, Hup	AFKPGK	ALKDAVK	IAASKVPAFKPGKALKDAVK
<i>S. aureus</i> MW2, HU	AFKAGK	ALKDAVK	IPASKVPAFKAGKALKDAVK
<i>S. epidermidis</i> RP62A Hup	AFKAGK	ALKDAVK	IPASKVPAFKAGKALKDAVK
<i>H. influenzae</i> KW20 Rd HupA	AFVSGK	ALKDAIK	IAASKVPAFVSGKALKDAIK
<i>Aggregatibacter actinomycetemcomitans</i> D11S-1 HU	AFVSGK	ALKDAIK	IAASKVPAFVSGKALKDAIK
<i>V. cholera</i> El Tor N16961, HupA	AFVAGK	ALKDAIK	IAAANVPAFVAGKALKDAIK
<i>E. coli</i> K12-MG1655 hupA	AFVSGK	ALKDAVK	IAAANVPAFVSGKALKDAVK
<i>P. aeruginosa</i> HupB	GFKAGK	ALKDAVN	IAAAKIPGFKAGKALKDAVN
<i>E. coli</i> K12-MG1655 hupB	SFRAGK	ALKDAVN	IAAAKVPFRAGKALKDAVN
<i>V. cholera</i> El Tor N16961 HupB	SFKAGK	ALKDACN	IAEAKVPFRAGKALKDACN
<i>Bordetella pertussis</i> Tohama 1 HupB	KFRPGK	ALKDAVN	IKKAKVPFRPGKALKDAVN
<i>Prevotella melanogenica</i> ATCC 25845 HupB	KFKAGA	ELADAVNK	AAKKVAKFKAGAEELADAVNK
<i>Prevotella intermedia</i> 17 Hup	KFKPGA	ELADAVNA	AAKKVAKFKPGAELADAVNA
<i>Moraxella catarrhalis</i> RH4 HupB	SFKAGK	VLKESVN	IAASKVPSFKAGKVLKESVN
<i>P. gingivalis</i> W83 Hup-1	RFKPGS	TLELK	ISIPARKVVRFPKPGSTLELK
<i>H. pylori</i> 2669 Hup	KFKPGK	TLKQKVEEGK	KRVPKFKPGKTLKQKVEEGK
<i>Prevotella melaninogenica</i> ATCC 25845 HupA	SFKPAK	TFIEDMKK	PAHDFPSFKPAKTFIEDMKK
<i>Prevotella intermedia</i> 17 Hup-2	SFKPAK	TFIEDMKK	PAHDFPSFKPAKTFIEDMKK
<i>P. gingivalis</i> W83 Hup-2	AFKPSK	IFMSQMKQD	KRNIPAFKPSKIFMSQMKQD
<i>Mycobacterium tuberculosis</i> CDC1551 HU	AFRPGA	QFKAVVSGAQRLPAEGPAVKRG	AKRPATKAPAKKATARRGRK
<i>Mycobacterium smegmatis</i> MC2 Hup	AFRPGA	QFKAVISGAQKLPAAGPAVKRG	TKAPAKKAAAKKAPAKKGRR
<i>Prevotella melaninogenica</i> ATCC 25845 Hima	NFKPAA	TIKGVKRGQDNG	NFKPAAITIKHVRKGGQDNG
<i>Prevotella intermedia</i> 17 Hima	NFRATA	SVKEKLKKGAE	VLNFRATASVKEKLKKGAE
<i>E. coli</i> K12-MG1655 Hima	TFRPGQ	KLKSRVENASPKDE	TFRPGQKLKSRVENASPKDE
<i>Salmonella enteric serovar typhi</i> CT18 Hima	TFRPGQ	KLKSRVENASPKDE	TFRPGQKLKSRVENASPKDE
<i>V. cholera</i> El Tor N1696 Hima	TFRPGQ	KLKARVENIKVEK	VTFRPGQKLKARVENIKVEK
<i>P. aeruginosa</i> Hima	TFRPGQ	KLKARVEAYAGTKS	TFRPGQKLKARVEAYAGTKS
<i>Burkholderia cenocepacia</i> HI2424 IHFA	TFHASQ	KLKALVENGAE	RVWTFHASQKLKALVENGAE
<i>Burkholderia pseudomallei</i> 668 IHFA	TFHASQ	KLKALVENGAE	HASQKLKALVENGAE
<i>Bordetella pertussis</i> Tohama 1 IHfa	TFHASQ	KLKSVVEQNSPPDPASAE	QKLKSVVEQNSPPDPASAE
<i>N. gonorrhoeae</i> FA1090 (Oklahoma) IHFa	TFHASQ	KLKGMVEHYDDKQR	TFHASQKLKGMVEHYDDKQR
<i>N. meningitides</i> MC58 Hima	TFHASQ	KLKSMVEHYDDKQR	TFHASQKLKSMVEHYDDKQR

TABLE 10 (CONTINUTED)

<i>H. influenza KW20 Rd Hima</i>	TFKPGQ	KLRLRVEKTK	RRVVTFKPGQKLRLRVEKTK
<i>Aggregatibacter actinomycetemcomitans D11S-1 Hima</i>	VFKEPGQ	KLRLRVEKVKPKA	VVFKEPGQKLRLRVEKVKPKA
<i>Moraxella catarrhalis RH4 Hima</i>	TFKAGQ	KLRLGWIDSQNEG	VVTFKAGQKLRLGWIDSQNEG
<i>Treponema palladium Nichols DNA binding protein_II</i>	VFRLPSK	RLKSAVRGYRSGEVGAD	PSKRLKSAVRGYRSGEVGAD
<i>Prevotella melaninogenica ATCC 25845 Hup</i>	SFTPD	VMKELVNKPFQFETVVINDGV	MQAGDTMKVPKVELRPEYRK
<i>Prevotella intermedia 17 hypothetical</i>	SFTPD	TMKELVNKPFQFETVVINDGV	SAGDTMKVPKVELRPEYRK
<i>E. coli K12-MG1655 HimD</i>	HFKEPGK	ELDRANIYG	KYVPHFKPGKELDRANIYG
<i>Salmonella enteric serovar typhi Ct18 bHimD</i>	HFKEPGK	ELDRANIYG	KYVPHFKPGKELDRANIYG
<i>V. cholera El Tor N1696 HipB</i>	HFKEPGK	ELDRVNL	EGKYVPHFKPGKELRVNL
<i>P. aeruginosa HimD</i>	HFKEPGK	ELDRVNEPE	KFVPHFKPGKELDRVNEPE
<i>H. influenza KW20 Rd HimD</i>	YFKAGK	ELKARVDVQA	KSVPYFKAGKELKARVDVQA
<i>Aggregatibacter actinomycetemcomitans D11S-1 IHFB</i>	YFKAGK	ELRERVDVYAA	CVPYFKAGKELRERVDVYAA
<i>N. gonorrhoeae FA1090 (Oklahoma) IHFβ</i>	HFKEPGK	ELRERVDLALKENAN	FKPGKELRERVDLALKENAN
<i>N. meningitides MC58 HimD</i>	HFKEPGK	ELRERVDLALKENAN	FKPGKELRERVDLALKENAN
<i>Burkholderia cenocepacia HI2424 IHFB</i>	HFKEPGK	ELRERVDGRAGEPLKADDPDDDR	ERVVDGRAGEPLKADDPDDDR
<i>Burkholderia pseudomallei 668 IHFB</i>	HFKEPGK	ELRERVDGRAGEPLKNDPEPDAQ	ERVVDGRAGEPLKNDPEPDAQ
<i>Bordetella pertusis Tohama 1 IhfB</i>	HFKEPGK	ELREWVDLVGNDQDDSSNGSS	DSSNGSSDPLQSVMDMHAMH
<i>Moraxella catarrhalis RH4 HimD</i>	YFKPGK	ALRESVNLVND	ATPYFKPGKALRESVNLVND
<i>B. burgdorferi B31 Hbb</i>	YFKPGK	DLKERVWGKIG	HVAYFRPGKDLKERVWGKIG
<i>Treponema denticola ATCC 35405 HU</i>	RFKEPGK	ELKEALHKIDTQELIES	PGKELKEALHKIDTQELIES

What is claimed is:

1. A method for inhibiting, competing or titrating the binding of a DNABII polypeptide or protein to a microbial DNA, comprising contacting the DNABII polypeptide or protein or the microbial DNA with an interfering agent, thereby inhibiting, competing or titrating the binding of the DNABII protein or polypeptide to the microbial DNA.
2. A method for inhibiting, preventing or breaking down a microbial biofilm, comprising contacting the biofilm with an interfering agent, thereby inhibiting, preventing or breaking down the microbial biofilm.
3. The method of claim 1 or 2, wherein the contacting is in vitro or in vivo.
4. A method of inhibiting, preventing or breaking down a biofilm in a subject, comprising administering to the subject an effective amount of an interfering agent, thereby inhibiting, preventing or breaking down the microbial biofilm.
5. A method for inhibiting, preventing or treating a microbial infection that produces a biofilm in a subject, comprising administering to the subject an effective amount of an interfering agent, thereby inhibiting, preventing or treating a microbial infection that produces the biofilm in the subject.
6. The method of any one of claims 1 to 5, wherein the interfering agent is of the group of:
 - (a) an isolated or recombinant integration host factor (IHF) polypeptide or a fragment or an equivalent of each thereof;
 - (b) an isolated or recombinant histone-like protein from *E.coli* strain U93 (HU) polypeptide or a fragment or an equivalent of each thereof;
 - (c) an isolated or recombinant protein or polypeptide identified in Table 8, Table 9A, 9B, Table 10 or a DNA binding peptide identified in Figure 6, or a fragment or an equivalent of each thereof;
 - (d) an isolated or recombinant polypeptide of SEQ ID NO. 1 through 340, or a fragment or an equivalent of each thereof;
 - (e) an isolated or recombinant C-terminal polypeptide of SEQ ID NO. 6 through 11, 28, 29, 42 through 100, Table 8 or those C-terminal polypeptides identified in Table 10 or a fragment or an equivalent of each thereof;

- (f) a polypeptide or polynucleotide that competes with an integration host factor on binding to a microbial DNA;
 - (g) a four-way junction polynucleotide resembling a Holliday junction, a 3 way junction polynucleotide resembling a replication fork, a polynucleotide that has inherent flexibility or bent polynucleotide;
 - (h) an isolated or recombinant polynucleotide encoding any one of (a) through (f) or an isolated or recombinant polynucleotide of SEQ ID NO. 36 or an equivalent of each thereof, or a polynucleotide that hybridizes under stringent conditions to the polynucleotide its equivalent or its complement;
 - (i) an antibody or antigen binding fragment that specifically recognizes or binds any one of (a) through (f), or an equivalent or fragment of each antibody or antigen binding fragment thereof;
 - (j) an isolated or recombinant polynucleotide encoding the antibody or antigen binding fragment of (i) or its complement; or
 - (k) a small molecule that competes with the binding of a DNABII protein or polypeptide to a microbial DNA.
7. The method of any one of claims 1 to 6, wherein the interfering agent is an isolated or recombinant DNABII polypeptide or a fragment or an equivalent of each thereof.
8. The method of claim 7, wherein the DNABII polypeptide is an IHF polypeptide or a fragment thereof, a C-terminal fragment of an IHF polypeptide or an equivalent of each thereof.
9. The method of claim 7, wherein the DNABII polypeptide is an HU polypeptide or a fragment thereof, a C-terminal fragment of HU polypeptide, or an equivalent of each thereof.
10. The method of any one of claims 4 to 9, further comprising administering to the subject an effective amount of one or more of an antimicrobial, a DNase, an antibody, an antigenic peptide or an adjuvant.
11. The method of any one of claims 4 to 10, wherein the subject is a non-human animal or a human patient.
12. The method of any one of claims 1 to 11, wherein the microbial DNA is from a microorganism identified in Table 8.

13. The method of any one of claims 4 to 12, wherein the agent is administered by a method comprising topically, transdermally, transcutaneously, sublingually, rectally, vaginally, ocularly, subcutaneous, intramuscularly, intraperitoneally, urethrally, intranasally, by inhalation or orally.
14. The method of any one of claims 4 to 12, wherein the subject is a pediatric patient and the agent is administered in a formulation for the pediatric patient.
15. A method for inducing an immune response in or conferring passive immunity on a subject in need thereof, comprising administering to the subject an effective amount of one or more agents of the group:
- (a) an isolated or recombinant integration host factor (IHF) polypeptide, or a fragment or an equivalent of each thereof;
 - (b) an isolated or recombinant histone-like protein from *E.coli* strain U93 (HU) polypeptide or a fragment or an equivalent of each thereof;
 - (c) an isolated or recombinant protein polypeptide identified in Table 8, Table 9A, Table 9B, Table 10 or a DNA binding peptide identified in Figure 6, or a fragment or an equivalent of each thereof;
 - (d) an isolated or recombinant polypeptide of SEQ ID NO. 1 through 340, or a fragment or an equivalent thereof;
 - (e) an isolated or recombinant C-terminal polypeptide of SEQ ID NO. 6 through 11, 28, 29, 42 through 100, Table 8 or those C-terminal polypeptides identified in Table 10 or a fragment or an equivalent of each thereof;
 - (f) an isolated or recombinant polynucleotide encoding any one of (a) through (e) or an isolated or recombinant polynucleotide of SEQ ID NO. 36 or an equivalent of each thereof, or a polynucleotide that hybridizes under stringent conditions to the polynucleotide, its equivalent or its complement;
 - (g) an antibody or antigen binding fragment that specifically recognizes or binds any one of (a) through (e), or an equivalent or fragment of each thereof;
 - (h) an isolated or recombinant polynucleotide encoding the antibody or antigen binding fragment of (g);
 - (i) an antigen presenting cell pulsed with any one of (a) through (e); and

(i) an antigen presenting cell transfected with one or more polynucleotides encoding any one of (a) through (e).

16. The method of claim 15, wherein the agent comprises an isolated or recombinant DNABII polypeptide or a fragment or an equivalent thereof.

17. The method of claim 16, wherein the DNABII polypeptide is an IHF polypeptide or a fragment thereof, a C-terminal fragment of an IHF polypeptide or an equivalent of each thereof..

18. The method of claim 16, wherein the DNABII polypeptide is an HU polypeptide or a fragment thereof, a C-terminal fragment of HU polypeptide, or an equivalent of each thereof.

19. The method of any one of claims 15 to 18, further comprising administering to the subject an effective amount of one or more of an antimicrobial, a DNase, an antibody, an antigenic peptide or an adjuvant.

20. The method of any one of claims 15 to 19, wherein the subject is a non-human animal or a human patient.

21. The method of any one of claims 15 to 20, wherein the isolated or recombinant polynucleotide derives from a microorganism identified in Table 8.

22. The method of any one of claims 15 to 21, wherein the agent is administered by a method comprising topically, transdermally, transcutaneously, sublingually, rectally, vaginally, ocularly, subcutaneous, intramuscularly, intraperitoneally, urethrally, intranasally, by inhalation or orally.

23. The method of any one of claims 15 to 22, wherein the subject is a pediatric patient and the agent is administered in a formulation for the pediatric patient.

24. An isolated or recombinant polypeptide consisting essentially of an amino acid sequence selected from SEQ ID NO. 1 to 5 or 12 to 27, 30 to 35, 101-340 or a DNA binding peptide identified in Figure 6 or a fragment or an equivalent of each thereof.

25. An isolated or recombinant polypeptide comprising SEQ ID NO. 1 or 2, with the proviso that the polypeptide is none of SEQ ID NOS. 6 to 11, 28, 29, or 42 through 100.

26. An isolated or recombinant polypeptide comprising SEQ ID NO. 3, 4 or 5, with the proviso that the polypeptide is none of SEQ ID NOS. 6 to 11, 28, 29, or 42 through 100.

27. An isolated or recombinant polypeptide comprising SEQ ID NO. 12, 14, 16, 18, 20, 22, 24, 26, 30 or 32, with the proviso that the polypeptide is none of SEQ ID NOS. 6 to 11, 28, 29, or 42 through 100.
28. An isolated or recombinant polypeptide comprising SEQ ID NO. 13, 15, 17, 19, 21, 23, 25, 27, 31 or 33, with the proviso that the polypeptide is none of SEQ ID NOS. 6 to 11, 28, 29, or 42 through 100.
29. An isolated or recombinant polypeptide of the group of:
- a polypeptide comprising SEQ ID NO. 12 and 13;
 - a polypeptide comprising SEQ ID NO. 14 and 15;
 - a polypeptide comprising SEQ ID NO. 16 and 17;
 - a polypeptide comprising SEQ ID NO. 18 and 19;
 - a polypeptide comprising SEQ ID NO. 20 and 21;
 - a polypeptide comprising SEQ ID NO. 23 and 24;
 - a polypeptide comprising SEQ ID NO. 25 and 26;
 - a polypeptide comprising SEQ ID NO. 30 and 31;
 - a polypeptide comprising SEQ ID NO. 32 and 33;
 - a polypeptide comprising SEQ ID NO. 34 and 35;
 - a polypeptide comprising SEQ ID NO. 337 and 338; or
 - a polypeptide comprising SEQ ID NO. 339 and 340;
- with the proviso that the polypeptide is none of wild-type of any one of IHF alpha, IHF beta or SEQ ID NO. 6 to 11, 28, 29, or 42 through 100.
30. An isolated or recombinant polypeptide of the group of:
- a polypeptide consisting essentially of SEQ ID NO. 12 and 13;
 - a polypeptide consisting essentially of SEQ ID NO. 14 and 15;
 - a polypeptide consisting essentially of SEQ ID NO. 16 and 17;
 - a polypeptide consisting essentially of SEQ ID NO. 18 and 19;
 - a polypeptide consisting essentially of SEQ ID NO. 20 and 21;

a polypeptide consisting essentially of SEQ ID NO. 23 and 24;
a polypeptide consisting essentially of SEQ ID NO. 25 and 26;
a polypeptide consisting essentially of SEQ ID NO. 30 and 31;
a polypeptide consisting essentially of SEQ ID NO. 32 and 33;
a polypeptide consisting essentially of SEQ ID NO. 34 and 35;
a polypeptide consisting essentially of SEQ ID NO. 337 and 338; or
a polypeptide consisting essentially of SEQ ID NO. 339 and 340;

with the proviso that the polypeptide is none of wild-type of any one of IHF alpha, IHF beta or SEQ ID NO. 6 to 11, 28, 29, or 42 through 100.

31. A fragment or an equivalent of the isolated or recombinant polypeptide of any one of claims 24 to 30.
32. An isolated or recombinant polypeptide comprising two or more of the isolated or recombinant polypeptides of any one of claims 22 to 31.
33. An isolated or recombinant polynucleotide encoding the polypeptide of any of claims 24 to 32 or its complement.
34. A vector comprising the isolated or recombinant polynucleotide of claim 33.
35. An isolated host cell comprising the isolated or recombinant polypeptide of any one of claims 24 to 32, the isolated or recombinant polynucleotide of claim 33 or the vector of claim 34.
36. An isolated antigen presenting cell comprising the isolated or recombinant polypeptide of any one of claims 24 to 32, wherein the polypeptide is present on the surface of the cell.
37. The isolated antigen presenting cell of claim 36, wherein the antigen presenting cell is a dendritic cell.
38. An antibody or antigen binding fragment that specifically recognizes or binds the isolated or recombinant polypeptide of any one of claims 24 to 32, or an isolated or recombinant polynucleotide encoding said antibody or antigen binding fragment.
39. The antibody of claim 34, wherein the antibody is selected from the group of a polyclonal antibody, a monoclonal antibody, a humanized antibody, a human antibody, an

antibody derivative, a veneered antibody, a diabody, an antibody derivative, a recombinant human antibody, a chimeric antibody, or an antibody fragment.

40. The antibody of claim 39, wherein the antibody is a monoclonal antibody.
41. A hybridoma cell line that produces the antibody of claim 40.
42. A composition comprising a carrier and one or more of:
 - (a) an isolated or recombinant polypeptide of any one of claims 24 to 32;
 - (b) an isolated or recombinant polynucleotide of claim 33;
 - (c) a vector of claim 34;
 - (d) an isolated host cell of any one of claims 35 to 37; or
 - (e) an antibody of any one of claims 38 to 40.
43. The composition of claim 42, further comprising one or more of an adjuvant, an antigenic peptide or an antimicrobial.
44. The composition of claim 42 or 43, wherein the carrier is selected from the group of a liquid carrier, a pharmaceutically acceptable carrier, a solid phase carrier, a pharmaceutically acceptable polymer, a liposome, a micelle, an implant, a stent, a paste, a gel, a dental implant or a medical implant.
45. The composition of any one of claims 42 to 44, formulated for administration to a human patient or a non-human animal.
46. The composition of any one of claims 42 to 44, formulated for administration by one or more method comprising topically, transdermally, transcutaneously, sublingually, rectally, vaginally, ocularly, subcutaneous, intramuscularly, intraperitoneally, urethrally, intranasally, by inhalation or orally.
47. The composition of any one of claims 42 to 46, formulated for a pediatric patient.
48. A kit comprising any one or more agent of the group
 - (a) an isolated or recombinant polypeptide of any one of claims 24 to 32;
 - (b) an isolated or recombinant polynucleotide of claim 33;
 - (c) a vector of claim 34;
 - (d) an isolated host cell of any one of claims 35 to 37;

- (e) an antibody of any one of claims 38 to 40;
 - (f) the composition of claim 42;
 - (g) an isolated or recombinant integration host factor (IHF) polypeptide or a fragment or an equivalent of each thereof;
 - (h) an isolated or recombinant histone-like protein from *E.coli* strain U93 (HU) polypeptide or a fragment or an equivalent of each thereof;
 - (i) an isolated or recombinant protein polypeptide identified in Table 8, Table 9A, Table 9B, Table 10 a DNA binding peptide identified in Figure 6, or a fragment or an equivalent of each thereof;
 - (j) an isolated or recombinant polypeptide of SEQ ID NO. 1 through 340, or a fragment or an equivalent of each thereof;
 - (k) an isolated or recombinant C-terminal polypeptide of SEQ ID NO. 6 through 11, 28, 29, 42 through 100, Table 8 or those C-terminal polypeptides identified in Table 10 or a fragment or an equivalent of each thereof;
 - (l) a polypeptide that competes with an integration host factor on binding to a microbial DNA;
 - (m) a four-way junction polynucleotide resembling a Holliday junction, a 3 way junction polynucleotide resembling a replication fork, a polynucleotide that has inherent flexibility or bent polynucleotide;
 - (n) an isolated or recombinant polynucleotide encoding any one of (g) through (l);
 - (o) an antibody or antigen binding fragment that specifically recognizes or binds any one of (g) through (l), or an equivalent or fragment thereof;
 - (p) an isolated or recombinant polynucleotide encoding the antibody or antigen binding fragment of (o); and
 - (q) a small molecule that competes with the binding of a DNABII protein or polypeptide to a microbial DNA, and instructions for use.
49. The kit of claim 48, further comprising one or more of an adjuvant, an antigenic peptide, a DNase, an antibody, or an antimicrobial.

50. The kit of claim 48 or 49, further comprising a carrier selected from the group of wherein the carrier is selected from the group of a liquid carrier, a pharmaceutically acceptable carrier, a solid phase carrier, a pharmaceutically acceptable polymer, a liposome, a micelle, an implant, a stent, a paste, a gel, a dental implant, or a medical implant.
51. A method of identifying an agent that modulates the binding of a DNABII protein or polypeptide to a microbial DNA in a sample containing the same, comprising contacting a candidate agent with the sample, and assaying for the binding of the protein or polypeptide to the DNA.
52. The method of claim 51, wherein the agent modulates the binding by inhibiting, preventing or titrating the binding of the DNABII protein or polypeptide to the microbial DNA.
53. Use of an agent of the group of:
- (a) an isolated or recombinant polypeptide of any one of claims 24 to 32;
 - (b) an isolated or recombinant polynucleotide of claim 33;
 - (c) a vector of claim 34;
 - (d) an isolated host cell of any one of claims 35 to 37;
 - (e) an antibody of any one of claims 38 to 40;
 - (f) the composition of claim 42;
 - (g) an isolated or recombinant integration host factor (IHF) polypeptide or a fragment or an equivalent of each thereof;
 - (h) an isolated or recombinant histone-like protein from *E.coli* strain U93 (HU) polypeptide or a fragment or an equivalent of each thereof;
 - (i) an isolated or recombinant protein polypeptide identified in Table 8, Table 9A, Table 9B, Table 10, a DNA binding peptide identified in Figure 6, or a fragment or an equivalent of each thereof;
 - (j) an isolated or recombinant polypeptide of SEQ ID NO. 1 through 340 or a fragment or an equivalent of each thereof;

(k) an isolated or recombinant C-terminal polypeptide of SEQ ID NO. 6 through 11, 28, 29, 42 through 100, Table 8 or those C-terminal polypeptides identified in Table 10 or a fragment or an equivalent of each thereof;

(l) a polypeptide that competes with an integration host factor on binding to a microbial DNA;

(m) a four-way junction polynucleotide resembling a Holliday junction, a 3 way junction polynucleotide resembling a replication fork, a polynucleotide that has inherent flexibility or bent polynucleotide;

(n) an isolated or recombinant polynucleotide encoding any one of (g) through (l);

(o) an antibody or antigen binding fragment that specifically recognizes or binds any one of (g) through (l), or an equivalent or fragment thereof; or

(p) an isolated or recombinant polynucleotide encoding the antibody or antigen binding fragment of (o); and

(q) a small molecule that competes with the binding of a DNABII protein or polypeptide to a microbial DNA.

54. The method of claims 6 or 15, wherein the antibody is a monoclonal antibody or fragment thereof.

55. The method of claim 54, wherein the monoclonal antibody or fragment thereof is a human antibody, a humanized antibody, a recombinant antibody, a chimeric antibody, a veneered antibody, a modified antibody or an antibody derivative.

56. The method of claim 55, wherein the antibody is modified by conservative amino acid mutations within the VH and/or VL CDR 1, CDR 2 and/or CDR 3 regions.

57. The method of claim 55, wherein the antibody is modified by altering the number of cysteine residues or by conservative amino acid mutations in the Fc hinge region.

58. The method of claim 55, wherein the monoclonal antibody or fragment thereof is chemically modified.

59. The method of claim 58, wherein the monoclonal antibody or fragment thereof is pegylated.

60. The method of claim 58, wherein the monoclonal antibody or fragment thereof is conjugated to serum protein.

61. The method of claim 60, wherein the serum protein is human serum albumin.
62. The method of claim 55, wherein the monoclonal antibody or fragment thereof is conjugated to a diagnostic agent.
63. The method of claim 62, wherein the diagnostic agent is selected from the group consisting of an enzyme, a prosthetic group complex, a fluorescent material, a luminescent material, a bioluminescent material, a radioactive material, a positron emitting metal and a nonradioactive paramagnetic metal ion.
64. The method of claim 63, wherein the enzyme is selected from the group consisting of horseradish peroxidase, alkaline phosphatase, beta-galactosidase and acetylcholinesterase.
65. The method of claim 63, wherein the prosthetic group complex is selected from the group consisting of streptavidin/biotin and avidin/biotin.
66. The method of claim 63, wherein the fluorescent material is selected from the group consisting of umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride and phycoerythrin.
67. The method of claim 63, wherein the luminescent material is luminol.
68. The method of claim 63, wherein the bioluminescent material is selected from the group consisting of luciferase, luciferin and aequorin.
69. The method of claim 63, wherein the radioactive material is selected from the group consisting of ^{125}I , ^{131}I , Indium-111, Lutetium-171, Bismuth-212, Bismuth-213, Astatine-211, Copper-62, Copper-64, Copper-67, Yttrium-90, Iodine-125, Iodine-131, Phosphorus-32, Phosphorus-33, Scandium-47, Silver-111, Gallium-67, Praseodymium-142, Samarium-153, Terbium-161, Dysprosium-166, Holmium-166, Rhenium-186, Rhenium-188, Rhenium-189, Lead-212, Radium-223, Actinium-225, Iron-59, Selenium-75, Arsenic-77, Strontium-89, Molybdenum-99, Rhodium-105, Palladium-109, Praseodymium-143, Promethium-149, Erbium-169, Iridium-194, Gold-198, Gold-199 and Lead-211.
70. The method of claim 55, wherein the monoclonal antibody or fragment thereof is conjugated to a therapeutic agent.
71. The method of claim 70, wherein the therapeutic agent is an antimicrobial.
72. The method of claim 70, wherein the therapeutic agent is selected from the group consisting of ribonuclease (RNase), DNase I, an antisense nucleic acid, an inhibitory RNA

molecule, an immunostimulatory nucleic acid, an aptamer, a ribozyme, a triplex forming molecule and an external guide sequence.

73. The method of claim 55, wherein the monoclonal antibody or fragment thereof is conjugated or fused to at least one additional functional molecule.

74. The method of claim 73, wherein the additional functional molecule is a second antibody.

75. The antibody of any one of claims 38-40, wherein the antibody is a monoclonal antibody or fragment thereof.

76. The antibody of claim 75, wherein the monoclonal antibody or fragment thereof is a human antibody, a humanized antibody, a recombinant antibody, a chimeric antibody, a veneered antibody, a modified antibody or an antibody derivative.

77. The antibody of claim 76, wherein the antibody is modified by conservative amino acid mutations within the VH and/or VL CDR 1, CDR 2 and/or CDR 3 regions.

78. The antibody of claim 76, wherein the antibody is modified by altering the number of cysteine residues or by conservative amino acid mutations in the Fc hinge region.

79. The antibody of claim 76, wherein the monoclonal antibody or fragment thereof is chemically modified.

80. The antibody of claim 79, wherein the monoclonal antibody or fragment thereof is pegylated.

81. The antibody of claim 79, wherein the monoclonal antibody or fragment thereof is conjugated to serum protein.

82. The antibody of claim 81, wherein the serum protein is human serum albumin.

83. The antibody of claim 76, wherein the monoclonal antibody or fragment thereof is conjugated to a diagnostic agent.

84. The antibody of claim 83, wherein the diagnostic agent is selected from the group consisting of an enzyme, a prosthetic group complex, a fluorescent material, a luminescent material, a bioluminescent material, a radioactive material, a positron emitting metal and a nonradioactive paramagnetic metal ion.

85. The antibody of claim 84, wherein the enzyme is selected from the group consisting of horseradish peroxidase, alkaline phosphatase, beta-galactosidase and acetylcholinesterase.

86. The antibody of claim 84, wherein the prosthetic group complex is selected from the group consisting of streptavidin/biotin and avidin/biotin.
87. The antibody of claim 84, wherein the fluorescent material is selected from the group consisting of umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride and phycoerythrin.
88. The antibody of claim 84, wherein the luminescent material is luminol.
89. The antibody of claim 84, wherein the bioluminescent material is selected from the group consisting of luciferase, luciferin and aequorin.
90. The antibody of claim 84, wherein the radioactive material is selected from the group consisting of ^{125}I , ^{131}I , Indium-111, Lutetium-171, Bismuth-212, Bismuth-213, Astatine-211, Copper-62, Copper-64, Copper-67, Yttrium-90, Iodine-125, Iodine-131, Phosphorus-32, Phosphorus-33, Scandium-47, Silver-111, Gallium-67, Praseodymium-142, Samarium-153, Terbium-161, Dysprosium-166, Holmium-166, Rhenium-186, Rhenium-188, Rhenium-189, Lead-212, Radium-223, Actinium-225, Iron-59, Selenium-75, Arsenic-77, Strontium-89, Molybdenum-99, Rhodium-105, Palladium-109, Praseodymium-143, Promethium-149, Erbium-169, Iridium-194, Gold-198, Gold-199 and Lead-211.
91. The antibody of claim 76, wherein the monoclonal antibody or fragment thereof is conjugated to a therapeutic agent.
92. The antibody of claim 91, wherein the therapeutic agent is an antimicrobial.
93. The antibody of claim 91, wherein the therapeutic agent is selected from the group consisting of ribonuclease (RNase), DNase I, an antisense nucleic acid, an inhibitory RNA molecule, an immunostimulatory nucleic acid, an aptamer, a ribozyme, a triplex forming molecule and an external guide sequence.
94. The antibody of claim 76, wherein the monoclonal antibody or fragment thereof is conjugated or fused to at least one additional functional molecule.
95. The antibody of claim 94, wherein the additional functional molecule is a second antibody.
96. The kit of claim 48, wherein the antibody is a monoclonal antibody or fragment thereof.

97. The kit of claim 96, wherein the monoclonal antibody or fragment thereof is a human antibody, a humanized antibody, a recombinant antibody, a chimeric antibody, a veneered antibody, a modified antibody or an antibody derivative.
98. The kit of claim 97, wherein the antibody is modified by conservative amino acid mutations within the VH and/or VL CDR 1, CDR 2 and/or CDR 3 regions.
99. The kit of claim 97, wherein the antibody is modified by altering the number of cysteine residues or by conservative amino acid mutations in the Fc hinge region.
100. The kit of claim 97, wherein the monoclonal antibody or fragment thereof is chemically modified.
101. The kit of claim 100, wherein the monoclonal antibody or fragment thereof is pegylated.
102. The kit of claim 100, wherein the monoclonal antibody or fragment thereof is conjugated to serum protein.
103. The kit of claim 102, wherein the serum protein is human serum albumin.
104. The kit of claim 97, wherein the monoclonal antibody or fragment thereof is conjugated to a diagnostic agent.
105. The kit of claim 104, wherein the diagnostic agent is selected from the group consisting of an enzyme, a prosthetic group complex, a fluorescent material, a luminescent material, a bioluminescent material, a radioactive material, a positron emitting metal and a nonradioactive paramagnetic metal ion.
106. The kit of claim 105, wherein the enzyme is selected from the group consisting of horseradish peroxidase, alkaline phosphatase, beta-galactosidase and acetylcholinesterase.
107. The kit of claim 105, wherein the prosthetic group complex is selected from the group consisting of streptavidin/biotin and avidin/biotin.
108. The kit of claim 105, wherein the fluorescent material is selected from the group consisting of umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride and phycoerythrin.
109. The kit of claim 105, wherein the luminescent material is luminol.
110. The kit of claim 105, wherein the bioluminescent material is selected from the group consisting of luciferase, luciferin and aequorin.

111. The kit of claim 105, wherein the radioactive material is selected from the group consisting of ^{125}I , ^{131}I , Indium-111, Lutetium-171, Bismuth-212, Bismuth-213, Astatine-211, Copper-62, Copper-64, Copper-67, Yttrium-90, Iodine-125, Iodine-131, Phosphorus-32, Phosphorus-33, Scandium-47, Silver-111, Gallium-67, Praseodymium-142, Samarium-153, Terbium-161, Dysprosium-166, Holmium-166, Rhenium-186, Rhenium-188, Rhenium-189, Lead-212, Radium-223, Actinium-225, Iron-59, Selenium-75, Arsenic-77, Strontium-89, Molybdenum-99, Rhodium-105, Palladium-109, Praseodymium-143, Promethium-149, Erbium-169, Iridium-194, Gold-198, Gold-199 and Lead-211.

112. The kit of claim 97, wherein the monoclonal antibody or fragment thereof is conjugated to a therapeutic agent.

113. The kit of claim 112, wherein the therapeutic agent is an antimicrobial.

114. The kit of claim 97, wherein the monoclonal antibody or fragment thereof is conjugated or fused to at least one additional functional molecule..

115. The kit of claim 114, wherein the additional functional molecule is a second antibody.

116. The use of claim 53 to vaccinate a subject in need thereof, wherein the antibody is a monoclonal antibody or fragment thereof.

117. The use of claim 116, wherein the monoclonal antibody or fragment thereof is a human antibody, a humanized antibody, a recombinant antibody, a chimeric antibody, a veneered antibody, a modified antibody or an antibody derivative.

118. The use of claim 117, wherein the antibody is modified by conservative amino acid mutations within the VH and/or VL CDR 1, CDR 2 and/or CDR 3 regions.

119. The use of claim 117, wherein the antibody is modified by altering the number of cysteine residues or by conservative amino acid mutations in the Fc hinge region.

120. The use of claim 117, wherein the monoclonal antibody or fragment thereof is chemically modified.

121. The use of claim 120, wherein the monoclonal antibody or fragment thereof is pegylated.

122. The use of claim 120, wherein the monoclonal antibody or fragment thereof is conjugated to serum protein.

123. The use of claim 122, wherein the serum protein is human serum albumin.

124. The use of claim 117, wherein the monoclonal antibody or fragment thereof is conjugated to a diagnostic agent.
125. The use of claim 124, wherein the diagnostic agent is selected from the group consisting of an enzyme, a prosthetic group complex, a fluorescent material, a luminescent material, a bioluminescent material, a radioactive material, a positron emitting metal and a nonradioactive paramagnetic metal ion.
126. The use of claim 125, wherein the enzyme is selected from the group consisting of horseradish peroxidase, alkaline phosphatase, beta-galactosidase and acetylcholinesterase.
127. The use of claim 125, wherein the prosthetic group complex is selected from the group consisting of streptavidin/biotin and avidin/biotin.
128. The use of claim 125, wherein the fluorescent material is selected from the group consisting of umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride and phycoerythrin.
129. The use of claim 125, wherein the luminescent material is luminol.
130. The use of claim 125, wherein the bioluminescent material is selected from the group consisting of luciferase, luciferin and aequorin.
131. The use of claim 125, wherein the radioactive material is selected from the group consisting of ^{125}I , ^{131}I , Indium-111, Lutetium-171, Bismuth-212, Bismuth-213, Astatine-211, Copper-62, Copper-64, Copper-67, Yttrium-90, Iodine-125, Iodine-131, Phosphorus-32, Phosphorus-33, Scandium-47, Silver-111, Gallium-67, Praseodymium-142, Samarium-153, Terbium-161, Dysprosium-166, Holmium-166, Rhenium-186, Rhenium-188, Rhenium-189, Lead-212, Radium-223, Actinium-225, Iron-59, Selenium-75, Arsenic-77, Strontium-89, Molybdenum-99, Rhodium-105, Palladium-109, Praseodymium-143, Promethium-149, Erbium-169, Iridium-194, Gold-198, Gold-199 and Lead-211.
132. The use of claim 117, wherein the monoclonal antibody or fragment thereof is conjugated to a therapeutic agent.
133. The use of claim 132, wherein the therapeutic agent is an antimicrobial.
134. The use of claim 132, wherein the therapeutic agent is selected from the group consisting of ribonuclease (RNase), DNase I, an antisense nucleic acid, an inhibitory RNA molecule, an immunostimulatory nucleic acid, an aptamer, a ribozyme, a triplex forming molecule and an external guide sequence.

135. The use of claim 117, wherein the monoclonal antibody or fragment thereof is conjugated or fused to at least one additional functional molecule.

136. The use of claim 135, wherein the additional functional molecule is a second antibody.

137. The method of claims 1-23, claims 51-52 or claims 54-74, the isolated or recombinant polypeptide of claims 24-33, the vector of claim 34, the isolated cell of claims 35-37, the antibody of claims 38-40 or 75-95, the hybridoma cell line of claim 41, the composition of claims 42-47, the kit of claims 48-50, or claims 96-115, the use of claims 53 or claims 116-136, wherein an equivalent of a recombinant polynucleotide comprises a polynucleotide that hybridizes under stringent conditions to the polynucleotide or its complement, wherein the stringent conditions comprise an incubation temperatures from about 25°C to about 37°C; hybridization buffer concentrations from about 6x SSC to about 10x SSC; formamide concentrations from about 0% to about 25%; and wash solutions of about 4x SSC to 8x SSC.

138. A computer-implemented method for identifying an agent that inhibits, competes or titrates the binding of a DNABII polypeptide or protein to a microbial DNA, that inhibits, prevents or breaks down a microbial biofilm, that inhibits, prevents or breaks down a biofilm in a subject, or that inhibits, prevents or treats a microbial infection that produces a biofilm in a subject, comprising positioning a three-dimensional structure of a candidate agent against a three-dimensional structure of an integration host factor (IHF) protein, wherein the three-dimensional structure of the IHF protein is based on X, Y and Z atomic structure coordinates determined from a crystalline form of an IHF and DNA complex, wherein interaction of the agent with the IHF at two or more IHF amino acids selected from T4, K5, A6, E28, Q43, K45, S47, G48, N51, R55, K57, R60, R63, N64, P65, K66, R76, T80, R82 or Q85 as represented in SEQ ID NO: 42, or the equivalent of each, identifies that the agent inhibits, competes or titrates the binding of a DNABII polypeptide or protein to a microbial DNA, inhibits, prevents or breaks down a microbial biofilm, inhibits, prevents or breaks down a biofilm, or inhibits, prevents or treats a microbial infection that produces a biofilm.

139. The method of claim 138, wherein interaction of the agent with the IHF at three or more IHF amino acids selected from T4, K5, A6, E28, Q43, K45, S47, G48, N51, R55, K57, R60, R63, N64, P65, K66, R76, T80, R82 or Q85 as represented in SEQ ID NO: 42, or the equivalent of each, identifies that the agent inhibits, competes or titrates the binding of a DNABII polypeptide or protein to a microbial DNA, inhibits, prevents or breaks down a

microbial biofilm, inhibits, prevents or breaks down a biofilm, or inhibits, prevents or treats a microbial infection that produces a biofilm.

140. The method of claim 138 or 139, wherein the X, Y and Z atomic structure coordinates comprise the coordinates as set forth in Protein Data Bank (PDB) Accession No.: 1IHF.

141. A custom computing apparatus comprising:

at least one processor;

a memory coupled to the at least one processor;

a storage medium in communication with the memory and the at least one processor, the storage medium containing a set of processor executable instructions that, when executed by the processor configure the custom computing apparatus to identify an agent that inhibits, competes or titrates the binding of a DNABII polypeptide or protein to a microbial DNA, that inhibits, prevents or breaks down a microbial biofilm, that inhibits, prevents or breaks down a biofilm in a subject, or that inhibits, prevents or treats a microbial infection that produces a biofilm in a subject, wherein the configuration comprises:

positioning a three-dimensional structure of a candidate agent against a three-dimensional structure of an integration host factor (IHF) protein, wherein the three-dimensional structure of the IHF protein is based on X, Y and Z atomic structure coordinates determined from a crystalline form of an IHF and DNA complex, wherein interaction of the agent with the IHF at two or more IHF amino acids selected from T4, K5, A6, E28, Q43, K45, S47, G48, N51, R55, K57, R60, R63, N64, P65, K66, R76, T80, R82 or Q85 as represented in SEQ ID NO: 42, or the equivalent of each, identifies that the agent inhibits, competes or titrates the binding of a DNABII polypeptide or protein to a microbial DNA, inhibits, prevents or breaks down a microbial biofilm, inhibits, prevents or breaks down a biofilm, or inhibits, prevents or treats a microbial infection that produces a biofilm.

142. A method of identifying an agent that modulates the binding of a DNABII protein or polypeptide to a microbial DNA in a sample containing the same, comprising contacting a candidate agent with the sample, and assaying for the binding of the protein or polypeptide to the DNA, wherein the agent modulates the binding by inhibiting, preventing or titrating the binding of the DNABII protein or polypeptide to the microbial DNA.

143. A method for identifying a patient having a microbial infection that is likely or is not likely to be treated by the administration of an interfering agent, comprising detecting the presence or absence of a DNABII polypeptide or protein in sample isolated from the patient

and containing the infection, wherein the presence of the DNABII polypeptide or protein identifies the patient as likely to be treated by the administration of the interfering agent and the absence of a DNABII polypeptide or protein identifies the patient as not likely to be treated by the administration of the interfering agent.

144. The method of claim 143, wherein the presence of the DNABII polypeptide or protein identifies that patient as likely to be treated by the administration of the interfering agent.

145. The method of claim 144, further comprising administering an effective amount of the interfering agent to the patient.

146. The method of claim 145, wherein the interfering agent is one or more of:

(a) an isolated or recombinant integration host factor (IHF) polypeptide or a fragment or an equivalent of each thereof;

(b) an isolated or recombinant histone-like protein from *E.coli* strain U93 (HU) polypeptide or a fragment or an equivalent of each thereof;

(c) an isolated or recombinant protein or polypeptide identified in Table 8, Table 9A, Table 9B, Table 10 or a DNA binding peptide identified in Figure 6, or a fragment or an equivalent of each thereof;

(d) an isolated or recombinant polypeptide of SEQ ID NO. 1 through 340, or an equivalent of each thereof, or a fragment or an equivalent of each fragment thereof;

(e) an isolated or recombinant C-terminal polypeptide of SEQ ID NO. 6 through 11, 28, 29, 42 through 100, Table 8 or those C-terminal polypeptides identified in Table 10 or a fragment or an equivalent of each thereof;

(f) a polypeptide or polynucleotide that competes with an integration host factor on binding to a microbial DNA;

(g) a four-way junction polynucleotide resembling a Holliday junction, a 3 way junction polynucleotide resembling a replication fork, a polynucleotide that has inherent flexibility or bent polynucleotide;

(h) an isolated or recombinant polynucleotide encoding any one of (a) through (f) or an isolated or recombinant polynucleotide of SEQ ID NO. 36 or an equivalent of each thereof, or a polynucleotide that hybridizes under stringent conditions to the polynucleotide or its complement;

(i) an antibody or antigen binding fragment that specifically recognizes or binds any one of (a) through (f), or an equivalent or fragment of each antibody or antigen binding fragment thereof;

(j) an isolated or recombinant polynucleotide encoding the antibody or antigen binding fragment of (i); or

(k) a small molecule that competes with the binding of a DNABII protein or polypeptide to a microbial DNA.