US 20230416311A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2023/0416311 A1**

YOUNG et al. (43) **Pub. Date: Dec. 28, 2023**

(54) **INFLUENZA VACCINES**

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(21) Appl. No.: **18/344,909**

(22) Filed: **Jun. 30, 2023**

Related U.S. Application Data

(63) Continuation of application No. 17/375,797, filed on Jul. 14, 2021, now Pat. No. 11,739,127, which is a continuation of application No. 15/857,436, filed on Dec. 28, 2017, now Pat. No. 11,111,277.

(60) Provisional application No. 62/439,865, filed on Dec. 28, 2016, provisional application No. 62/550,167, filed on Aug. 25, 2017.

Publication Classification

(51) **Int. Cl.**

C07K 14/11	(2006.01)
C07K 14/075	(2006.01)
A61P 31/16	(2006.01)
A61K 39/145	(2006.01)
A61K 39/12	(2006.01)
C07K 14/005	(2006.01)
C12N 15/86	(2006.01)

(52) **U.S. Cl.**

CPC ***C07K 14/11*** (2013.01); ***C07K 14/075*** (2013.01); ***A61P 31/16*** (2018.01); ***A61K 39/145*** (2013.01); ***A61K 39/12*** (2013.01); ***C07K 14/005*** (2013.01); ***C12N 15/86*** (2013.01); *A61K 2039/57* (2013.01)

(57) **ABSTRACT**

Provided herein are compositions related to vaccines, e.g., influenza vaccines, including, peptide based vaccines, nucleic acid based vaccines, recombinant virus based vaccines, antibody based vaccines, and virus based vaccines. Also provided herein are methods related to vaccines, e.g., influenza vaccines, including methods of identifying epitopes for the vaccines, producing, formulating, and administering the vaccines

Specification includes a Sequence Listing.

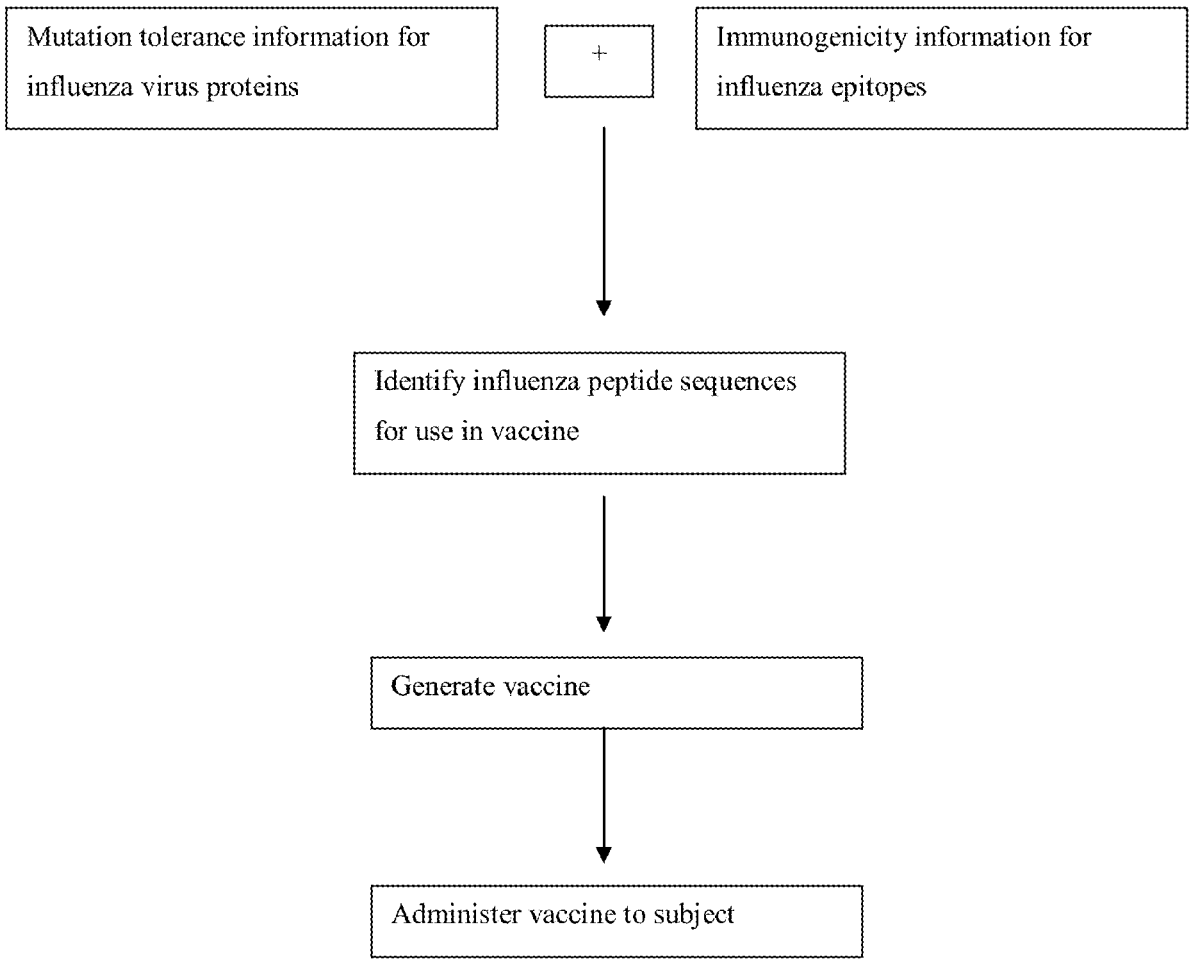


Fig. 1

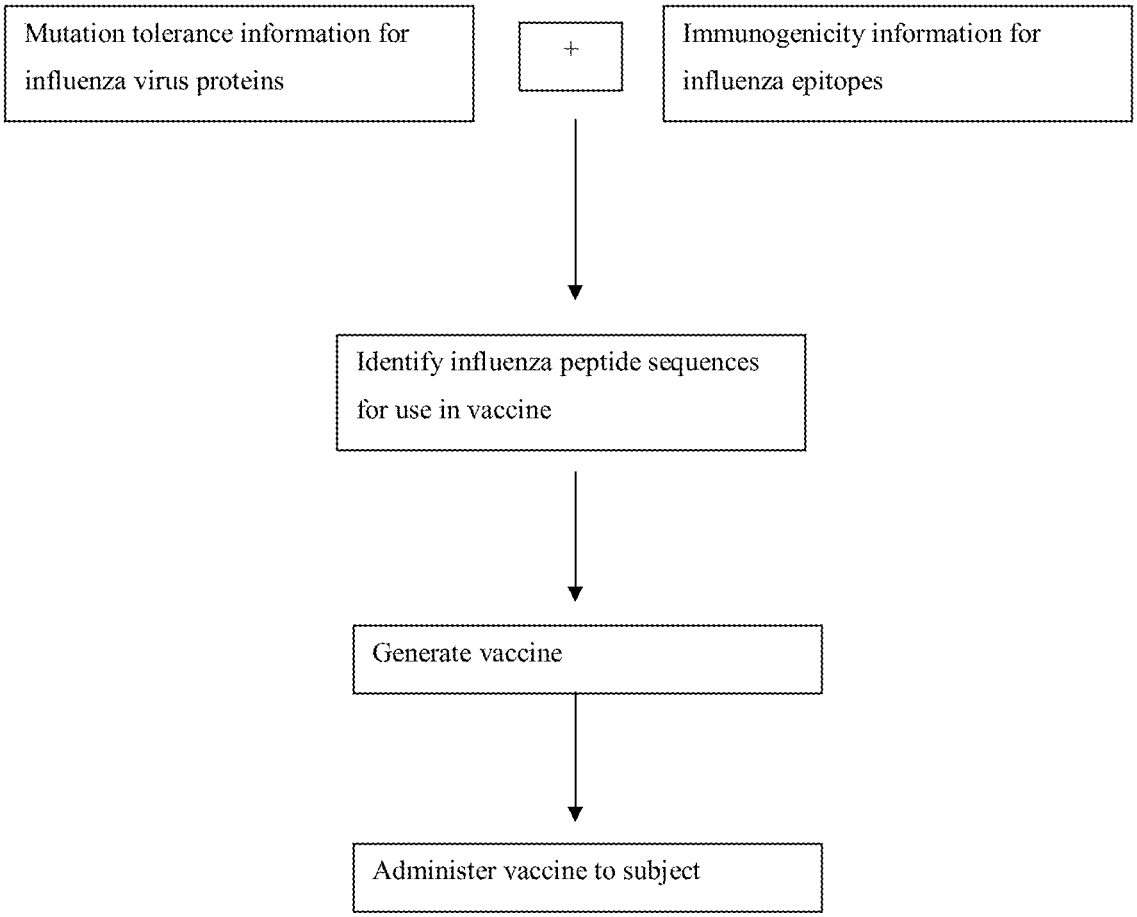

Mutation tolerance information for influenza virus proteins
+
Immunogenicity information for influenza epitopes
Identify influenza peptide sequences for use in vaccine
Generate vaccine
Administer vaccine to subject

Fig. 2

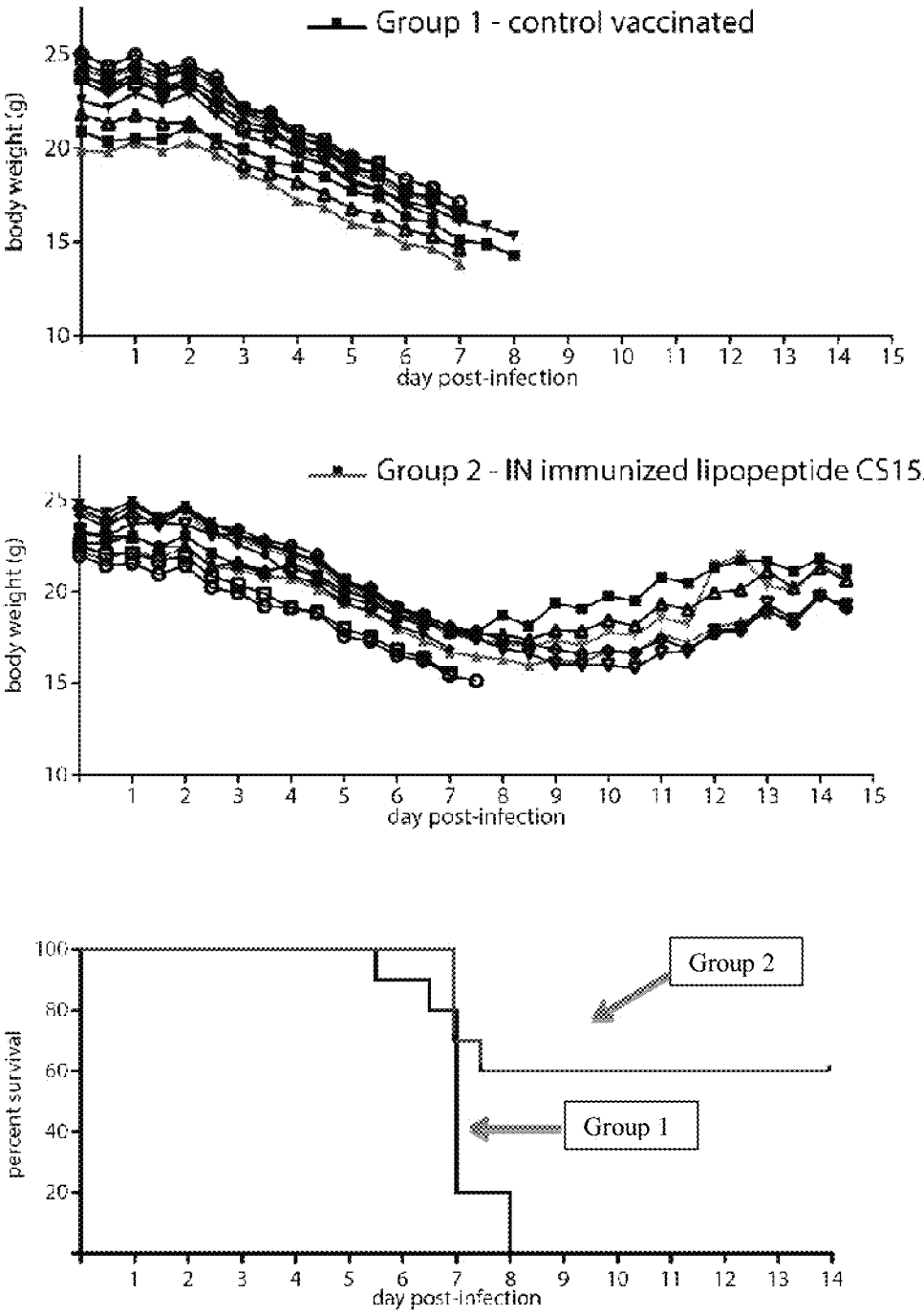

Group 1 - control vaccinated
body weight (g)
day post-infection
Group 2 - IN immunized lipopeptide CS15226
body weight (g)
day post-infection
percent survival
Group 2
Group 1
day post-infection

Fig. 3A

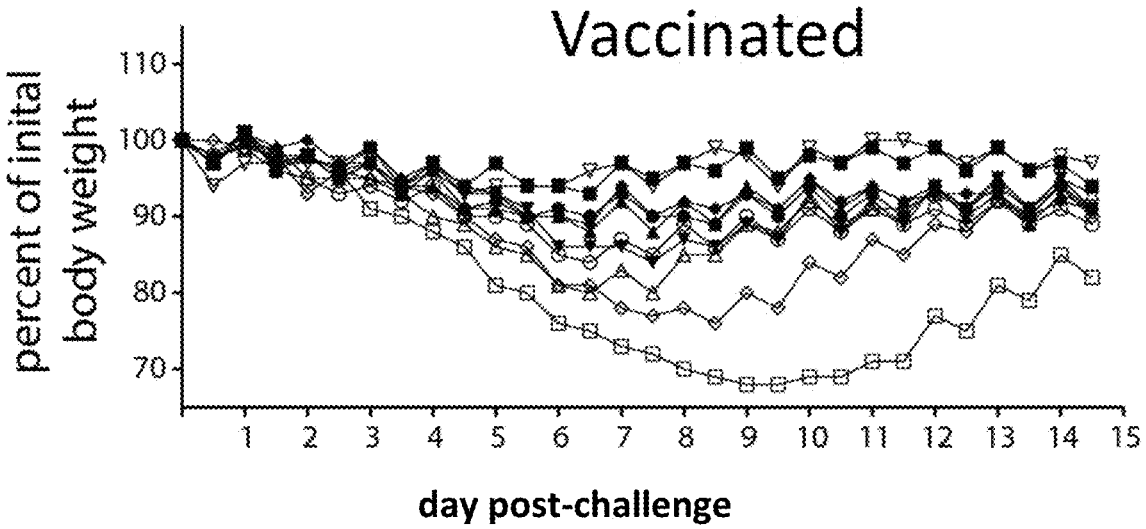

Vaccinated
percent of inital body weight
110
100
90
80
70
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
day post-challenge

Fig. 3B

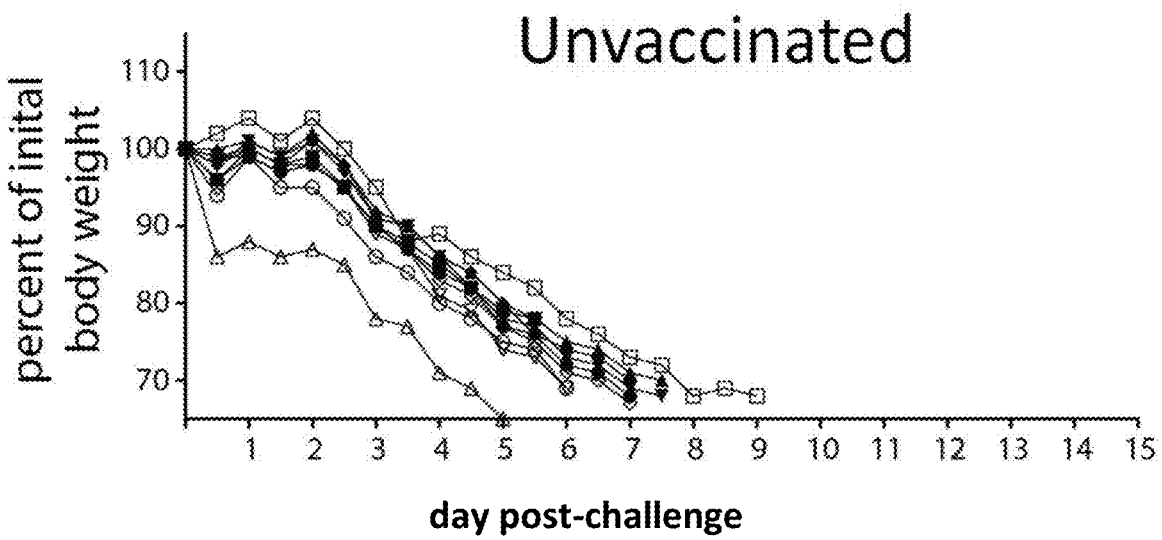

Unvaccinated
percent of inital body weight
110
100
90
80
70
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
day post-challenge

Fig. 3C

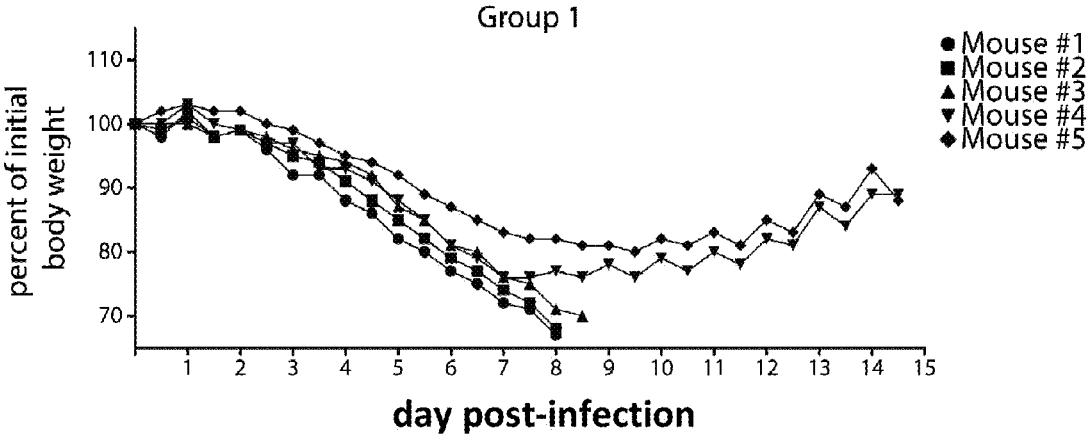

Group 1
percent of initial body weight
110
100
90
80
70
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
day post-infection
Mouse #1
Mouse #2
Mouse #3
Mouse #4
Mouse #5

Fig. 3D

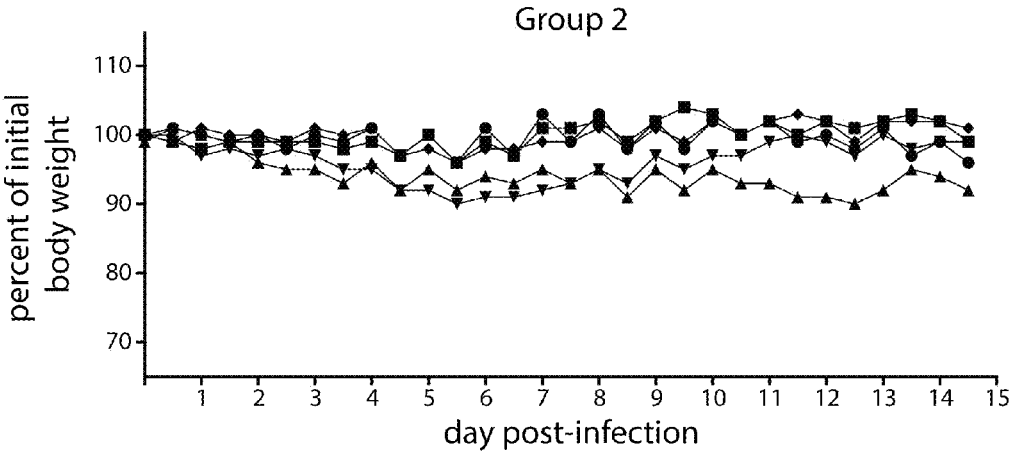

Group 2
percent of initial body weight
110
100
90
80
70
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
day post-infection

Fig. 4

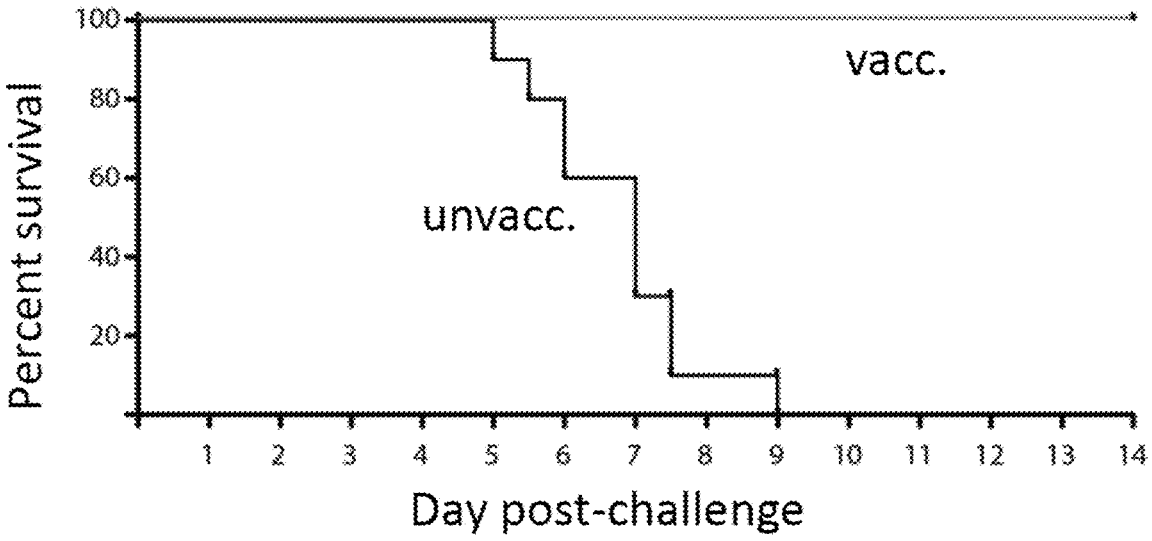
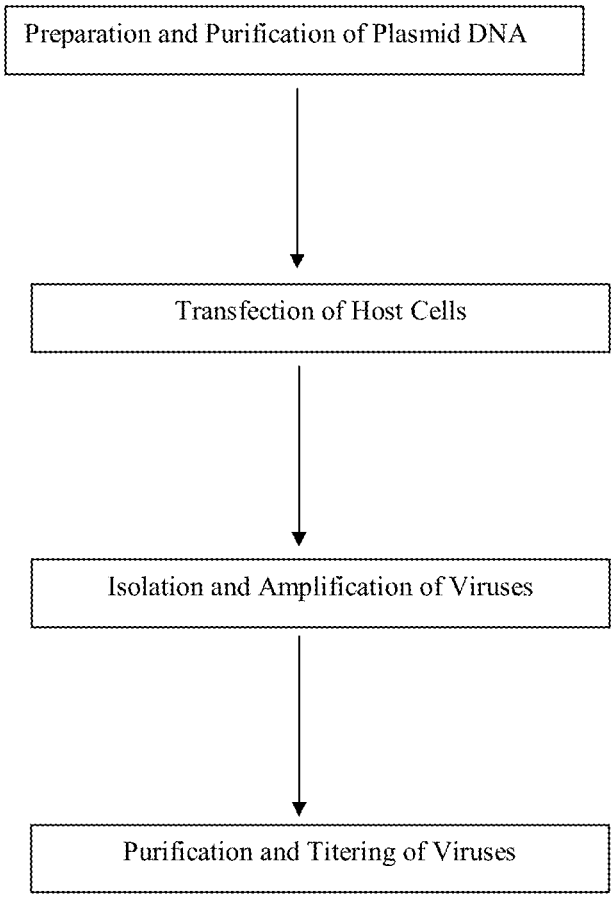

Percent survival
100
80
60
40
20
vacc.
unvacc.
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Day post-challenge

Fig. 5


Preparation and Purification of Plasmid DNA
Transfection of Host Cells
Isolation and Amplification of Viruses
Purification and Titering of Viruses

INFLUENZA VACCINES

CROSS-REFERENCE

[0001] This application is a continuation of U.S. patent application Ser. No. 17/375,797, filed on Jul. 14, 2021, which is a continuation of U.S. patent application Ser. No. 15/857,436, filed on Dec. 28, 2017, which issued as U.S. Pat. No. 11,111,277 on Sep. 7, 2021, which claims the benefit of U.S. provisional application No. 62/439,865, filed Dec. 28, 2016, and U.S. provisional application No. 62/550,167, filed Aug. 25, 2017, which are herein incorporated by reference in their entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing XML which has been submitted electronically in XML file is hereby incorporated by reference in its entirety. Said XML copy, created on Jun. 23, 2023, is named US001CON797_SL.xml and is 10,977,503 bytes in size.

[0003] The instant application contains a Sequence Listing which has been submitted electronically in ASCII text file and PDF file is hereby incorporated by reference in its entirety. Sequence Listing content of the PDF copy and the ASCII text file copy electronically are identical to each other and the Sequence Listing XML. Said ASCII copy, created on Jun. 24, 2023, is named US001CON797_SL.txt and is 152,120 bytes in size. Said PDF copy, created on Jun. 24, 2023, is named US001CON797_SL.pdf and is 173,462 bytes in size.

BACKGROUND

[0004] Vaccines greatly promote human health by providing active adapted immunity to a particular disease. There is a need for improved vaccines, e.g., influenza vaccines. Improved vaccines may exhibit higher safety, increased immunogenicity, coverage of broader range of pathogens, or any combination thereof.

SUMMARY

[0005] One aspect of the present disclosure provides a polypeptide that comprises a first sequence selected from the group consisting of SEQ ID NOs: 2, 3, 8, 11, 12, 40, 41, 43, 51, 52, 58, 59, 61, 62, 84, and 92; and a second sequence selected from the group consisting of SEQ ID NOs: 17, 20, 21, 22, 24, 26, 29, 30, 32, 33, 34, 44, 45, 49, 53, 60, 70, 73, 74, 75, 76, 77, 78, 82, 83, 85, 86, 87, 88, 89, 90, 91, 93, and 94.

[0006] Another aspect of the present disclosure provides a polypeptide that comprises a first sequence selected from the group consisting of SEQ ID NOs: 2, 8, 11, 12, 40, 41, 43, 52, 58, 59, 61, 62, 84, and 92; and a second sequence selected from the group consisting of SEQ ID NOs: 17, 20, 21, 22, 24, 26, 29, 30, 31, 32, 33, 34, 44, 45, 49, 53, 60, 70, 73, 74, 75, 76, 77, 78, 82, 83, 85, 86, 87, 88, 89, 90, 91, 93, and 94.

[0007] Another aspect of the present disclosure provides a polypeptide that comprises: (a) a first sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 8, 11, 12, 40, 41, 58, 59, 61, 62, 84, or 92, and a second sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 2, 3, 17, 20, 21, 22, 24, 26, 29, 30, 31, 32, 33, 34, 43, 44, 45, 49, 51, 52, 53, 60, 70, 73, 74, 75, 76, 77, 78, 82, 83, 85, 86, 87, 88, 89, 90, 91, 93, or 94; or (b) a first sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 2 or 43 and a second sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 8, 11, 12, 17, 20, 21, 22, 24, 26, 29, 30, 31, 32, 33, 34, 40, 41, 44, 45, 49, 53, 58, 59, 60, 61, 62, 70, 73, 74, 75, 76, 77, 78, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, or 94; or (c) a first sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 21, 22, 24, 26, 30, 32, 49, 53, 60, 70, 85, 86, 93, or 94, and a second sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 2, 3, 8, 11, 12, 40, 41, 43, 51, 52, 58, 59, 61, 62, 84, or 92; or (d) a first sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 17, 20, 29, 31, 33, 34, 44, 45, 73, 74, 75, 76, 77, 78, 82, 83, 87, 88, 89, 90, or 91 and a second sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 2, 8, 11, 12, 40, 41, 43, 58, 59, 61, 62, 84, or 92; or (e) a first sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 2 or 43 and a second sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 8, 11, 12, 21, 22, 24, 26, 30, 32, 40, 41, 49, 53, 58, 59, 60, 61, 62, 70, 84, 85, 86, 92, 93, or 94; or (f) a first sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 3, 51, or 52, and a second sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 8, 11, 12, 21, 22, 24, 26, 30, 32, 40, 41, 49, 53, 58, 59, 60, 61, 62, 70, 84, 85, 86, 92, 93, 94; or (g) a first sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 2, 8, 11, 12, 40, 41, 43, 58, 59, 61, 62, 84, or 92, and a second sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 21, 22, 24, 26, 30, 32, 49, 53, 60, 70, 85, 86, 93, or 94; or (h) a first sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 2, 8, 11, 12, 21, 22, 24, 26, 30, 32, 40, 41, 43, 49, 53, 58, 59, 60, 61, 62, 70, 84, 85, 86, 92, 93, or 94, and a second different sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 2, 3, 8, 11, 12, 17, 20, 21, 22, 24, 26, 29, 30, 31, 32, 33, 34, 40, 41, 43, 44, 45, 49, 51, 52, 53, 58, 59, 60, 61, 62, 70, 73, 74, 75, 76, 77, 78, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, or 94, wherein a contiguous sequence comprising the first sequence and the second different sequence is not found in a PB1, PA, or NP.

[0008] Another aspect of the present disclosure provides a polypeptide that comprises a first sequence, second sequence, third sequence, fourth sequence, and a fifth sequence, wherein each of the first sequence, second sequence, third sequence, fourth sequence, and fifth sequence comprises at least 75% sequence identity to a

different sequence selected from the group consisting of SEQ ID NOs: 2, 3, 8, 11, 12, 17, 20-22, 24, 26, 29-34, 40, 41, 43-45, 49, 51-53, 58-62, 70, 73-78, and 82-94, wherein the polypeptide is not naturally occurring.

[0009] In some cases, at least one of the first sequence, second sequence, third sequence, fourth sequence, and fifth sequence can comprise at least 75% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 17, 20-22, 24, 26, 29-34, 44, 45, 49, 53, 60, 70, 72-78, 82, 83, 85-91, 93, and 94. In some cases, at least one of the first sequence, second sequence, third sequence, fourth, and fifth sequence can comprise at least 75% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 2, 3, 43, 51, and 52. In some cases, at least one of the first sequence, second sequence, third sequence, fourth sequence, and fifth sequence can comprise at least 75% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 8, 11, 12, 40, 41, 58, 59, 61, 62, and 92.

[0010] A polypeptide provided herein can further comprise sequence with at least 50% sequence identity to at least 10% of the amino acid sequence of an NP protein of influenza B. A polypeptide provided herein can further comprise sequence with at least 50% sequence identity to at least 10% of the amino acid sequence of SEQ ID NO: 116, 117, or 118. In some cases, each sequence can be at most 10, 12, 15, 20, 25, 30, 35, 40, 45, or 50 amino acids in length. In some cases, the first sequence and second sequence can be directly linked. In some cases, the first sequence and the second sequence can be linked by a linker. In some cases, the linker can comprise a plurality of glycines, alanines, arginines, valines, or lysines. A linker can comprise the sequence RVKR (SEQ ID NO: 110). A polypeptide provided herein can further comprise sequence GALNNRFQIKGVELKSK (SEQ ID NO: 103). SEQ ID NO: 103 can be linked to an amino terminal portion of the polypeptide. A polypeptide provided herein can comprise SEQ ID NOs: 2, 3, 8, 11, 12, 17, 20, 21, 22, 24, 26, 29, 30, 31, 32, 33, 34, 40, 41, 43, 44, 45, 49, 51, 52, 53, 58, 59, 60, 61, 62, 70, 73, 74, 75, 76, 77, 78, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, and 94.

[0011] A polypeptide provided herein can be an isolated polypeptide.

[0012] Another aspect of the present disclosure provides a composition that comprises a polypeptide provided herein.

[0013] Another aspect of the present disclosure provides a polynucleotide encoding a polypeptide provided herein. A polynucleotide provided herein can be isolated.

[0014] Another aspect of the present disclosure provides a composition that comprises a polynucleotide provided herein.

[0015] Another aspect of the present disclosure provides a vector that comprises a polynucleotide provided herein. A vector provided herein can be a non-human primate vector. A vector provided herein can be an adenovirus vector. A vector provided herein can be a chimpanzee adenovirus vector. In some cases, the chimpanzee adenovirus vector can comprise at least 50% sequence identity to least 50% of the sequence of C68 (AdC68) (SEQ ID NO: 104), C7 (AdC7), C6 (AdC6) (SEQ ID NO: 105), Pan7, or Pan9.

[0016] A vector provided herein can be isolated.

[0017] Another aspect of the present disclosure provides a composition that comprises a vector provided herein.

[0018] Another aspect of the present disclosure provides a virus that comprises a polynucleotide provided herein.

[0019] Another aspect of the present disclosure provides a virus that comprises a vector provided herein.

[0020] A virus provided herein can be isolated. A virus can be an adenovirus.

[0021] Another aspect of the present disclosure provides a composition that comprises a virus provided herein.

[0022] Another aspect of the present disclosure provides a composition that comprises at least five different peptides, wherein each of the at least five different peptides comprises, consists of, or consists essentially of a sequence comprising at least 75% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 2, 3, 8, 11, 12, 17, 20-22, 24, 26, 29-34, 40, 41, 43-45, 49, 51-53, 58-62, 70, 73-78, and 82-94. In some cases of the composition, each peptide can be at most 10, 12, 15, 20, 25, 30, 35, 40, 45, or 50 amino acids in length. A composition provided herein can further comprise a peptide comprising a sequence with at least 50% sequence identity to at least 10% of the amino acid sequence of an NP protein of influenza B. A composition provided herein can further comprise a peptide comprising a sequence with at least 50% sequence identity to at least 10% of the amino acid sequence of SEQ ID NOs: 116, 117, or 118. A composition provided herein can further comprise a pharmaceutically acceptable excipient. A composition provided herein can be formulated for subcutaneous, intranasal, or intramuscular administration.

[0023] Another aspect of the present disclosure provides a method that comprises administering to a subject a composition provided herein. In some cases, the administration can be subcutaneous. In some cases, the administration can be intranasal. In some cases, the administration can be intramuscular. A method provided herein can further comprise administering the composition to the subject a second time.

[0024] In some cases, an immune response can be induced following the administration. The immune response can be a systemic immune response. In some cases, the subject can be a human. In some cases, the subject can be infected with a virus. The virus can be an influenza virus. The influenza virus can be influenza A virus, influenza B virus, or influenza C virus. In some cases, the influenza virus can be influenza A virus. In some cases, the composition when administered can induce cross-protection against one or more subtypes of influenza A strains in the subject.

INCORPORATION BY REFERENCE

[0025] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0027] FIG. **1** illustrates a method for discovering vaccine epitopes.

[0028] FIG. **2** illustrates a result of an influenza challenge of vaccinated mice.

[0029] FIG. **3**A illustrates the percent of initial body weight of vaccinated mice.

[0030] FIG. **3**B illustrates the percent of initial body weight of unvaccinated mice.

[0031] FIG. **3**C illustrates the percent of initial body weight of Group 1 (AdCre-injected) mice.

[0032] FIG. **3**D illustrates the percent of initial body weight of Group 2 vaccinated mice.

[0033] FIG. **4** illustrates percent survival of vaccinated and unvaccinated mice in FIGS. **3**A and **3**B.

[0034] FIG. **5** illustrates a method of producing an adenovirus-based vaccine provided herein.

DETAILED DESCRIPTION

[0035] Provided herein are methods and compositions for forming vaccines, e.g., influenza vaccines.

[0036] The methods can comprise, e.g., making a recombinant viral vaccine, e.g., recombinant adenoviral vaccine, using sequence encoding one or more influenza epitopes, e.g., one or more influenza A epitopes, e.g., one or more influenza A peptide epitopes. The one or more epitopes can comprise an "invariant" sequence, e.g., a sequence with a low tolerance for mutations. The one or more epitopes can comprise an experimentally verified human CD8 T cell influenza A virus epitope. The recombinant adenovirus can express at least 1, 5, 8, 10, 25, 50, 100, or 1000 peptide epitopes. In some cases, the expressed epitopes are linked, e.g., through covalent bonds, e.g., in a single polypeptide. In some cases, the expressed epitopes are not linked, e.g., each epitope can be expressed from a separate promoter, separate nucleic acid, or separate virus. In some cases, the one or more epitopes are described in the Immune Epitope Database and Analysis Resource (worldwideweb.iedb.org). A single polypeptide comprising one or more epitopes can be linked to one, two, or more copies of a full-length viral protein (or at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% of a full-length viral protein, or a sequence having at least 50%, 60%, 70%, 80%, 90%, 95%, or 100% sequence identity to such proteins) from, e.g., influenza A, influenza B, or influenza C.

Definitions

[0037] The terminology used herein is for the purpose of describing particular cases only and is not intended to be limiting. As used herein, the singular forms "a", "an" and "the" can include the plural forms as well, unless the context clearly indicates otherwise. Furthermore, to the extent that the terms "contains," "containing," "including," "includes," "having," "has," "with," or variants thereof are used in either the detailed description and/or the claims, such terms can be inclusive in a manner similar to the term "comprising."

[0038] The term "about" or "approximately" can mean within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which can depend in part on how the value is measured or determined, e.g., the limitations of the measurement system. For example, "about" can mean within 1 or more than 1 standard deviation, per the practice in the given value. Where particular values are described in the application and claims, unless otherwise stated the term "about" can mean an acceptable error range for the particular value, such as +10% of the value modified by the term "about."

[0039] The term "polypeptide" and its grammatical equivalents, as used herein, can refer to a continuous and unbranched chain of amino acid monomers linked by peptide (amide) bonds, which can be covalent chemical bonds formed when the carboxyl group of one amino acid reacts with the amino group of another. Unless indicated otherwise, the terms "polypeptide" and "peptide" are interchangeable as used herein. The shortest polypeptide can be dipeptide with only two amino acids joined by a single peptide bond. For the purposes of the present disclosure, these terms should not to be construed as limiting with respect to an upper length. The terms can also encompass analogues of natural amino acids, as well as amino acids that are modified in the side chain, chirality, or properties.

[0040] The terms "nucleic acid," "polynucleotide," "polynucleic acid," and "oligonucleotide" and their grammatical equivalents can be used interchangeably and can refer to a deoxyribonucleotide or ribonucleotide polymer, in linear or circular conformation, and in either single- or double-stranded form. For the purposes of the present disclosure, these terms should not to be construed as limiting with respect to an upper length. The terms can also encompass analogues of natural nucleotides, as well as nucleotides that are modified in the base, sugar and/or phosphate moieties (e.g., phosphorothioate backbones). Modifications of the terms can also encompass demethylation, addition of CpG methylation, removal of bacterial methylation, and/or addition of mammalian methylation. In general, an analogue of a particular nucleotide can have the same base-pairing specificity, i.e., an analogue of A can base-pair with T.

[0041] The term "antigen" and its grammatical equivalents as used herein can refer to a molecule that contains one or more epitopes capable of being bound by one or more receptors. For example, an antigen can stimulate a host's immune system to make a cellular antigen-specific immune response when the antigen is presented, or a humoral antibody response. An antigen can also have the ability to elicit a cellular and/or humoral response by itself or when present in combination with another molecule. For example, an influenza A viral protein can be recognized by a TCR.

[0042] The term "epitope" and its grammatical equivalents as used herein can refer to a part of an antigen that can be recognized by antibodies, B cells, T cells or engineered cells. For example, an epitope can be an influenza A viral epitope that is recognized by a TCR. Multiple epitopes within an antigen can also be recognized. The epitope can also be mutated.

[0043] The term "mutation" and its grammatical equivalents, as used herein, can refer to a deletion, an insertion of a heterologous nucleic acid, an inversion or a substitution, including an open reading frame ablating mutation as commonly understood in the art.

[0044] The term "gene" and its grammatical equivalents, as used herein, can refer to a segment of nucleic acid that encodes an individual polypeptide, protein or RNA (also referred to as a "coding sequence" or "coding region"), optionally together with associated regulatory regions such as promoters, operators, terminators and the like, which can be located upstream or downstream of the coding sequence.

[0045] The term "naturally-occurring" and its grammatical equivalents, as used herein with reference to a virus, can indicate that the virus can be found in nature, i. e., it can be isolated from a source in nature and has not been intentionally modified.

[0046] The terms "inhibiting," "reducing" or "prevention," or any variation of these terms, referred to herein, can include any measurable decrease or complete inhibition to achieve a desired result.

[0047] A "promoter" and its grammatical equivalents, as used herein, can be a control sequence that is a region of a nucleic acid sequence at which initiation and rate of transcription are controlled. A promoter can contain genetic elements at which regulatory proteins and molecules can bind such as RNA polymerase and other transcription factors. The terms "operatively positioned," "operatively linked," "under control" and "under transcriptional control" can mean that a promoter is in a correct functional location and/or orientation in relation to a nucleic acid sequence to control transcriptional initiation and/or expression of that sequence. In some cases, a promoter may or may not be used in conjunction with an "enhancer," which can refer to a cis-acting regulatory sequence involved in the transcriptional activation of a nucleic acid sequence.

[0048] The term "subject" and its grammatical equivalents can refer to an animal, including, but not limited to, a primate (e.g., human), cow, sheep, goat, horse, dog, cat, rabbit, rat, or mouse. The terms "subject" and "patient" can be used interchangeably herein in reference, for example, to a mammalian subject, such as a human subject.

[0049] Methods of Identifying Epitopes for Use in a Vaccine

[0050] One aspect of the present disclosure provides methods of identifying epitopes for use in a vaccine. The methods can be used to identify an epitope sequence of a pathogen, e.g., a virus, e.g., an RNA virus, e.g., an influenza virus, e.g., an influenza A, influenza B, or influenza C virus. In some cases, the methods provided herein are used in connection with identification of epitope sequences of a pathogen, e.g., a virus, e.g., an RNA virus, e.g., an influenza virus, e.g., an influenza A influenza B, or influenza C virus, for use in a vaccine. The epitope sequences can be from one or more of, or have homology to one or more of, PB1, PB1-F2, PB2, PA, HA, NP, NA, M1, M2, NS1, or NEP/NS2.

[0051] Invariance, or invariant peptide regions, can be determined, e.g., as described in International PCT application publication WO/2015/157189, filed Apr. 6, 2015, which is herein incorporated by reference in its entirety. Invariance can describe the functional importance of an amino acid residue in the context of the fitness of a pathogen. Invariance can be a measurement of the fitness of a pathogen. At an amino acid residue level, invariance can be associated with how tolerant an amino acid residue is to a mutation and how adverse this mutation is to the ability of the pathogen to propagate, that is, fitness of the pathogen. Invariance can be correlated with the role of an amino acid residue in a pathogen's survival. For example, a mutation in a pathogen that exerts a deleterious effect on the proliferation of the pathogen can be considered a destructive mutation and would not be propagated within a pathogen population. An associated amino acid position correlated with the deleterious mutation can be characterized as invariant as its mutation would not be tolerated.

[0052] The methods can comprise generating a nucleic acid library. In some instances, the nucleic acid library can allow simulation of all possible mutations that can occur in a particular pathogen strain, for example, by generating a pool of mutant influenza A viruses or influenza B viruses. In some cases, the methods can further comprise introducing the library to cells to support production of a pool of pathogens, e.g. influenza A viruses. Sometimes, the methods can further comprise infection of cells with the pool of pathogens for a number of rounds (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more rounds), obtaining the resultant pool of pathogens, e.g. influenza A viruses, and generating a second sequencing library. The methods can further comprise obtaining the nucleic acid sequences of the second sequencing library. In some instances, the methods can further comprise comparing the sequences (e.g., comparing sequences from the library to sequences from the second library, or a library from the last round of infection) to obtain invariant sequence regions, e.g. invariant peptide regions. An invariant sequence region can have an average invariance ratio (frequency of mutant in final population/frequency in initial population) of <0.05, <0.08, <0.1, <0.2, <0.3, or <0.4 among all possible mutations in the stretch. The methods can further comprise evaluating the invariant sequence regions. In some cases, the methods can further comprise HLA affinity binding analysis of the invariant sequence regions, some additional immunogenicity analysis for vaccine development and treatment of patient, or any combination thereof. The term "immunogenicity," as used herein, can refer to the capability of a particular substance, e.g., antigen or epitope, to induce an immune response. In some cases, a first screen for epitope sequences for use in a vaccine comprises identifying immunogenicity peptide sequences and then evaluating the epitope sequences for sequence invariance.

[0053] HLA affinity binding analysis can be carried out using analysis programs such as NetMHCpan4.0 from the Center for Biological Sequences Analysis (CBS) at the Technical University of Denmark, HLA Peptide Binding Predictions server from the National Institute of Health, MHC-1 binding predictions server from the Immune Epitope Database (IEDB), and the like.

[0054] In some cases, candidate invariant sequences identified by a method provided herein can be further analyzed. For instance, the candidate invariant sequences can be compared against experimentally tested immunogenicity data of influenza virus epitopes, e.g., in various databases, such as Influenza Research Database (https://www.fludb.org). Upon the comparison, candidate invariant sequences with experimentally proven immunogenicity (e.g., deposited at https://fludb.org) can be identified and subject to either further analysis, or vaccine development. For instance, such candidate invariant sequences, or the variants thereof, can be chosen to be one of the constituent epitope sequences of a polypeptide as described herein, which can be expressed from a transgene as described herein, and the transgene can be part of a vector, e.g., a viral vector, for producing a virus-based vaccine, e.g., an adenoviral-based vaccine.

[0055] Additional Analysis

[0056] Additional analysis can be carried out to select candidate invariant sequences or peptides for vaccine development and for administration of the vaccine to a patient for treatment or prevention of a condition, e.g., influenza. These additional analysis or screenings can involve analysis of an immune response based on immunological assays. In some cases, test animals are first immunized (prime) with or without a second immunization (boost) following weeks after the prime and blood or tissue samples are collected, for example, two to four weeks after the last immunization. These studies can allow measurement of immune parameters

that correlate to protective immunity, such as induction of specific antibodies (e.g., IgA, IgD, IgE, IgG, or IgM) and induction of specific T lymphocyte responses, in addition to determining whether an antigen or pools of antigens provides protective immunity.

[0057] Spleen cells, lung cells. cells from mediastinal lymph nodes, or peripheral blood mononuclear cells can be isolated from immunized test animals and measured for the presence of antigen-specific T cells and induction of cytokine synthesis. ELISA, ELISPOT, or cytoplasmic cytokine staining, alone or combined with flow cytometry, can provide such information on a single-cell level.

[0058] Immunological tests that can be used to identify the efficacy of immunization include antibody measurements, neutralization assays and analysis of activation levels or frequencies of antigen presenting cells or lymphocytes that are specific for the antigen or pathogen. The test animals that can be used in such studies include mice, rats, guinea pigs, hamsters, rabbits, cats, dogs, pigs, monkeys, or humans.

[0059] Monkey can be a useful test animal, e.g., due to the similarities of the MHC molecules between monkeys and humans. Virus neutralization assays can be useful for detection of antibodies that not only specifically bind to a pathogen, but also neutralize the function of the pathogen (e.g., virus). These can be based on detection of antibodies in the sera of immunized animal and analysis of these antibodies for their capacity to inhibit pathogen (e.g., virus) growth in tissue culture cells. Such assays are known to those skilled in the art. Virus neutralization assays can be used to screen for antigens that also provide protective immunity.

[0060] Polypeptides

[0061] One aspect of the present disclosure provides polypeptides that comprise, consist of, or consist essentially of, one or more influenza virus epitope sequences, for example, influenza A virus epitope sequences.

[0062] As used herein, a peptide or polypeptide that "comprises" a sequence according to a specified sequence or formula can be a peptide or polypeptide that can include additional amino acid residues, amino acid isomers, and/or amino acid analogs at its N-terminus, C-terminus, or both. The additional residues may or may not change its activity or function, e.g., increase or decrease the activity of the peptide as compared to the activity of a peptide consisting solely of the specified sequence or formula. As used herein, a peptide that "consists essentially of" a specified sequence or formula can mean that the peptide can include additional amino acid residues, amino acid isomers, and/or amino acid analogs at its N-terminus, C-terminus, or both, so long as the additional residues do not materially change its activity or function, e.g., increase or decrease the activity of the peptide as compared to the activity of a peptide consisting solely of the specified sequence or formula. As used herein, a peptide that "consists of" a specified sequence or formula can mean that the peptide does not include additional amino acid residues, amino acid isomers, and/or amino acid analogs at both its N-terminus and C-terminus.

[0063] The polypeptide can comprise, consist of, or consist essentially of, epitope sequences identified by the exemplary methods provided by the present disclosure. For instance, the polypeptide can comprise, consist of, or consist essentially of, one or more epitope sequences selected from the group consisting of: SEQ ID NOs: 1-94, or one or more epitope sequences selected from Table 1, Table 2, or Table 3. Tables 1, 2, and 3 below list epitope sequences, and viral proteins, which the epitope sequences are derived from, or variations of. Each of the polypeptides described herein can be chemically synthesized, or expressed in vivo, or in vitro. The polypeptide can be encoded by a nucleic acid, and the nucleic acid can be within a vector, e.g., viral vector, e.g., adenoviral vector. The vector, e.g. adenoviral vector, can be used to generate a recombinant virus, e.g. a recombinant adenovirus. Any of the polypeptides herein can be different than a full length viral protein. A polypeptide provided herein can be non-naturally occurring. A "non-naturally occurring polypeptide," as the term is used herein, can refer to a polypeptide whose primary sequence does not occur in nature, e.g., cannot be found in a single molecule in nature.

TABLE 1

Protein	SEQ ID NO:	Sequences
PB1	SEQ ID NO: 1	GPATAQMAL
PB1	SEQ ID NO: 2	GTFEFTSFFY
PB1	SEQ ID NO: 3	YSHGTGTGY
PB1	SEQ ID NO: 4	GLPVGGNEKKAKLANVVR
PB1	SEQ ID NO: 5	GMMMGMFNMLSTVLGVS
PB1	SEQ ID NO: 6	LQLFIKDYRYTYRCHRG
PB1	SEQ ID NO: 7	RRAIATPGM
PA	SEQ ID NO: 8	FMYSDFHFI
PA	SEQ ID NO: 9	MRRNYFTAEVSHCRATEY
PA	SEQ ID NO: 10	QLMWALGENMA
PA	SEQ ID NO: 11	DVVNFVSMEFSLTDPRL
PA	SEQ ID NO: 12	KWGMEMRRCLLQSLQQI
NP	SEQ ID NO: 13	AEIEDLIFLA
NP	SEQ ID NO: 14	CTELKLSDY
NP	SEQ ID NO: 15	CTELKLTDQ
NP	SEQ ID NO: 16	CTELKLTDY
NP	SEQ ID NO: 17	ELRSRYWAIRTRSG
NP	SEQ ID NO: 18	ELKSRYWAIRTRSG
NP	SEQ ID NO: 19	GMDPRMCSL
NP	SEQ ID NO: 20	ILKGKFQTA
NP	SEQ ID NO: 21	ILRGSIAHK
NP	SEQ ID NO: 22	ILRGSVAHK
NP	SEQ ID NO: 23	LELRSRYWAI
NP	SEQ ID NO: 24	LIFLARSAL
NP	SEQ ID NO: 25	RGINDRNFW
NP	SEQ ID NO: 26	FLARSALILRGSVAHK
NP	SEQ ID NO: 27	RMVLSAFDER
NP	SEQ ID NO: 28	TLELRSGYWAIRTRSGGN
NP	SEQ ID NO: 29	IAYERMCNILKGKFQTAA

TABLE 1-continued

Protein	SEQ ID NO:	Sequences
NP	SEQ ID NO: 30	FLARSALILRGSVAHKS
NP	SEQ ID NO: 31	FQGRGVFEL
NP	SEQ ID NO: 32	GQISIQPTFS
NP	SEQ ID NO: 33	WHSNLNDATYQRTRALVRTGMDPRM

TABLE 1-continued

Protein	SEQ ID NO:	Sequences
NP	SEQ ID NO: 34	WHSNLNDTTYQRTRALVRTGMDPRM
NA	SEQ ID NO: 35	CVNGSCFTV
M1	SEQ ID NO: 36	RMVLASTTAK

TABLE 2

SEQ ID NO:	Peptide	Protein	HLA (Experimentally Validated)	Additional HLA (NetMHCpan3.0 prediction)	Inv. Ratio
35	CVNGSCFTV	NA	A2		0.044
38	CVNGSCYTV	NA			
17	ELRSRYWAIRTRSG	NP	B27	A3, A26, B8	0.053
39	ELKSRYWAIRTRSG	NP			
8	FMYSDFHFI	PA	A2	A24, B8, B39, B15	0.05
40	FMYSDLHFI	PA			
41	FMYTDFHFI	PA			
19	GMDPRMCSL	NP	A2	B39	0.052
42	GRDPRMCSL	NP			
2	GTFEFTSFFY	PB1	A3	A1, A24, A26, B58, B15	0.04
43	GTFEFTSYFY	PB1			
20	ILKGKFQTA	NP	B8		0.056
44	IIKGKFQTA	NP			
45	ILKGKFQIA	NP			
21	ILRGSIAHK	NP	A3		0.03
46	ILRGSVAHK	NP			
47	LQLRSRYWAI	NP		B8	
48	LELRSRHWAI	NP			
24	LIFLARSAL	NP	A2	B7, B8, B15	0.056
49	LVFLARSAL	NP			
50	RWINDRNFW	NP			
3	YSHGTGTGY	PB1	A1	A26, B15	0.055
51	YSHWTGTGY	PB1			
52	YSHGSGTGY	PB1			
26	FLARSALILRGSVAHK	NP		A2, A3, B7, B27, B8, B39, B15	0.033
53	FLARSALVLRGSVAHK	NP			
29	IAYERMCNILKGKFQTAA	NP	B40	A3, A24, B8, B27, B15	0.058

TABLE 2-continued

SEQ ID NO:	Peptide	Protein	HLA (Experimentally Validated)	Additional HLA (NetMHCpan3.0 prediction)	Inv. Ratio
54	IAYERMCNIIKGKFQ TAA	NP			
55	IAYERMCNILKVKFQ TAA	NP			
56	IAYERMCNILKGKFK TAA	NP			
57	IAYERMCNILKGKFQ IAA	NP			
11	DVVNFVSMEFSLTDP RL	PA		A1, A3, A24, A26, B8, B39, B40, B58, B15	0.021
58	DVVNFVSMEFSLTYP RL	PA			
59	DVVNFVSMEFSLTD QRL	PA			
30	FLARSALILRGSVAH KS	NP	A3	A2, B7, B8, B27, B39, B15	0.049
60	FLARSALVLRGSVA HKS	NP			
12	KWGMEMRRCLLQS LQQI	PA		A2, A24, B7, B8, B27, B39, B40	0.031
61	KLGMEMRRCLLQSL QQI	PA			
62	KWGMEMRRCLLQS LQQV	PA			
6	LQLFIKDYRYTYRCH RG	PB1		A26, B27, B15	0.05
63	LQLFIKDFRYTYRCH RG	PB1			
64	LQLFIKDYRYTYRCL RG	PB1			
65	LQLFIKDYRYTYRCP RG	PB1			
66	LQLFIKDYRYTYRCH RV	PB1			
31	FQGRGVFEL	NP	A2	B39, B40	0.04
67	FQGPGVFEL	NP			
32	GQISIQPTFS	NP		B40, B15	0.053
68	SQISIQPTFS	NP			
69	GQVSIQPTFS	NP			
70	GQISVQPTFS	NP			
71	GQNSIQPTFS	NP			
33	WHSNLNDATYQRTR ALVRTGMDPRM	NP	B27	A1, A3, A24, A26, B7, B8, B39, B58, B15	N/A
72	WHSNLNDTTYQRTR ALVRTGMDPRM	NP			

TABLE 2-continued

SEQ ID NO:	Peptide	Protein	HLA (Experimentally Validated)	Additional HLA (NetMHCpan3.0 prediction)	Inv. Ratio
73	WHSNLNDSTYQRTR ALVRTGMDPRM	NP			
74	WHSNLNDATYQRKR ALVRTGMDPRM	NP			
75	WHSNLNDATYQRTR SLVRTGMDPRM	NP			
76	WHSNLNDATYQRTR AIVRTGMDPRM	NP			
77	WHSNLNDATYQRTR ALVRSGMDPRM	NP			
78	WHSNLNDATYQRTR ALVRTGRDPRM	NP			

TABLE 3

SEQ ID NO:	Peptide	Protein	HLA (Experimentally Validated)	Additional HLA (NetMHCpan3.0 prediction)
17	ELRSRYWAIRTRSG	NP	B27	B8
82	ELRSRHWAIRTRSG	NP	B27	B8
83	ELRSRYWASRTRSG	NP		
8	FMYSDFHFI	PA	A2	A24, B8, B39, B15
40	FMYSDLHFI	PA	A2	B8 4.00 pctl, A26 1.9 pctl
41	FMYTDFHFI	PA	A2	A26 3.5 pctl, A24, B8, B39, B15
84	FMFSDFHFI	PA		
2	GTFEFTSFFY	PB1	A3	A1, A24, A26, B58, B15
43	GTFEFTSYFY	PB1	A3	A1, A24, A26, B58, B15
20	ILKGKFQTA	NP	B8	
44	IIKGKFQTA	NP	B8	
45	ILKGKFQIA	NP	B8	
21	ILRGSIAHK	NP	A3	
22	ILRGSVAHK	NP	A3	
85	VLRGSIAHK	NP		
24	LIFLARSAL	NP	A2 (negative on NetMHC)	B7, B8, B15
49	LVFLARSAL	NP		B7, B8, B15, B39 1.9 pctl
86	LTFLARSAL	NP		
3	YSHGTGTGY	PB1	A1	A26, B15
51	YSHWTGTGY	PB1	A1	A26, B15
52	YSHGSGTGY	PB1	A1	A26, B15
26	FLARSALILRGSVAHK	NP		A2, A3, B7, B8, B39, B15

TABLE 3-continued

SEQ ID NO:	Peptide	Protein	HLA (Experimentally Validated)	Additional HLA (NetMHCpan3.0 prediction)
53	FLARSALVLRGSVAH K	NP		A2, A3, B7, B8, B39, B15
29	IAYERMCNILKGKFQT AA	NP	B40	A3, A24, B8, B27
87	VAYERMCNILKGKFQ TAA	NP		
88	VAYERMCNIIKGKFQT AA	NP	B40	A3, A24, B8, B27
89	VAYERMCNILKGKFK TAA	NP	B40	A3, A24, B8, B27
90	VAYERMCNILKGKFQI AA	NP	B40	A3, A24, B8, B27
91	VAYERMCNILKGKFQ TAV	NP		
11	DVVNFVSMEFSLTDP RL	PA		A1, A3, A24, A26, B8, B39, B40, B58, B15
58	DVVNFVSMEFSLTYP RL	PA		A1, A3, A24, A26, B8, B39, B40, B58, B15
59	DVVNFVSMEFSLTDQ RL	PA		A3 2.5 pctl
30	FLARSALILRGSVAHK S	NP	A3	A2, B7, B8, B39, B15
60	FLARSALVLRGSVAH KS	NP	A3	A2, B7, B8, B39, B15
12	KWGMEMRRCLLQSL QQI	PA		A2, B8, B27, B39
61	KLGMEMRRCLLQSLQ QI	PA		A2, B8, B27, B39
62	KWGMEMRRCLLQSL QQV	PA		A2, B8, B27, B39
92	KWGMELRRCLLQSLQ QI	PA		
31	FQGRGVFEL	NP	A2	B39, B40
32	GQISIQPTFS	NP		B40, B15
93	SQISVQPTFS	NP		B39, B40, A24 (WB), B39 (WB)
94	GQVSVQPTFS	NP		B39, B40
70	GQISVQPTFS	NP		B39, B40
33	WHSNLNDATYQRTRA LVRTGMDPRM	NP	B27	A1, A3, A24, A26, B7, B8, B39, B58, B15
34	WHSNLNDTTYQRTRA LVRTGMDPRM	NP	B27	A1, A3, A24, A26, B7, B8, B39, B58, B15
73	WHSNLNDSTYQRTRA LVRTGMDPRM	NP	B27	A1, A3, A24, A26, B7, B8, B39, B58, B15
74	WHSNLNDATYQRKR ALVRTGMDPRM	NP	B27	A1, A3, A24, A26, B7, B8, B39, B58, B15
75	WHSNLNDATYQRTRS LVRTGMDPRM	NP	B27	A1, A3, A24, A26, B7, B8, B39, B58, B15

TABLE 3-continued

SEQ ID NO:	Peptide	Protein	HLA (Experimentally Validated)	Additional HLA (NetMHCpan3.0 prediction)
76	WHSNLNDATYQRTRA IVRTGMDPRM	NP	B27	A3 2.5 pctl
77	WHSNLNDATYQRTRA LVRSGMDPRM	NP	B27	A1, A3, A24, A26, B7, B8, B39, B58, B15
78	WHSNLNDATYQRTRA LVRTGRDPRM	NP	B27	A1, A3, A24, A26, B7, B8, B39, B58, B15

[0064] A polypeptide as described herein can comprise, consist of, or consist essentially of one or more epitope sequences. Sometimes, a polypeptide as described herein can comprise, consist of, or consist essentially of, more than one epitope sequence, among which some of the epitope sequences are the same, while others of the epitope sequences are different, or all the epitope sequences are the same, or all the epitope sequences are different. In some cases, one or more epitope sequences are repeated in a polypeptide, e.g., about, at least, or at most 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20 times. Sometimes, the polypeptide can comprise, consist of, or consist essentially of, one or more different epitope sequences. The polypeptide can comprise, consist of, or consist essentially of only one epitope sequence, and the one epitope sequence can be present in the polypeptide about, at least, or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20 times.

[0065] In some cases, a polypeptide can comprise, consist of, or consist essentially of one or more different epitope sequences, each of the one or more different epitope sequences comprising at least 70% sequence identity to at least 8 contiguous amino acids of a sequence selected from the group consisting of SEQ ID NOs: 1-94. A polypeptide provided herein can comprise, consist of, or consist essentially of one or more different epitope sequences, each of the one or more different epitope sequences comprising at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to at least 8 contiguous amino acids of a sequence selected from the group consisting of SEQ ID NOs: 1-94. In some embodiments, the polypeptide comprises, consists of, or consists essentially of one or more different epitope sequences, each of the one or more different epitope sequences comprising at least 70% sequence identity to at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 12, at least 14, at least 16, at least 18, at least 20, at least 22, at least 24, or at least 25 contiguous amino acids of a sequence selected from the group consisting of SEQ ID NOs: 1-94. A polypeptide can comprise, consist of, or consist essentially of one or more different epitope sequences, each of the one or more different epitope sequences comprising at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 12, at least 14, at least 16, at least 18, at least 20, at least 22, at least 24, or at least 25 contiguous amino acids of a sequence selected from the group consisting of SEQ ID NOs: 1-94.

[0066] In some cases, a polypeptide can comprise, consist of, or consist essentially of one or more different epitope sequences, each of the one or more different epitope sequences comprises at least 70% sequence identity to at least 8 contiguous amino acids of a sequence selected from Table 1, Table 2, Table 3, or any combination thereof. A polypeptide provided herein can comprise, consist of, or consist essentially of one or more different epitope sequences, each of the one or more different epitope sequences comprising at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to at least 8 contiguous amino acids of a sequence selected from Table 1, Table 2, Table 3, or any combination thereof. The polypeptide can comprise, consist of, or consist essentially of one or more different epitope sequences, each of the one or more different epitope sequences comprising at least 70% sequence identity to at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 12, at least 14, at least 16, at least 18, at least 20, at least 22, at least 24, or at least 25 contiguous amino acids of a sequence selected from Table 1, Table 2, Table 3, or any combination thereof. A polypeptide can comprise, consist of, or consist essentially of one or more different epitope sequences, each of the one or more different epitope sequences comprising at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 12, at least 14, at least 16, at least 18, at least 20, at least 22, at least 24, or at least 25 contiguous amino acids of a sequence selected from Table 1, Table 2, Table 3, or any combination thereof.

[0067] A polypeptide can comprise, consist of, or consist essentially of at least two different epitope sequences, each of the at least two different epitope sequences comprises at least 70% sequence identity to at least 8 contiguous amino acids of a sequence selected from the group consisting of SEQ ID NOs: 1-94 or of a sequence selected from Table 1, Table 2, or Table 3. The polypeptide can comprise, consist of, or consist essentially of at least three different epitope sequences, each of the at least three different epitope sequences comprising at least 70% sequence identity to at least 8 contiguous amino acids of SEQ ID NOs: 1-94 or of a sequence selected from Table 1, Table 2, or Table 3. The polypeptide can comprise, consist of, or consist essentially

of at least four different epitope sequences, each of the at least four different epitope sequences comprising at least 70% sequence identity to at least 8 contiguous amino acids of SEQ ID NOs: 1-94 or of a sequence selected from Table 1, Table 2, or Table 3. The polypeptide can comprise, consist of, or consist essentially of, at least five different epitope sequences, each of the at least five different epitope sequences comprises at least 70% sequence identity to at least 8 contiguous amino acids of SEQ ID NOs: 1-94 or of a sequence selected from Table 1, Table 2, or Table 3. The polypeptide can comprise, consist of, or consist essentially of at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 50, or at least 51 different epitope sequences, each of the different epitope sequences comprising at least 70% sequence identity to at least 8 contiguous amino acids of SEQ ID NOs: 1-94 or of a sequence selected from Table 1, Table 2, or Table 3.

[0068] One non-limiting example relates to a polypeptide that comprises, consists of, or consists essentially of, at least 51 different epitope sequences, each of the 51 different epitope sequences comprises at least 70% sequence identity to at least 8 contiguous amino acids of a different sequence selected from Table 3. Sometimes, a polypeptide can comprise, consist of, or consist essentially of, at least 51 different epitope sequences, each of the 51 different epitope sequences comprises at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 12, at least 14, at least 16, at least 18, at least 20, at least 22, at least 24, or at least 25 contiguous amino acids of a different sequence selected from Table 3. In some cases, a polypeptide comprises, consists of, or consists essentially of each sequence from Table 3.

[0069] In some cases, the polypeptide comprises at least 8 amino acids. The polypeptide can comprise at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 120, at least 140, at least 160, at least 180, at least 200, at least 250, at least 300, at least 400, at least 500, at least 1000, at least 1500, at least 2000, at least 2500, at least 3000, at least 4000, at least 5000, or more amino acids. In some cases, the polypeptide comprises, or consists of, at most 20, at most 30, at most 40, at most 50, at most 60, at most 70, at most 80, at most 90, at most 100, at most 150, at most 200, at most 250, at most 300, at most 500, at most 800, at most 1000, at most 1500, at most 2000, at most 2500, at most 3000, at most 4000, or at most 5000 amino acids. The polypeptide can comprise, consist of, or consist essentially of about 8 to about 5000 amino acids, about 8 to about 4000 amino acids, about 8 to about 3000 amino acids, about 8 to about 2000 amino acids, about 8 to about 1000 amino acids, about 8 to about 500 amino acids, about 100 to about 5000 amino acids, about 100 to about 2500 amino acids, about 100 to about 1000 amino acids, about 1500 to about 3000 amino acids, about 1000 to about 3000 amino acids, or about 1000 to about 2500 amino acids. The polypeptide can consist of less than 5000, 4000, 3000, 2900, 2800, 2700, 2600, 2500, 2400, 2300, 2200, 2100, 2000, 1900, 1800, 1700, 1600 1500, 1400, 1300, 1200, 1100, 1000, 750, 500, 250, or 100 amino acids, e.g., when synthesized or initially expressed, e.g., in a cell.

[0070] Provided herein is an engineered polypeptide that comprises, consists of, or consists essentially of one or more different epitope sequences that can be derived from, or variants of, or fragments of, at least a portion of a viral protein, e.g., an influenza virus protein, e.g. an influenza A virus protein. In some embodiments, the influenza A virus protein can be PB1, PB1-F2, PB2, PA, HA, NP, NA, M1, M2, NS1, or NEP/NS2. PB2 can be a part of an RNA-dependent RNA polymerase complex, which can facilitate "cap-snatching" from host pre-mRNA molecules to initiate transcription, and can be conducive for replication. In certain situations, PB1 can be a RNA-dependent RNA polymerase, which can bind to terminal ends of vRNA and cRNA for initiation of transcription and replication and can catalyze the sequential addition of nucleotides during RNA chain elongation. PA can be used for viral transcription and replication and can have endonuclease activity. In some instances, PA does not correlate with polymerase activity. HA can bind sialic acid on cell surface for attachment, and can undergo conformational change with low pH exposing fusion peptide which can interact with the endosomal membrane, forming a pore through which the viral RNPs can be released into the cytoplasm. NP can coat viral RNA to form viral ribonucleoprotein (vRNP) complex, which can be critical for the trafficking of vRNPs into the nucleus. NA can be needed for the final release of virus through cleavage of the HA-sialic acid bond which can anchor virus to cell membrane. NA can also prevent virus particles from aggregating. M1 can form intermediate core of a virion and tether NP w/vRNPs, and can drive budding of virus from the cell membrane. M2 can have ion channel activity, and can conduct protons from acidified endosomes into viral particle resulting in pH dependent dissociation of vRNP from the remainder of viral components. NS1 can inhibit cellular antiviral Type 1 Interferon response, and can be dependent on binding to dsRNA. NEP/NS2 can be necessary for nuclear export of vRNP through recruitment of cellular export machinery. The influenza A virus protein can be NP, PB1, or PA.

[0071] One non-limiting example of the polypeptide provided herein comprises, consists of, or consists essentially of one or more different epitope sequences having at least 70%, 75%, 80%, 85%, 90%, or 100% sequence identity to at least 8 contiguous amino acids of a sequence derived from or variants of PB1 protein, such as, but not limited to a sequence selected from the group consisting of SEQ ID NOs: 1-7, 43, 51, 52, and 63-66, or a sequence selected from the group consisting of SEQ ID Nos: 2, 3, 43, 51, and 52. In some cases, the polypeptide further comprises one or more different epitope sequences having at least 70%, 75%, 80%, 85%, 90%, or 100% sequence identity to at least 8 contiguous amino acids of a sequence selected from the group consisting of SEQ ID NOs: 8-42, 44-50, 53-62, 67-80, and 82-94. The polypeptide can comprise, consist of, or consist essentially of SEQ ID NOs: 2, 3, 8, 11, 12, 17, 20-22, 24, 26, 29-34, 40, 41, 43-45, 49, 51-53, 58-62, 70, 73-78, and 82-94. In some cases, the polypeptide does not comprise full-length PB1 sequence.

[0072] Another non-limiting example of the polypeptide provided herein comprises, consists of, or consists essentially of one or more different epitope sequences having at

least 70% sequence identity to at least 8 contiguous amino acids of a sequence derived from or variants of PA protein, such as, but limited to a sequence selected from the group consisting of SEQ ID NOs: 8-12, 40, 41, 58, 59, 61, 62, 84, and 92, or a sequence selected from the group consisting of SEQ ID NOs: 8, 11, 12, 40, 41, 58, 59, 61, 62, 84, and 92. Sometimes, the polypeptide can further comprise one or more different epitope sequences having at least 70% sequence identity to at least 8 contiguous amino acids of a sequence selected from the group consisting of SEQ ID NOs: 1-7, 13-39, 42-57, 60, 63-80, 82, 83, 85-91, and 94. The polypeptide can comprise, consist of, or consist essentially of SEQ ID NOs: 2, 3, 8, 11, 12, 17, 20-22, 24, 26, 29-34, 40, 41, 43-45, 49, 51-53, 58-62, 70, 73-78, and 82-94. In some cases, the polypeptide does not comprise full-length PA sequence.

[0073] Another non-limiting example of the polypeptide provided herein comprises, consists of, or consists essentially of, one or more different epitope sequences having at least 70%, 75%, 80%, 85%, 90%, or 100% sequence identity to at least 8 contiguous amino acids of a sequence derived from or variants of NP protein, such as, but not limited to a sequence selected from the group consisting of SEQ ID NOs: 13-34, 37, 39, 42, 44-50, 53-57, 60, 67-80, 82, 83, 85-91, 93, and 94, or a sequence from the group consisting of SEQ ID NOs: 17, 20-22, 24, 26, 29-34, 44, 45, 49, 53, 60, 70, 73-78, 82, 83, 85-91, 93, and 94. Sometimes, the polypeptide can further comprise one or more different epitope sequences having at least 70%, 75%, 80%, 85%, 90%, or 100% sequence identity to at least 8 contiguous amino acids of a sequence selected from the group consisting of SEQ ID NOs: 1-12, 35, 36, 38, 43, 51, 52, 58, 59, 61-66, 84, and 92. The polypeptide can comprise, consist of, or consist essentially of SEQ ID NOs: 2, 3, 8, 11, 12, 17, 20-22, 24, 26, 29-34, 40, 41, 43-45, 49, 51-53, 58-62, 70, 73-78, and 82-94. In some cases, the polypeptide does not comprise full-length NP sequence.

[0074] Another non-limiting example of the polypeptide provided herein comprises, consists of, or consists essentially of one or more different epitope sequences having at least 70%, 75%, 80%, 85%, 90%, or 100% sequence identity to at least 8 contiguous amino acids of a sequence derived from or variants of NA protein, such as, but not limited to, a sequence selected from SEQ ID NOs: 35 and 38. Sometimes, the polypeptide can further comprise one or more different epitope sequences having at least 70%, 75%, 80%, 85%, 90%, or 100% sequence identity to at least 8 contiguous amino acids of a sequence selected from the group consisting of SEQ ID NOs: 1-34, 36, 37, 39-80, and 82-94. The polypeptide can comprise, consist of, or consist essentially of SEQ ID NOs: 2, 3, 8, 11, 12, 17, 20-22, 24, 26, 29-34, 40, 41, 43-45, 49, 51-53, 58-62, 70, 73-78, and 82-94. In some cases, the polypeptide does not comprise full-length NA sequence.

[0075] Another non-limiting example of the polypeptide provided herein comprises, consists of, or consists essentially of, one or more different epitope sequences having at least 70%, 75%, 80%, 85%, 90%, or 100% sequence identity to at least 8 contiguous amino acids of a sequence derived from or variants of M1 protein, such as, but not limited to SEQ ID NOs: 36. Sometimes, the polypeptide further comprises one or more different epitope sequences having at least 70%, 75%, 80%, 85%, 90%, or 100% sequence identity to at least 8 contiguous amino acids of a sequence selected from the group consisting of SEQ ID NOs: 1-35, 37-80, and 82-94. The polypeptide can comprise, consist of, or consist essentially of SEQ ID NOs: 2, 3, 8, 11, 12, 17, 20-22, 24, 26, 29-34, 40, 41, 43-45, 49, 51-53, 58-62, 70, 73-78, and 82-94. In some cases, the polypeptide does not comprise full-length M1 sequence.

[0076] One non-limiting example of a polypeptide provided herein can comprise, consist of, or consist essentially of a first sequence selected from the group consisting of SEQ ID NOs: 2, 3, 8, 11, 12, 40, 41, 43, 51, 52, 58, 59, 61, 62, 84, and 92; and a second sequence selected from the group consisting of SEQ ID NOs: 17, 20, 21, 22, 24, 26, 29, 30, 32, 33, 34, 44, 45, 49, 53, 60, 70, 73, 74, 75, 76, 77, 78, 82, 83, 85, 86, 87, 88, 89, 90, 91, 93, and 94.

[0077] Another non-limiting example of a polypeptide provided herein can comprise, consist of, or consist essentially of a first sequence selected from the group consisting of SEQ ID NOs: 2, 8, 11, 12, 40, 41, 43, 52, 58, 59, 61, 62, 84, and 92; and a second sequence selected from the group consisting of SEQ ID NOs: 17, 20, 21, 22, 24, 26, 29, 30, 31, 32, 33, 34, 44, 45, 49, 53, 60, 70, 73, 74, 75, 76, 77, 78, 82, 83, 85, 86, 87, 88, 89, 90, 91, 93, and 94.

[0078] Another non-limiting example of a polypeptide provided herein can comprise, consist of, or consist essentially of (a) a first sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 8, 11, 12, 40, 41, 58, 59, 61, 62, 84, or 92, and a second sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 2, 3, 17, 20, 21, 22, 24, 26, 29, 30, 31, 32, 33, 34, 43, 44, 45, 49, 51, 52, 53, 60, 70, 73, 74, 75, 76, 77, 78, 82, 83, 85, 86, 87, 88, 89, 90, 91, 93, or 94; or (b) a first sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 2 or 43 and a second sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 8, 11, 12, 17, 20, 21, 22, 24, 26, 29, 30, 31, 32, 33, 34, 40, 41, 44, 45, 49, 53, 58, 59, 60, 61, 62, 70, 73, 74, 75, 76, 77, 78, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, or 94; or (c) a first sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 21, 22, 24, 26, 30, 32, 49, 53, 60, 70, 85, 86, 93, or 94, and a second sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 2, 3, 8, 11, 12, 40, 41, 43, 51, 52, 58, 59, 61, 62, 84, or 92; or (d) a first sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 17, 20, 29, 31, 33, 34, 44, 45, 73, 74, 75, 76, 77, 78, 82, 83, 87, 88, 89, 90, or 91 and a second sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 2, 8, 11, 12, 40, 41, 43, 58, 59, 61, 62, 84, or 92; or (e) a first sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 2 or 43 and a second sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 8, 11, 12, 21, 22, 24, 26, 30, 32, 40, 41, 49, 53, 58, 59, 60, 61, 62, 70, 84, 85, 86, 92, 93, or 94; or (f) a first sequence comprising at least 60%, at least 75%, at least 85%, or 100%

sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 3, 51, or 52, and a second sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 8, 11, 12, 21, 22, 24, 26, 30, 32, 40, 41, 49, 53, 58, 59, 60, 61, 62, 70, 84, 85, 86, 92, 93, 94; or (g) a first sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 2, 8, 11, 12, 40, 41, 43, 58, 59, 61, 62, 84, or 92, and a second sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 21, 22, 24, 26, 30, 32, 49, 53, 60, 70, 85, 86, 93, or 94; or (h) a first sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 2, 8, 11, 12, 21, 22, 24, 26, 30, 32, 40, 41, 43, 49, 53, 58, 59, 60, 61, 62, 70, 84, 85, 86, 92, 93, or 94, and a second different sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 2, 3, 8, 11, 12, 17, 20, 21, 22, 24, 26, 29, 30, 31, 32, 33, 34, 40, 41, 43, 44, 45, 49, 51, 52, 53, 58, 59, 60, 61, 62, 70, 73, 74, 75, 76, 77, 78, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, or 94, wherein a contiguous sequence comprising the first sequence and the second different sequence is not found in a PB1, PA, or NP.

[0079] Another non-limiting example of a polypeptide provided herein can comprise, consist of, or consist essentially of a sequence selected from at least two of the following groups: (a) a sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 8, 40, 41, or 84; (b) a sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids SEQ ID NO: 11, 58, or 59; (c) a sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids SEQ ID NO: 12, 61, 62, or 92; (d) a sequence comprising at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 2 or 43; (e) a sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 3, 51, or 52; (f) a sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 32, 93, 94, or 70; (g) a sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 21, 22, or 85; (h) a sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 24, 49, or 86; (i) a sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 26, 53, 30, or 60; (j) a sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 17, 82, or 83; (k) a sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids SEQ ID NO: 20, 44, or 45; (l) a sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 29, 87, 88, 89, 90, or 91; (m) a sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 31; and (n) a sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 33, 34, 73, 74, 75, 76, 77, or 78; wherein at least one sequence is selected from groups (a)-(d) and at least one sequence is selected from groups (f)-(n).

[0080] Another non-limiting example of a polypeptide provided herein can comprise, consist of, or consist essentially of: (o) at least 1, 2, 3, or 4 sequences, each comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of any of SEQ ID NOs: 8, 40, 41, or 84, or least 1, 2, 3, or 4 sequences, each comprising at least 55%, at least 66%, at least 77%, at least 88%, or 100% sequence identity to any of SEQ ID NOs: 8, 40, 41, or 84; (p) at least 1, 2, or 3 sequences, each comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of any of SEQ ID NOs: 11, 58, or 59, or at least 1, 2, or 3 sequences, each comprising at least 52%, at least 58%, at least 64%, at least 70%, and least 76%, at least 82%, at least 88%, at least 94%, or 100% sequence identity to at least 9, 10, 11, 12, 13, 14, 15, 16, or 17 contiguous amino acids of any of SEQ ID NOs: 11, 58, or 59; (q) at least 1, 2, 3, or 4 sequences, each comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NOs: 12, 61, 62, or 92, or at least 1, 2, 3, or 4 sequences, each comprising at least 52%, at least 58%, at least 64%, at least 70%, and least 76%, at least 82%, at least 88%, at least 94%, or 100% sequence identity to at least 9, 10, 11, 12, 13, 14, 15, 16, or 17 contiguous amino acids of any of SEQ ID NOs: 12, 61, 62, or 92; (r) at least 1 or 2 sequences, each comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of any of SEQ ID NOs: 2 or 43, or at least 1 or 2 sequences, each comprising at least 60%, at least 70%, at least 80%, at least 90%, or 100% sequence identity to at least 9 or 10 contiguous amino acids of any of SEQ ID NOs: 2 or 43; (s) at least 1, 2, or 3 sequences, each comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of any of SEQ ID NOs: 3, 51, or 52, or at least 1, 2, or 3 sequences, each comprising at least 55%, at least 66%, at least 77%, at least 88%, or 100% sequence identity to at least 9 or 10 contiguous amino acids of any of SEQ ID NOs: 3, 51, or 52; (t) at least 1, 2, 3, or 4 sequences, each comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids to any of SEQ ID NOs: 32, 93, 94, or 70, or at least 1, 2, 3, or 4 sequences, each comprising at least 60%, 70%, 80%, 90%, or 100% sequence identity to 9 or 10 contiguous amino acids of any of SEQ ID NOs: 32, 93, 94, or 70; (u) at least 1, 2, or 3 sequences, each comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids any of SEQ ID NOs: 21, 22, or 85, or at least 1, 2, 3, or 4 sequences, each comprising at least 55%, at least 66%, at least 77%, at least 88%, or 100% sequence identity to 9 contiguous amino acids of any of SEQ ID NOs: 21, 22, or 85; (v) at least 1, 2 or 3 sequences, each comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of any of SEQ ID NOs: 24, 49, or 86, or at least 55%, at least 66%, at least 77%, at least 88%, or 100% sequence identity to 9 contiguous amino acids of any of SEQ ID NOs: 24, 49, or 86; (w) at least 1, 2, 3, or 4 sequences, each comprising at least 60%, at least 75%, at least 85%, or 100% sequence

identity to at least 8 contiguous amino acids of any of SEQ ID NOs: 26, 53, 30, or 60, or at least 1, 2, 3, or 4 sequences, each comprising at least 50%, at least 56%, at least 62%, at least 68%, at least 75%, at least 81%, at least 87%, at least 93%, or at least 100% sequence identity to at least 9, 10, 11, 12, 13, 14, 15, or 16 contiguous amino acids of any of SEQ ID NOs: 26, 53, 30, or 60, or at least 9, 10, 11, 12, 13, 14, 15, 16, or 17 contiguous amino acids of any of SEQ ID NOs: 30 or 60; (x) at least 1, 2, or 3 sequences, each comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of any of SEQ ID NOs: 17, 82, or 83, or at least 1, 2, or 3 sequences, each comprising at least 50%, at least 57%, at least 60%, at least 71%, at least 78%, at least 85%, at least 92%, or 100% sequence identity to at least 9, 10, 11, 12, 13, or 14 contiguous amino acids of any of SEQ ID NOs: 17, 82, or 83; (y) at least 1, 2, or 3 sequences, each comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of any of SEQ ID NOs: 20, 44, or 45 or at least 1, 2, or 3 sequences, each comprising at least 55%, at least 66%, at least 77%, at least 88%, or 100% sequence identity to 9 contiguous amino acids of any of SEQ ID NOs: 20, 44, or 45; (z) at least 1, 2, 3, 4, 5, or 6 sequences, each comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of any of SEQ ID NOs: 29, 87, 88, 89, 90, or 91, or at least 1, 2, 3, 4, 5, or 6 sequences, each comprising at least 50%, at least 55%, at least 61%, at least 66%, at least 72%, at least 83%, at least 88%, at least 94%, or 100% sequence identity to at least 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18 contiguous amino acids of any of SEQ ID NOs: 29, 87, 88, 89, 90, or 91; (aa) a sequence comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of SEQ ID NO: 31, or a sequence comprising at least 55%, at least 66%, at least 77%, at least 88%, or 100% sequence identity to 9 contiguous amino acids of SEQ ID NO: 31; (bb) at least 1, 2, 3, 4, 5, 6, 7, or 8 sequences, each comprising at least 60%, at least 75%, at least 85%, or 100% sequence identity to at least 8 contiguous amino acids of any of SEQ ID NOs: 33, 34, 73, 74, 75, 76, 77, or 78, or at least 1, 2, 3, 4, 5, 6, 7, or 8 sequences, each comprising at least 52%, at least 56%, at least 60%, at least 64%, at least 68%, at least 72%, at least 76%, at least 80%, at least 84%, at least 88%, at least 92%, at least 96%, or 100% sequence identity to at least 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 contiguous amino acids of any of SEQ ID NOs: 33, 34, 73, 74, 75, 76, 77, or 78; or any combination of (o)-(bb).

[0081] Another non-limiting example of a polypeptide provided herein can comprise, consist of, consist essentially of: (cc) at least two sequences from the group consisting of SEQ ID NOs: 8, 40, 41, and 84; (dd) at least two sequences from the group consisting of SEQ ID NOs: 11, 58, and 59; (ee) at least two sequences from the group consisting of SEQ ID NO: 12, 61, 62, and 92; (ff) at least two sequences from the group consisting of SEQ ID NOs: 2 and 43; (gg) at least three sequences from the group consisting of SEQ ID NOs: 3, 51, and 52; (hh) at least two sequences from the group consisting of SEQ ID NOs: 32, 93, 94, and 70; (ii) at least two sequences from the group consisting of SEQ ID NOs: 21, 22, and 85; (jj) at least two sequences from the group consisting of SEQ ID NOs: 24, 49, and 86; (kk) at least two sequences from the group consisting of SEQ ID NOs: 26, 53, 30, and 60; (ll) at least two sequences from the group consisting of SEQ ID NOs: 17, 82, and 83; (mm) at least two sequences from the group consisting of SEQ ID NOs: 20, 44, and 45; (nn) at least two sequences from the group consisting of SEQ ID NOs: 29, 87, 88, 89, 90, and 91; or (oo) at least two sequences from the group consisting of SEQ ID NOs: 33, 34, 73, 74, 75, 76, 77, and 78.

[0082] In some cases, a polypeptide provided herein can further comprise a full-length amino acid sequence (or at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% of the full-length sequence) of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) virus proteins, e.g., one or more influenza virus proteins, e.g., one or more influenza A, influenza B, or influenza C virus proteins, e.g., PB1, PB1-F2, PB2, PA, HA, NP, NA, M1, M2, NS1, or NEP/NS2 from influenza A, influenza B, or influenza C. In some cases, a vaccine provided herein comprises a polypeptide comprising a full-length amino acid sequence (or at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% of the full-length sequence) of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) virus proteins, e.g., one or more influenza virus proteins, e.g., one or more influenza A, influenza B, or influenza C virus proteins, e.g., PB1, PB1-F2, PB2, PA, HA, NP, NA, M1, M2, NS1, or NEP/NS2 from influenza A, influenza B, or influenza C, e.g., or the polypeptide is expressed from a separate nucleic acid or virus in the vaccine, e.g., an adenovirus, A polypeptide provided herein can comprise 1, 2, 3, 4, or 5 or more copies of a sequence of a full-length amino acid sequence (or at least 50%, 60%, 70%, 80%, 90%, or 95% of the full-length sequence) of the viral protein. The full-length amino acid sequence (or at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% of the full-length sequence) can be at the N-terminus of the polypeptide, the C-terminus of the polypeptide, internal in the polypeptide, or, e.g., if multiple copies are present, the N-terminus and C-terminus of the polypeptide. For example, a polypeptide can comprise at least 5, 10, 20, 30, 40, 50, or 51 sequences from Table 1, Table 2, or Table 3 (e.g., each sequence is from one of Table 1, Table 2, or Table 3), each sequence separated or not by a linker, and 1, 2, or 3 copies of a full-length amino acid sequence (or at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% of the full-length sequence) of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) virus proteins, e.g., one or more influenza virus proteins, e.g., one or more influenza A, influenza B, or influenza C virus proteins, e.g., PB1, PB1-F2, PB2, PA, HA, NP, NA, M1, M2, NS1, or NEP/NS2 from influenza A, influenza B, or influenza C.

[0083] In some cases, a polypeptide provided herein can further comprise a full length amino acid sequence (or at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% of the full-length sequence) of NP protein of influenza, e.g., influenza A, or Influenza B, influenza C. In some cases, NP protein sequence (or at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% of the full-length sequence of NP) can be from any appropriate strain of Influenza B. For instance, the sequence can be selected based the prevalent strain, or expected prevalent strain, for an influenza season. A strain of Influenza B from which an NP sequence is chosen can be chosen randomly. For instance, a polypeptide can comprise amino acid sequence of NP protein sequence (or at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% of the full-length sequence of NP) from a particular strain of Influenza B, like B/Brisbane/60/2008-like that belong to B/Victoria lineage or B/Phuket/3073/2013-like or B/Pennsylvania/49/2015 that belongs to B/Yamagata lin-

eage. A derivative (or fragment) of a full length amino acid sequence of NP protein of Influenza B can comprise at least 60% identity, at least 75% identity, at least 80% identity, at least 85% identity, at least 90% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity, at least 99.5% identity, at least 99.8% identity, at least 99.9% identity, at least 99.99% identity to the full length amino acid sequence of NP protein (or at least 10%, 20%, 30%, 40% 50%, 60%, 70%, 80%, 90%, or 95% of the full-length sequence of NP) of Influenza B. A derivative (or fragment) of a full length amino acid sequence of NP protein of Influenza B can comprise or consist of at most 100 amino acids, at most 80 amino acids, at most 60 amino acids, at most 50 amino acids, at most 40 amino acids, at most 30 amino acids, at most 20 amino acids, at most 15 amino acids, at most 10 amino acids, at most 9 amino acids, at most 8 amino acids, at most 7 amino acids, at most 6 amino acids, at most 5 amino acids, at most 4 amino acids, at most 3 amino acids, at most 2 amino acids, or only 1 amino acid different than the full length amino acid sequence of NP protein of Influenza B. A polypeptide can comprise all of the sequences in Table 1, with or without a linker between each sequence, and one or two copies of a full-length protein (or at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% of the full-length sequence of the protein), e.g., NP protein from an influenza B strain. A polypeptide can comprise all of the sequences in Table 2, with or without a linker between each sequence, and one or two copies of a full-length protein (or at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% of the full-length sequence of a protein), e.g., NP protein from an influenza B strain. A polypeptide can comprise all of the sequences in Table 3, with or without a linker between each sequence, and one or two copies of a full-length protein (or at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% of the full-length sequence of a protein), e.g., NP protein from an influenza B strain.

[0084] In some cases, a polypeptide can comprise amino acid sequence (full-length or fragment) of NP protein from a virus that belongs to B/Victoria lineage. For example, a polypeptide can comprise amino acid sequence with at least 50%, 60%, 70%, 80%, 90%, 95%, or 100% sequence identity to full-length, or fragment, e.g., comprising amino acids 1 to 560, 38-557, 50 to 500, 100 to 500, 200 to 400, 1 to 100, 100 to 200, 200 to 300, 300 to 400, or 500 to 560, of NP protein (Accession NO.: AGK63064.1, SEQ ID NO: 116) from Influenza B/Brisbane/60/2008. In some cases, a polypeptide can comprise amino acid sequence (full-length or fragment) of NP protein from a virus that belongs to B/Yamagata lineage. For example, a polypeptide can comprise amino acid sequence with at least 50%, 60%, 70%, 80%, 90%, 95%, or 100% sequence identity to full-length, or fragment, e.g., comprising amino acids 1 to 560, 2 to 560, 50 to 500, 100 to 500, 200 to 400, 1 to 100, 2 to 100, 100 to 200, 200 to 300, 300 to 400, or 500 to 560, of NP protein (Accession NO.: ABL77260.1, SEQ ID NO: 117) from Influenza B/Yamagata/16/1988, or NP protein (Accession NO.: AOZ82278.1, SEQ ID NO: 118) from Influenza B/Pennsylvania/49/2015. In some cases, a polypeptide can comprise both an amino acid sequence (full-length or fragment) of NP protein from a virus that belongs to B/Victoria lineage, or a derivative thereof, and an amino acid sequence (full-length or fragment) of NP protein from a virus that belongs to B/Yamagata lineage, or a derivative thereof.

[0085] A polypeptide provided herein can comprise, consist of, or consist essentially of, one or more epitope sequences arranged in order. In some cases, epitope sequences are arranged in a particular order with the consideration of promoting immunogenicity, increasing expression, facilitating polypeptide stability, increasing polypeptide solubility, facilitating the in vivo cleavage of the polypeptide chain, or any other factors that may affect the vaccine performance, or any combination thereof. It is also possible to manipulate the order of the epitope sequences in the polypeptide in order to finely tune certain aspects of a vaccine, either upregulating or downregulating one or more certain parameters as one skilled in the art would be able to achieve.

[0086] In some embodiments, the polypeptide comprises more than one epitope sequence that are linked together directly, e.g., "back-to-back". Alternatively, the polypeptide can comprise, consist of, or consist essentially of, more than one epitope sequence, at least two neighboring epitope sequences among which are linked with a linker sequence. The polypeptide can comprise a single type of linker sequence throughout. The polypeptide can comprise more than one different type of linker sequence. The choice of linker sequence can vary depending on the selection of peptide sequences, the specific requirement for a number of different parameters, such as, but not limited to, expression level, folding and stability, solubility, cellular and subcellular targeting, immunogenicity, half-life in vitro and in vivo.

[0087] Linkers can be short amino acid sequences to separate multiple domains in a single polypeptide. In some cases, the linker sequence can comprise 3, 4, 5, 6, 7, 8, 9, 10, or more amino acids. The linker sequence can comprise at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20, or at least 50 amino acids. The linker sequence can comprise at most 4, at most 5, at most 6, at most 7, at most 8, at most 9, at most 10, at most 11, at most 12, at most 15, at most 20, at most 30, at most 40, at most 50, or at most 100 amino acids.

[0088] The linker sequence can comprise sequences occurring in natural multi-domain proteins that link the domains therein. The linker sequence can comprise an artificially created linker. The linker can also be a joined product of both a natural linker protein and an artificially created sequence. In some cases, specific linker sequences can be selected for in vivo cleavability of the polypeptide. For example, it can be desirable to cleave between certain epitope sequences, rendering the separated two or more parts of the polypeptide presented to the antigen-presenting cells separately. A linker can comprise a plurality of glycines, alanines, or any combinations thereof. A linker can comprise a plurality of arginines, valines, lysines, or any combinations thereof. Under such exemplary circumstances, linker sequences such as, LEAGCKNFFPRSFTSCGSLE (SEQ ID NO: 95), CRRRRRREAEAC (SEQ ID NO: 96), can be chosen. Sometimes, it can be desirable to use flexible linker sequences, such as, but not limited to, stretches of Gly and Ser residues ("GS" linker) like $(GGGGS)_n$ (n=1 to 10) (SEQ ID NO: 107), $(Gly)_8$ (SEQ ID NO: 97), GSAGSAAGSGEF (SEQ ID NO: 98), $(GGGGS)_4$ (SEQ ID NO: 99). In some cases, it can be desirable to use rigid linker sequences, such as, but not limited to, $(EAAAK)_n$ (SEQ ID NO: 108), Pro-rich sequences like $(XP)_n$ (SEQ ID NO: 109), with X designating any amino acid can be used (n=1 to 20). In some cases, the linker sequence RVKR (SEQ ID NO: 110) can be

chosen. The linker sequence RVKR (SEQ ID NO: 110) can be immunostimulatory in some situations. The polypeptide can comprise, consist of, or consist essentially of, each sequence from Table 1, Table 2, or Table 3, wherein each sequence is separated by a linker. The polypeptide can comprise, consist of, or consist essentially of, each sequence from Table 1, Table 2, or Table 3, wherein each sequence is not separated by a linker. The polypeptide can comprise, consist of, or consist essentially of, each sequence from Table 1, Table 2, or Table 3, wherein some of the sequences are separated by a linker and some are not separated by a linker.

[0089] In certain aspects of the present disclosure, a polypeptide provided herein further comprises a CD4+ (helper) T cell epitope that is connected to one or more of the epitope sequences described above. A "connection" can be, e.g., a direct or indirect covalent linkage, or a direct or indirect non-covalent linkage. The CD4+ (helper) T cell epitope can be ISQAVHAAHAEINEAGR (SEQ ID NO: 100). In some cases, the CD4+ (helper) T cell epitope is AKFVAAWTLKAAA (HLA DR-binding Epitope, PADRE) (SEQ ID NO: 101), or a non-natural amino acid derivative of the PADRE sequence, AKXVAAWTLKAAAZC (SEQ ID NO: 102), wherein X is L-cyclohexylalanine and Z is aminocaproic acid. In some cases, the CD4+ (helper) T cell epitope can be GALNNRFQIKGVELKSK (SEQ ID NO: 103). In some embodiments, the C-terminus of a polypeptide provided herein, e.g., a polypeptide comprising a sequence selected from SEQ ID NOs: 1-94 or a sequence selected from Table 1, Table 2, or Table 3, is attached to a lysine and the lysine is attached to the N-terminus of a CD4+ T cell epitope. The C-terminus of a CD4+ (helper) T cell epitope can be attached to a lysine and the lysine can be attached to the N-terminus of a peptide comprising a sequence selected from SEQ ID NOs: 1-94 or a sequence selected from Table 1, Table 2, or Table 3.

[0090] A polypeptide can be linked to a full length viral protein, e.g. full length PB1, PB1-F2, PB2, PA, HA, NP, NA, M1, M2, NS1, or NEP/NS2 protein, or the polypeptide can be link to a protein with at least 50%, 60%, 70%, 80%, 90%, 95%, or 100% sequence identity to at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% of the full-length sequence of any of these proteins, from, e.g., influenza A, influenza B, or influenza C, e.g., via a linker described herein, or the connection can be without a linker.

[0091] A polypeptide provided herein can comprise one or more natural amino acids, unnatural amino acids, or a combination thereof. An amino acid residue can be a molecule containing both an amino group and a carboxyl group. Suitable amino acids for use in the peptides described include, without limitation, both the D- and L-isomers (amino acid isomer) of the naturally-occurring amino acids, as well as non-naturally occurring amino acids prepared by organic synthesis or other metabolic routes. An amino acid can be an α-amino acid, β-amino acid, natural amino acid, non-natural amino acid, or amino acid analog. An α-amino acid can be molecule containing both an amino group and a carboxyl group bound to a carbon which is designated the α-carbon. A α-amino acid can be a molecule containing both an amino group and a carboxyl group in a β configuration. A naturally occurring amino acid can be any one of the twenty amino acids commonly found in peptides synthesized in nature, and known by the one letter abbreviations A, R, N, C, D, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y and V.

[0092] A polypeptide provided herein can comprise one or more hydrophobic, hydrophilic, polar, or charged amino acids. A hydrophobic amino acid can include small hydrophobic amino acids and large hydrophobic amino acids. A small hydrophobic amino acid can be glycine, alanine, proline, and analogs and isomers thereof. A large hydrophobic amino acid can be a valine, leucine, isoleucine, phenylalanine, methionine, tryptophan, and analogs and isomers thereof. A polar amino acid can be a serine, threonine, asparagine, glutamine, cysteine, tyrosine, and analogs and isomers thereof. A charged amino acid can be a lysine, arginine, histidine, aspartate, glutamate, or analog thereof.

[0093] A polypeptide as provided herein can comprise one or more amino acid analogs. An amino acid analog can be a molecule which is structurally similar to an amino acid and which can be substituted for an amino acid in the formation of a peptidomimetic macrocycle. Amino acid analogs include β-amino acids and amino acids where the amino or carboxyl group is substituted by a similarly reactive group (e.g., substitution of the primary amine with a secondary or tertiary amine, or substitution of the carboxyl group with an ester).

[0094] A polypeptide provided herein can comprise one or more non-natural amino acids. A non-natural amino acid can be an amino acid which is not one of the twenty amino acids commonly found in peptides synthesized in nature, and known by the one letter abbreviations A, R, N, C, D, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y and V.

[0095] Amino acid analogs can include β-amino acid analogs. Amino acid analogs can include analogs of alanine, valine, glycine, leucine, arginine, lysine, aspartic acids, glutamic acids, cysteine, methionine, phenylalanine, tyrosine, proline, serine, threonine, and/or tryptophan.

[0096] Amino acid analogs can be racemic. In some embodiments, the D isomer of the amino acid analog is used. In some cases, the L isomer of the amino acid analog is used. In some embodiments, the amino acid analog comprises chiral centers that are in the R or S configuration. Sometimes, the amino group(s) of a β-amino acid analog is substituted with a protecting group, e.g., tert-butyloxycarbonyl (BOC group), 9-fluorenylmethyloxycarbonyl (FMOC), tosyl, and the like. Sometimes, the carboxylic acid functional group of a β-amino acid analog is protected, e.g., as its ester derivative. In some cases, the salt of the amino acid analog is used.

[0097] A polypeptide provided herein can comprise a non-essential amino acid. A non-essential amino acid residue can be a residue that can be altered from the wild-type sequence of a peptide without abolishing or substantially altering its essential biological or biochemical activity (e.g., receptor binding or activation). A peptide provided herein can comprise an essential amino acid. An essential amino acid residue can be a residue that, when altered from the wild-type sequence of the peptide, results in abolishing or substantially abolishing the peptide's essential biological or biochemical activity.

[0098] A polypeptide provided herein can comprise a conservative amino acid substitution. A conservative amino acid substitution can be one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families can include amino acids with basic side chains (e.g., K, R, H), acidic side chains (e.g., D, E), uncharged polar side chains

(e.g., G, N, Q, S, T, Y, C), nonpolar side chains (e.g., A, V, L, I, P, F, M, W), beta-branched side chains (e.g., T, V, I) and aromatic side chains (e.g., Y, F, W, H). Thus, a predicted nonessential amino acid residue in a peptide, for example, can be replaced with another amino acid residue from the same side chain family. Other examples of acceptable substitutions can be substitutions based on isosteric considerations (e.g., norleucine for methionine) or other properties (e.g., 2-thienylalanine for phenylalanine, or 6-Cl-tryptophan for tryptophan).

[0099] Vaccine Compositions

[0100] Individual epitope sequences provided herein, polypeptides in which individual epitope sequences are linked, as provided herein, nucleic acid expressing individual epitope sequences or polypeptides provided herein, vectors comprising nucleic acid expressing individual epitope sequences or polypeptides provided herein, or viruses comprises such nucleic acid or vectors, can be formulated as vaccines. Vaccines provided herein can be any substance used to stimulate the production of antibodies and provide immunity against one or more diseases, e.g., influenza. The vaccines can be prepared from live pathogens, live attenuated pathogens, or inactivated pathogens that have been inactivated by e.g., chemicals, heat, or radiation. The vaccines can contain subunits or portions of a pathogen, in which these subunits can be optionally conjugated. The vaccine can also be prepared as a peptide-based vaccine, a nucleic acid-based vaccine, a viral vector-based vaccine, an antibody based vaccine, or an antigen-presenting cell based vaccine.

[0101] The vaccines can protect against pathogens, for example. A pathogen can be any infectious organism, including bacteria, fungi, viruses, protozoa, and others. The vaccines can also include tumor or cancer vaccines. A composition, e.g., a vaccine, provided herein can induce a systemic immune response, when administered into a subject body, e.g. human body. A composition, e.g., a vaccine, provided herein can induce a mucosal immune response, e.g., in the respiratory tract, in addition to systemic immune response, when administered into a subject body, e.g. human body.

[0102] The vaccines can be a traditional vaccine or a universal vaccine. A traditional vaccine can be a vaccine that can target a specific pathogen. Measles vaccine is one example of a traditional vaccine. It can target epitopes present on the hemagglutinin (H) protein of the Measles virus that have remained conserved over 50 years.

[0103] Seasonal vaccines can be another type of traditional vaccine. For example, an influenza vaccine can be modified annually and is tailored to the population of influenza viruses present at a given year. In some cases, an influenza vaccine is generated as a trivalent vaccine, which can include two subtypes of the influenza A virus, H1N1 and H3N2, and one strain of the influenza B virus. Sometimes, the influenza vaccine is generated as a quadrivalent vaccine, which can include two subtypes of influenza A virus and two strains of influenza B virus. The specific strains of the influenza A and B viruses can be chosen based on surveillance-based forecasts that can predict the pathogenicity of the circulating strains each year and can vary from country to country.

[0104] A universal vaccine can be a vaccine that offers broad-based protection against multiple strains of a pathogen, and/or against multiple pathogens within the same family. Exemplary universal vaccines include SynCon® influenza vaccines from Inovio Pharmaceuticals, M-001 from BiondVax, and FP-01 from Immune Targeting Systems. These universal vaccines can target conserved regions or epitopes that exist within the influenza viral proteins. Conserved regions or epitopes can exhibit at least 70%, 80%, 90%, 95%, 99% sequence homology or sequence identity.

[0105] Vaccine compositions can be formulated using one or more physiologically acceptable carriers including excipients and auxiliaries which facilitate processing of one or more active agents, such as one or more peptides, nucleic acids, proteins (e.g., antibodies or fragments thereof), APCs, or viruses described herein, into preparations which can be used pharmaceutically. Proper formulation can be dependent upon the route of administration chosen.

[0106] In some cases, the vaccine composition is formulated as a peptide-based vaccine, a nucleic acid-based vaccine, an antibody based vaccine, a cell based vaccine, or a virus-based vaccine. For example, a vaccine composition can include naked cDNA in cationic lipid formulations; lipopeptides (see e.g., Vitiello, A. et al, J. Clin. Invest. 95:341, 1995), naked cDNA or peptides, encapsulated e.g., in poly(DL-lactide-co-glycolide) ("PLG") microspheres (see, e.g., Eldridge, et al, Molec. Immunol. 28:287-294, 1991: Alonso et al, Vaccine 12:299-306, 1994; Jones et al, Vaccine 13:675-681, 1995); peptide composition contained in immune stimulating complexes (ISCOMS) (see, e.g. Takahashi et al, Nature 344:873-875, 1990; Hu et al, Clin Exp Immunol. 113:235-243, 1998); or multiple antigen peptide systems (MAPs) (see e.g., Tarn, J. P., Proc. Natl Acad. Sci. U.S.A. 85:5409-5413, 1988; Tarn, J. P., J. Immunol. Methods 196: 17-32, 1996). Sometimes, a vaccine is formulated as a peptide-based vaccine, or nucleic acid based vaccine in which the nucleic acid encodes the peptides. Sometimes, a vaccine is formulated as an antibody based vaccine. Sometimes, a vaccine is formulated as a cell based vaccine.

[0107] Vaccine compositions can be formulated using one or more physiologically acceptable carriers including excipients and auxiliaries which facilitate processing of one or more active agents, such as one or more peptides, nucleic acids, proteins (e.g., antibodies or fragments thereof), APCs, or viruses described herein, into preparations which can be used pharmaceutically. Proper formulation can be dependent upon the route of administration chosen.

[0108] Peptide-Based Vaccine

[0109] Provided herein is a peptide-based vaccine that comprises one or more epitope sequences or one or more polypeptides described herein. For instance, the polypeptide can comprise, consist of, or consist essentially of, one or more epitope sequences selected from the group consisting of: SEQ ID NOs: 1-94, or one or more epitope sequences selected from Table 1, Table 2, or Table 3. The peptide-based vaccine can comprise one polypeptide. The peptide-based vaccine can comprises at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 51, or more different peptide sequences, e.g., each peptide can have at least 70%, 75%, 80%, 85%, 90%, 95%, or 100% sequence identity to at least 8, 9, 10, 14, 16, 17, 18, or 25 amino acids of SEQ ID NOs: 2, 3, 8, 11, 12, 17, 20, 21, 22, 24, 26, 29, 30, 31, 32, 33, 34, 40, 41, 43, 44, 45, 49, 51, 52, 53, 58, 59, 60, 61, 62, 70, 73, 74, 75, 76, 77, 78, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92,

93, or 94. The peptide-based vaccine can be used to treat or prevent an influenza infection.

[0110] In some embodiments, a composition comprises, consists essentially of, or consists of one or more peptides or polypeptides, which may or may not be purified peptides or polypeptides as described herein. As used herein, a composition "comprising" one or more peptides or polypeptides as described herein can mean that the composition can contain other compounds, including one or more proteins that are not described herein. As used herein, a composition "consisting essentially of" one or more peptides or polypeptides can mean that the composition can comprise other compounds in addition to the peptides or polypeptides described herein so long as the additional compounds do not materially change the activity or function of the one or more peptides or polypeptides that are contained in the composition. As used herein, a composition "consisting of" one or more peptides or polypeptides as described herein can mean that the composition does not contain other proteins in addition to the one or more peptides or polypeptides described herein. Compositions consisting of one or more peptides or polypeptides described herein can comprise ingredients other than proteins, e.g., pharmaceutically acceptable carriers, surfactants, preservatives, etc. In some embodiments, compositions consisting of one or more peptides or polypeptides described herein can contain insignificant amounts of contaminants, which can include peptide or polypeptide contaminants, e.g., smaller fragments of the one or more peptides or polypeptides described herein, which can result from, for example, the synthesis of the one or more peptides or polypeptides described herein, subsequent processing, storage conditions, and/or protein degradation.

[0111] Peptide-based vaccine can be formulated using techniques, carriers, and excipients as suitable. The peptide-based vaccines can be formulated to improve their biological half-life, stability, efficacy, bioavailability, bioactivity, or a combination thereof.

[0112] Sometimes, a vaccine can comprises a cocktail of multiple polypeptides described herein containing the same sequence, or a cocktail of multiple copies of different polypeptides described herein. The polypeptides can be modified, such as by lipidation, or attachment to a carrier protein. Lipidation can be the covalent attachment of a lipid group to a polypeptide. Lipidated polypeptides can stabilize structures and can enhance efficacy of the vaccine treatment.

[0113] Lipidation can be classified into several different types, such as N-myristoylation, palmitoylation, GPI-anchor addition, prenylation, and several additional types of modifications. N-myristoylation can be the covalent attachment of myristate, a C14 saturated acid, to a glycine residue. Palmitoylation can be thioester linkage of long-chain fatty acids (CI 6) to cysteine residues. GPI-anchor addition can be glycosyl-phosphatidylinositol (GPI) linkage via amide bond. Prenylation can be the thioether linkage of an isoprenoid lipid (e.g., farnesyl (C-15), geranylgeranyl (C-20)) to cysteine residues. Additional types of modifications can include attachment of S-diacylglycerol by a sulfur atom of cysteines, 0-octanoyl conjugation via serine or threonine residues, S-archaeol conjugation to cysteine residues, and cholesterol attachment.

[0114] Fatty acids for generating a lipidated polypeptide can include C2 to C30 saturated, monounsaturated, or polyunsaturated fatty acyl groups. Exemplary fatty acids can include palmitoyl, myristoyl, stearoyl, and decanoyl groups.

[0115] In some embodiments, a lipid moiety that has adjuvant property is attached to a peptide of interest to elicit or enhance immunogenicity in the absence of an extrinsic adjuvant. A lipidated peptide or lipopeptide can be referred to as a self-adjuvant lipopeptide.

[0116] Any of the fatty acids described above and elsewhere herein can elicit or enhance immunogenicity of a peptide of interest. A fatty acid that can elicit or enhance immunogenicity can include palmitoyl, myristoyl, stearoyl, lauroyl, octanoyl, and decanoyl groups. In some cases, a fatty acid that can elicit or enhance immunogenicity can include palmitoyl groups. Non-limiting examples of palmitoyl group include Pam_2Cys, Pam_3Cys, or Pam_3OH.

[0117] Pam_2Cys, also known as dipalmitoyl-S-glyceryl-cysteine or S-[2,3 bis(palmitoyloxy) propyl]cysteine, corresponds to the lipid moiety of MALP-2, a macrophage-activating lipopeptide isolated from *Mycoplasma fermentans.*

[0118] Pam_3Cys, also known as Pam_3OH or N-palmitoyl-S-[2,3-bis(palmitoyloxy)propyl]cysteine, is a synthetic version of the N-terminal moiety of Braun's lipoprotein that spans the inner and outer membranes of Gram negative bacteria.

[0119] Other fatty acid groups contemplated for use include Set2Cys (also known as S-(2,3-bis(stearoyloxy) propyl) cysteine or distearoyl-5-glyceryl-cysteine), Lau2Cys (also known as S-[2,3-bis(lauroyloxy) propyl] cysteine or dilauroyl-S-glyceryl-cysteine); and Oct2Cys (also known as S-[2,3-bis(octanoyloxy)propyl]cysteine or dioctanoyl-S-glyceryl-cysteine).

[0120] Additional suitable fatty acid groups include synthetic triacylated and diacylated lipopeptides, FSL-I (a synthetic lipoprotein derived from *Mycoplasma salivarium* I), Pam_3Cys (tripaltnitoyl-S-glyceryl cysteine) and S-[2,3-bis (palmitoyloxy)-(2RS)-propyl]-N-palmitoyl-(R)-cysteine, where "Pam_3" is "tripalmitoyl-S-glyceryl". Derivatives of Pam_3Cys are also suitable for use, in which derivatives include S-[2,3-bis(palmitoyloxy)-(2-R,S)-propyl]-N-palmitoyl-(R)-Cys-(S)-Ser-(Lys)4-hydroxytrihydrochloride("(R)-Cys-(S)-Ser-(Lys)4" disclosed as SEQ ID NO: 111); Pam3Cys-Ser-Ser-Asn-Ala (SEQ ID NO: 112); PaM3Cys-Ser-(Lys)4 (SEQ ID NO: 113); Pam3Cys-Ala-Gly; PamsCys-Ser-Gly; Pam3Cys-Ser; PaM3CyS-OMe; Pam3Cys-OH; PamCAG, palmitoyl-Cys((RS)-2,3-di(palmitoyloxy)-propyl)-Ala-Gly-OH; and the like. Another non-limiting examples include Pam2CSK4 (SEQ ID NO: 114) (dipalmitoyl-S-glyceryl cysteine-serine-(lysine)4; or Pam2Cys-Ser-(Lys)4 (SEQ ID NO: 114)).

[0121] Peptides such as naked peptides or lipidated peptides can be incorporated into a liposome. For example, the lipid portion of the lipidated peptide can spontaneously integrate into the lipid bilayer of a liposome. Thus, a lipopeptide can be presented on the "surface" of a liposome. A lipidated peptide can be a peptide that is encapsulated within a liposome.

[0122] Exemplary liposomes suitable for incorporation in the formulations include, and are not limited to, multilamellar vesicles (MLV), oligolamellar vesicles (OLV), unilamellar vesicles (UV), small unilamellar vesicles (SUV), medium-sized unilamellar vesicles (MUV), large unilamellar vesicles (LUV), giant unilamellar vesicles (GUV), multivesicular vesicles (MVV), single or oligolamellar vesicles made by reverse-phase evaporation method (REV), multilamellar vesicles made by the reverse-phase evaporation

method (MLV-REV), stable plurilamellar vesicles (SPLV), frozen and thawed MLV (FATMLV), vesicles prepared by extrusion methods (VET), vesicles prepared by French press (FPV), vesicles prepared by fusion (FUV), dehydration-rehydration vesicles (DRV), and bubblesomes (BSV).

[0123] Depending on the method of preparation, liposomes can be unilamellar or multilamellar, and can vary in size with diameters ranging from about 0.02 μm to greater than about 10 μm. Sometimes, the liposomes can be small unilamellar vesicles (25-50 nm), large unilamellar vesicles (100-200 nm), giant unilamellar vesicles (1-2 μm), and multilamellar vesicles (MLV; 1 μm-2 μm). The peptides being delivered can be either encapsulated into liposomes or adsorbed on the surface. The size and surface properties of liposomes can be optimized for a desired result. For example, unilamellar and multilamellar liposomes provide sustained release from several hours to days after intravascular administration. The prolonged drug release can be achieved by multivesicular liposomes, also known as DepoFoam® technology. Unlike ULV and MLV, multivesicular liposomes are composed of nonconcentric multiple aqueous chambers surrounded by a network of lipid layers which confers an increased level of stability and longer duration of drug release. The liposomes can be further modified to achieve a desired result. For example, the liposomes can be PEGylated or have other surface modifications in order to interfere with recognition and uptake by the reticuloendothelial system and provide increased circulation times.

[0124] Liposomes can adsorb many types of cells and then release an incorporated agent (e.g., a polypeptide described herein). In some cases, the liposomes fuse with the target cell, whereby the contents of the liposome then empty into the target cell. A liposome can be endocytosed by cells that are phagocytic. Endocytosis can be followed by intralysosomal degradation of liposomal lipids and release of the encapsulated agents.

[0125] The liposomes provided herein can also comprise carrier lipids. In some embodiments the carrier lipids are phospholipids. Carrier lipids capable of forming liposomes include, but are not limited to dipalmitoylphosphatidylcholme (DPPC), phosphatidylcholine (PC; lecithin), phosphatidic acid (PA), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidylserine (PS). Other suitable phospholipids further include distearoylphosphatidylcholine (DSPC), dimyristoylphosphatidylcholine (DMPC), dipalmitoylphosphatidyglycerol (DPPG), distearoylphosphatidyglycerol (DSPG), dimyristoylphosphatidylglycerol (DMPG), dipalmitoylphosphatidic acid (DPPA); dimyristoylphosphatidic acid (DMPA), distearoylphosphatidic acid (DSP A), dipalmitoylphosphatidylserine (DPPS), dimyristoylphosphatidylserine (DMPS), distearoylphosphatidylserine (DSPS), dipalmitoylphosphatidyethanolamine (DPPE), dimyristoylphosphatidylethanolamine (DMPE), distearoylphosphatidylethanolamine (DSPE) and the like, or combinations thereof. In some embodiments, the liposomes further comprise a sterol (e.g., cholesterol) which modulates liposome formation. The carrier lipids can be any known non-phosphate polar lipids.

[0126] A polypeptide as described herein can also be attached to a carrier protein for delivery as a vaccine. The carrier protein can be an immunogenic carrier element and can be attached by any recombinant technology. Exemplary carrier proteins include Mariculture keyhole limpet hemocyanin (mcKLH), PEGylated mcKLH, Blue Carrier® Proteins, bovine serum albumin (BSA), cationized BSA, ovalbumin, and bacterial proteins such as tetanus toxoid (TT).

[0127] A polypeptide as described herein can also be prepared as multiple antigenic peptides (MAPs). Polypeptides can be attached at the N-terminus or the C-terminus to small non-immunogenic cores. Polypeptides built upon this core can offer highly localized peptide density. The core can be a dendritic core residue or matrix composed of bifunctional units. Suitable core molecules for constructing MAPs can include ammonia, ethylenediamine, aspartic acid, glutamic acid, and lysine. For example, a lysine core molecule can be attached via peptide bonds through each of its amino groups to two additional lysines. This second generation molecule has four free amino groups, each of which can be covalently linked to an additional lysine to form a third generation molecule with 8 free amino groups. A polypeptide can be attached via its C-terminus to each of these free groups to form an octavalent multiple antigenic peptide (also referred to as a "MAP8" structure). The second generation molecule having four free amino groups can be used to form a tetravalent or tetrameric MAP, e.g., a MAP having four peptides covalently linked to the core (also referred to as a "MAP4" structure). The carboxyl group of the first lysine residue can be left free, amidated, or coupled to β-alanine or another blocking compound. As used herein, the "linear portion or molecule" of a MAP system structure can refer to antigenic peptides that are linked to the core matrix. Thus, a cluster of antigenic epitopes can form the surface of a MAP and a small matrix forms its core. The dendritic core, and the entire MAP can be synthesized on a solid resin using a classic Merrifield synthesis procedure.

[0128] The polypeptides used for MAP preparation can be identical or can comprise multiple different sequences and lengths. The polypeptides can be derived from a bacterium, a virus, or a fungus. The peptides can be derived from a virus, such as influenza A virus, influenza B virus, influenza C virus, hepatitis B virus, hepatitis C virus, or HIV.

[0129] Sometimes, a polypeptide as described herein can be subjected to cyclization to result in a cyclic peptide which is resistant to proteolytic degradation. Cyclization can be carried out between side chains or ends of the peptide sequences through disulfide bonds, lanthionine, dicarba, hydrazine, or lactam bridges using methods known in the art.

[0130] In some embodiments, the polypeptide as described herein are conjugated to a molecule such as vitamin B12, a lipid, or an ethylene oxide compound, e.g., polyethylene glycol (PEG), polyethylene oxide (PEO), and polyoxyethylene (POE), methoxypolyethylene glycol (MPEG), mono-methoxy PEG (mPEG), and the like. The ethylene oxide compound can be further functionalized with, for example, amine binding terminal functional groups such as N-hydroxysuccinimide esters, N-hydroxysuccinimide carbonates, and aliphatic aldehyde, or thiol binding groups such as maleimide, pyridyl disulphides, and vinyl sulfonates. Since amino groups (a-amino and ε-lysine amino) and cysteine residues are well suited for conjugation, the peptides provided herein can further include one or more amino acid residues for conjugation to an ethylene oxide molecule or a carrier compound known in the art. The pharmacokinetic and pharmacodynamic properties of a conjugated peptide can be further modified by the use of a particular linker. For example, propyl and amyl linkers can be used to provide a conjugate having a loose conformation

whereas a phenyl linker can be used to provide a denser conformation as well as shield domains adjacent to the C-terminus. In some instances, dense conformations can be more efficient in maintaining bioactivity, prolonging plasma half-life, lowering proteolytic sensitivity, and immunogenicity relative to loose conformations.

[0131] In some embodiments, the polypeptides as described herein can be hyperglycosylated using methods known in the art, e.g., in situ chemical reactions or site-directed mutagenesis. Hyperglycosylation can result in either N-linked or O-linked protein glycosylation. The clearance rate of a given peptide can be optimized by the selection of the particular saccharide. For example, polysialic acid (PSA) is available in different sizes and its clearance depends on type and molecular size of the polymer. Thus, for example, PSAs having high molecular weights can be suitable for the delivery of low-molecular-weight peptides, and PSAs having low molecular weights can be suitable for the delivery of peptides having high molecular weights. The type of saccharide can be used to target the peptide to a particular tissue or cell. For example, polypeptides conjugated with mannose can be recognized by mannose-specific lectins, e.g., mannose receptors and mannose binding proteins, and are taken up by the liver. In some embodiments, the polypeptides can be hyperglycosylated to improve their physical and chemical stability under different environmental conditions, e.g., to inhibit inactivation under stress conditions and reduce aggregation resulting from production and storage conditions.

[0132] In some embodiments, a drug delivery system, such as microparticles, nanoparticles (particles having sizes ranging from 10 to 1000 nm), nanoemulsions, liposomes, and the like, can be used to provide protection of sensitive proteins, prolong release, reduce administration frequency, increase patient compliance, and control plasma levels. Various natural or synthetic microparticles and nanoparticles, which can be biodegradable and/or biocompatible polymers, can be used. Microparticles and nanoparticles can be fabricated from lipids, polymers, and/or metal. Polymeric microparticles and nanoparticles can be fabricated from natural or synthetic polymers, such as starch, alginate, collagen, chitosan, polycaprolactones (PCL), polylactic acid (PLA), poly (lactide-co-glycolide) (PLGA), and the like. In some embodiments, the nanoparticles are solid lipid nanoparticles (SLNs), carbon nanotubes, nanospheres, nanocapules, and the like. In some embodiments, the polymers are hydrophilic. In some embodiments, the polymers are thiolated polymers.

[0133] Since the rate and extent of drug release from microparticles and nanoparticles can depend on the composition of polymer and fabrication methods one can select a given composition and fabrication method, e.g., spray drying, lyophilization, microextrusion, and double emulsion, to confer a desired drug release profile. Since peptides incorporated in or on microparticles or nanoparticles can be prone to denaturation at aqueous-organic interface during formulation development, different stabilizing excipients and compositions can be used to prevent aggregation and denaturation. For example, PEG and sugars, e.g., PEG (MW 5000) and maltose with a-chymotrypsin, can be added to the composition to reduce aggregation and denaturation. Additionally, chemically modified peptides, e.g., conjugated peptides and hyperglycosylated peptides, as described herein, can be employed.

[0134] Protein stability can also be achieved by the selected fabrication method. For example, to prevent degradation at aqueous-organic interface, non-aqueous methodology called ProLease® technology can be used. Peptides in solid state can also be encapsulated using solid-in-oil-in-water (s/o/w) methods, e.g., spray- or spray-freeze-dried peptides or peptide-loaded solid nanoparticles can be encapsulated in microspheres using s/o/w methods.

[0135] Hydrophobic ion-pairing (HIP) complexation can be used to enhance protein stability and increase encapsulation efficiency into microparticles and nanoparticles. In hydrophobic ion-pairing (HIP) complexation, ionizable functional groups of a peptide are complexed with ion-pairing agents (e.g., surfactant or polymer) containing oppositely charged functional groups leading to formation of HIP complex where hydrophilic protein molecules exist in a hydrophobic complex form.

[0136] A polypeptide described herein can be chemically synthesized, or recombinantly expressed in a cell system or a cell-free system. A polypeptide can be synthesized, such as by a liquid-phase synthesis, a solid-phase synthesis, or by microwave assisted peptide synthesis. A polypeptide as described herein can be modified, such as by acylation, alkylation, amidation, arginylation, polyglutamylation, polyglycylation, butyrylation, gamma-carboxylation, glycosylation, malonylation, hydroxylation, iodination, nucleotide addition (e.g., ADP-ribosylation), oxidation, phosphorylation, adenylylation, propionylation, S-glutathionylation, S-nitrosylation, succinylation, sulfation, glycation, palmitoylation, myristoylation, isoprenylation or prenylation (e.g., farnesylation or geranylgeranylation), glypiation, lipoylation, attachement of flavin moiety (e.g., FMN or FAD), attachment of heme C, phosphopantetheinylation, retinylidene Schiff base formation, diphthamide formation, ethanolamine phosphoglycerol attachment, hypusine formuation, biotinylation, pegylation, ISGylation, SUMOylation, ubiquitination, Neddylation, Pupylation, citrullination, deamidation, eliminylation, carbamylation, or a combination thereof.

[0137] After generation of a polypeptide, the polypeptide can be subjected to one or more rounds of purification steps to remove impurities. The purification step can be a chromatographic step utilizing separation methods such as affinity-based, size-exclusion based, ion-exchange based, or the like. In some cases, the peptide is at most 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, 99.9%, or 100% pure or without the presence of impurities. In some cases, the peptide is at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, 99.9%, or 100% pure or without the presence of impurities. In some cases, the amount of the peptides in the peptide composition is at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, 99.9%, or 100% by weight of the total composition. As used herein, a "purified" peptide of polypeptide can mean that an amount of the macromolecular components that are naturally associated with the peptide have been removed from the peptide. As used herein, a composition comprising, consisting essentially of, or consisting of one or more purified peptides of the present invention can mean that the composition does not contain an amount of the macromolecular components that are naturally associated with the one or more peptides or polypeptides and/or the reagents used to synthesize the peptides or polypeptides. In some embodiments, the compositions described herein consist solely of one or more peptides or

polypeptides described herein, e.g., one or more peptides or polypeptides in a solid or crystalized form.

[0138] In some embodiments, the peptides or polypeptides or nucleic acid molecules of described herein can be isolated. As used herein, an "isolated" compound (e.g., peptide, polypeptide, nucleic acid molecule) can refer to a compound which is isolated from its native environment. For example, an isolated peptide or polypeptide can be one which does not have its native amino acids which correspond to the full length polypeptide, flanking the N-terminus, C-terminus, or both. As another example, an isolated peptide can be one which is immobilized to a substrate with which the peptide is not naturally associated. As a further example, an isolated peptide or polypeptide can be one which is linked to another molecule, e.g., a PEG compound, with which the peptide is not naturally associated. Similarly, an "isolated" nucleic acid molecule can be one which does not have its native nucleic acid basses which correspond to the full length nucleic acid molecule, flanking its 5' end, 3' end, or both. As another example, an isolated nucleic acid molecule can be one which is bound to a substrate or a compound, e.g., a label such as a fluorescent tag, with which the nucleic acid molecule is not naturally associated. As a further example, with respect to nucleic acid molecules, the term isolated can mean that it is separated from the nucleic acid and cell in which it naturally occurs.

[0139] A peptide-based vaccine can comprise about, at least, or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 51, 55, 60, 65, 70, of 75 different peptide sequences. The different peptide sequences can include any polypeptide described herein.

[0140] Nucleic Acid-Based Vaccine

[0141] Provided herein is a nucleic acid-based vaccine that codes for about, at least, or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 51, 55, 60, 65, 70, of 75 polypeptides as described herein. The nucleic acid-based vaccine can be used to treat or prevent an influenza infection.

[0142] A nucleic acid-based vaccine can be formulated using techniques, carriers, and excipients as suitable. The nucleic acid can be DNA, both genomic and cDNA, RNA, or a hybrid, where the nucleic acid can contain combinations of deoxyribo- and ribo-nucleotides, and combinations of bases including uracil, adenine, thymine, cytosine, guanine, inosine, xanthine hypoxanthine, isocytosine and isoguanine. Nucleic acids can be obtained by chemical synthesis methods or by recombinant methods. The vaccine can be a DNA-based vaccine, an RNA-based vaccine, a hybrid DNA/RNA based vaccine, or a hybrid nucleic acid/peptide based vaccine. The peptide can be a polypeptide that has a sequence with at least 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% sequence homology or identity to a peptide selected from the group consisting of SEQ ID NOs: 1-94 or of a sequence selected from Table 1, Table 2, or Table 3. The peptide can be a polypeptide that has a sequence with at most 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% sequence homology or identity to a peptide selected from the group consisting of SEQ ID NOs: 1-94 or of a sequence selected from Table 1, Table 2, or Table 3.

[0143] Nucleic acid molecules can refer to at least two nucleotides covalently linked together. A nucleic acid described herein can contain phosphodiester bonds, although in some cases, as outlined below (for example in the construction of primers and probes such as label probes), nucleic acid analogs are included that can have alternate backbones, comprising, for example, phosphoramide, phosphorothioate, phosphorodithioate, O-methylphosphoroamidite linkages, and peptide nucleic acid (also referred to herein as "PNA") backbones and linkages. Other analog nucleic acids include those with bicyclic structures including locked nucleic acids (also referred to herein as "LNA"); positive backbones; non-ionic backbones and non-ribose backbones. Nucleic acids containing one or more carbocyclic sugars are also included within the definition of nucleic acids (see e.g., Jenkins et al, Chem. Soc. Rev. (1995) pp 169 176). Several nucleic acid analogs are described, e.g., in Rawls, C & E News Jun. 2, 1997 page 35. "Locked nucleic acids" are also included within the definition of nucleic acid analogs. LNAs are a class of nucleic acid analogues in which the ribose ring is "locked" by a methylene bridge connecting the 2-0 atom with the 4'-C atom. All of these references are hereby expressly incorporated by reference. These modifications of the ribose-phosphate backbone can be done to increase the stability and half-life of such molecules in physiological environments. For example, PNA:DNA and LNA-DNA hybrids can exhibit higher stability and thus can be used in some embodiments. The target nucleic acids can be single stranded or double stranded, as specified, or contain portions of both double stranded or single stranded sequence. Depending on the application, the nucleic acids can be DNA (including, e.g., genomic DNA, mitochondrial DNA, and cDNA), RNA (including, e.g., mRNA and rRNA) or a hybrid, where the nucleic acid contains any combination of deoxyribo- and ribo-nucleotides, and any combination of bases, including uracil, adenine, thymine, cytosine, guanine, inosine, xathanine hypoxathanine, isocytosine, isoguanine, etc.

[0144] Provided herein is a vector that comprises a polynucleotide that codes for a polypeptide as described herein. In some cases, the vector can be used to treat or prevent influenza infection. In some cases, the vector can be used to produce one or more of the polypeptides described herein.

[0145] A polynucleotide encoding a polypeptide provided herein can be codon optimized for a target subject, such as human being, mouse, pig, or dog. For example, a polynucleotide provided herein can be codon optimized for mice for pre-clinical animal experiments. In some cases, the subject matter may find use in preventing influenza for agriculture, for example, for preventing swine flu, or avian flu. This type of optimization can entail the mutation of foreign-derived (e.g., recombinant) DNA to mimic the codon preferences of the intended host organism or cell while encoding the same protein.

[0146] The vector can be a circular plasmid or a linear nucleic acid. The circular plasmid or linear nucleic acid can be capable of directing expression of a particular nucleotide sequence in an appropriate subject cell. The vector can have a promoter operably linked to the peptide-encoding nucleotide sequence, which can be operably linked to termination signals. The vector can also contain sequences required for proper translation of the nucleotide sequence. The vector comprising the nucleotide sequence of interest can be chimeric, meaning that at least one of its components is heterologous with respect to at least one of its other components. The expression of the nucleotide sequence in the expression cassette can be under the control of a constitutive

promoter or of an inducible promoter, which can initiate transcription only when the host cell is exposed to some particular external stimulus.

[0147] The vector can be a plasmid. The plasmid can be useful for transfecting cells with nucleic acid encoding the peptide, which the transformed host cells can be cultured and maintained under conditions wherein expression of the peptide takes place.

[0148] The plasmid can comprise a nucleic acid sequence that encodes one or more of the various polypeptides disclosed herein. A single plasmid can contain coding sequence for a single polypeptide, or coding sequence for more than one polypeptide. Sometimes, the plasmid can further comprise coding sequence that encodes an adjuvant, such as an immune stimulating molecule, such as a cytokine.

[0149] The plasmid can further comprise an initiation codon, which can be upstream of the coding sequence, and a stop codon, which can be downstream of the coding sequence. The initiation and termination codon can be in frame with the coding sequence. The plasmid can also comprise a promoter that is operably linked to the coding sequence, and an enhancer upstream of the coding sequence. The enhancer can be human actin, human myosin, human hemoglobin, human muscle creatine, or a viral enhancer such as one from CMV, FMDV, RSV, or EBV.

[0150] The plasmid can also comprise a mammalian origin of replication in order to maintain the plasmid extrachromosomally and produce multiple copies of the plasmid in a cell. The plasmid can be pVAXI, pCEP4, or pREP4 from Invitrogen (San Diego, CA).

[0151] The plasmid can also comprise a regulatory sequence, which can be well suited for gene expression in a cell into which the plasmid is administered. The coding sequence can comprise a codon that can allow more efficient transcription of the coding sequence in the host cell.

[0152] The plasmid can be pSE420 (Invitrogen, San Diego, CA), pYES2 (Invitrogen, San Diego, CA), MAX-BAC™ complete baculovirus expression system (Invitrogen, San Diego, CA), pcDNA I or pcDNA3 (Invitrogen, San Diego, CA).

[0153] The vector can be circular plasmid, which can transform a target cell by integration into the cellular genome or exist extrachromosomally (e.g., autonomous replicating plasmid with an origin of replication). Exemplary vectors include pVAX, pcDNA3.0, or provax, or any other expression vector capable of expressing DNA encoding the antigen and enabling a cell to translate the sequence to an antigen that is recognized by the immune system.

[0154] The nucleic acid based vaccine can also be a linear nucleic acid vaccine, or linear expression cassette ("LEC"), that can be efficiently delivered to a subject via electroporation and expressing one or more peptides disclosed herein. The LEC can be any linear DNA devoid of any phosphate backbone. The DNA can encode one or more peptides disclosed herein. The LEC can contain a promoter, an intron, a stop codon, and/or a polyadenylation signal. The expression of the peptide can be controlled by the promoter. It is also possible that the LEC does not contain any antibiotic resistance genes and/or a phosphate backbone. The LEC cannot contain other nucleic acid sequences unrelated to the polypeptide expression.

[0155] The LEC can be derived from any plasmid capable of being linearized. The plasmid can express the peptide. Exemplary plasmids include: pNP (Puerto Rico/34), pM2 (New Caledonia/99), WLV009, pVAX, pcDNA3.0, provax, or any other expression vector capable of expressing DNA encoding the antigen and enabling a cell to translate the sequence to an antigen that is recognized by the immune system.

[0156] The nucleic acid based vaccine can be delivered to a subject through a parenteral delivery method. A parenteral delivery can include intravenous, transdermal, oral, intrabiliary, intraparenchymal, intra-hepatic artery, intra-portal vein, intratumoral, or transvenous delivery. Sometimes, a parenteral delivery can utilize a needle (e.g., a hypodermic needle) for delivery of the nucleic acid based vaccine. The nucleic acid based vaccine can be formulated in an aqueous solution, e.g., saline. The delivery can be further assisted by electroporation. Sometimes, a parenteral delivery can utilize a gene gun as a delivery method. The nucleic acid based vaccine can be formulated as a DNA-coated microparticle, e.g., a DNA-coated gold or tungsten bead. The gene gun delivery method can use a ballistical delivery method to accelerate nucleic acid into target cells. Sometimes, a parenteral delivery can utilize a pneumatic injection as a delivery method. The nucleic acid based vaccine can be formulated as an aqueous solution.

[0157] The nucleic acid based vaccine can also be delivered to a subject through a topical delivery method. Topical nucleic acid based vaccine can be formulated as aerosol instillation of naked DNA to be delivered onto mucosal surfaces, such as the nasal and lung mucosa, ocular administration, or vaginal mucosa.

[0158] The nucleic acid based vaccine can further be delivered to a subject through a lipid-mediated delivery method. Sometimes, the lipid-mediated delivery method can be a cytofectin-mediated delivery method. Cytofectin can be cationic lipids that can bind and transport nucleic acid molecules across cell membranes. The nucleic acid can be incorporated by cytofectin-based liposomes. Sometimes, the lipid-mediated delivery method can be a neutral lipid-mediated delivery method.

[0159] A composition provided herein, e.g., a vaccine, can comprise at least 5, 10, 25, 50, 100, or 1000 different nucleic acids.

[0160] Recombinant Virus-Based Vaccine

[0161] Provided herein is a recombinant virus-based vaccine.

[0162] A vector as described above can be a viral vector, e.g. a recombinant viral vector. In some cases, a nucleic acid-based vaccine as described above can be in the form of a recombinant virus. The recombinant virus can comprise a recombinant viral vector as described herein that is encapsulated by a capsid protein, typically derived from the viral vector and from other viral origin than influenza virus.

[0163] The viral vector can be based on a range of different viruses, such as, but not limited to, adenoviruses, adeno-associated viruses (AAV), alphaviruses, baculoviruses, Newcastle Disease viruses (NDV), poxviruses. Parainfluenza Virus 5 (PIV5), and Vesicular Stomatitis Viruses (VSV). In some cases, the vector can be a recombinant viral vector that comprises polynucleotide that codes for one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10) of the polypeptides described herein and polynucleotide sequences that code for viral proteins from viruses other than influenza virus. A vaccine can comprise one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10) different viral vectors, each vector expressing a polypeptide with a different sequence.

[0164] An adenovirus based vaccine can infect broad range of hosts. In some cases, an adenovirus vaccine can induce high levels of transgene expression without the potential of viral genes being integrated into the host genome. Due to their ability to grow in high titers in cell culture, an adenovirus vaccine described herein can be manufactured safely and inexpensively. In some cases, adenoviral vectors that are used for a vaccine provided herein can inherently stimulate innate immune responses via Toll-like receptor-dependent and Toll-like receptor-independent pathways. In some cases, an adenovirus vaccine can also infect dendritic cells (DCs), thereby leading to more effective antigen presentation to immune cells, through e.g., up-regulation of co-stimulatory molecules, increased cytokine and chemokine production by the infected DCs, or both.

[0165] An adenoviral vector described herein can be generated in two different forms: replication-defective or replication-competent. Replication-defective adenoviral vectors can be rendered by deletion of the E1 genes, which can be essential for replication. Sometimes, replication-defective adenoviral vectors can be rendered to lack E3 genes as well in order to create more space for foreign gene inserts. An expression cassette with desired transgene, e.g., a polynucleotide that encodes one or more polypeptide described herein, can be inserted. Replication-competent adenoviral vectors can be rendered with the deletion of E3 genes. Sometimes, replication-competent Ad-vectors can mimic the natural viral infection, thereby a potent adjuvant effect can be exerted due to the inherent stimulation of various elements of innate and adaptive immunity.

[0166] In some embodiments of the present disclosure, the vector can be an adenoviral vector. In some instances, the vector is a non-human adenoviral vector. In some cases, the vector can be a non-human primate adenoviral vector. In some cases, the vector can be a chimpanzee adenoviral vector.

[0167] In certain embodiments, Chimpanzee adenovirus vector can be used for expressing one or more polypeptides, such as C68 (AdC68) (SEQ ID NO: 104), e.g., that is disclosed in U.S. Pat. No. 6,083,716, C7 (AdC7), e.g., that is disclosed in Tatsis, et al., "Chimpanzee-origin adenovirus vectors as vaccine carriers," Gene Therapy 13: 421-429 (2006), C6(AdC6) (SEQ ID NO: 105) that is disclosed in Haut et al., "A Partial E3 Deletion in Replication-Defective Adenoviral Vectors Allows for Stable Expression of Potentially Toxic Transgene Products", Human Gene Therapy Methods DOI: 10.1089/hgtb.2016.044 (2016), Pan7 and Pan9, which are both disclosed in Roy, et al., "Rescue of chimeric adenoviral vectors to expand the serotype repertoire," J Virol Methods 141(a): 14-21 (2007).

[0168] Alternatively, the vector can be based on AAV. Recombination AAV can have broad tropism infecting a variety of hosts, tissues, and proliferating and non-proliferating cell types. AAVs that can be used in connection with the present disclosure can include, but not limited to, AAV serotype 2 (AAV2), AAV5, AAV7, AAV1, and AAV6.

[0169] The vector can also be based on a baculovirus. Baculoviruses that can be used as vector for the vaccine provided herein, e.g., influenza vaccine, can include, but not limited to, alphabaculoviruses, betabaculoviruses, gammabaculoviruses, and deltabaculoviruses.

[0170] Alternatively, the vector can be based on a poxvirus. Poxviruses can be double-stranded DNA viruses. Poxvirus genome can be very large; mammalian poxviruses can possess a genome of approximately 130 kb, and avian poxvirus genome is even larger at approximately 300 kb. Such large genome size can enable the insertion of more than 10 kb of foreign DNA without compromising the infectivity or other essential viral functions. Poxviruses can have their own transcription machinery, viral DNA-dependent RNA polymerase and post-transcriptional modifying enzymes, thereby allowing self-sufficient cytoplasmic replication. As a result, inserted transgene products can be expressed at high levels, resulting in potent cellular immune responses.

[0171] Recombinant vaccinia virus can be created to express the polypeptide as described herein. Non-replicating poxviral vectors that can be used in connection with the present disclosure include, but not limited to, modified vaccinia virus Ankara (MVA), NYVAC, and ALVAC strains. MVA was rendered replication-deficient by loss of approximately 15% of its original genome resulting from repetitive passaging in chick embryo fibroblasts. NYVAC strain, derived from the Copenhagen strain of vaccinia, was rendered replication-defective by deletion of 18 different open reading frames from the original viral genome. ALVAC is a canarypoxviral vector that does not replicate in human cells with further attenuation induced via over 200 passages in chicken embryo fibroblasts.

[0172] Alternatively, the vector can be based on an alphavirus. Alphaviruses can be single-stranded positive-sense RNA viruses that can replicate in the cytoplasm of infected cells. Alphaviruses that can be used in connection with the present disclosure include, but are not limited to, Venezuelan equine encephalitis virus (VEE), Sindbis virus (SIN), Semliki forest virus (SFV), and VEE-SIN chimeras.

[0173] Alphaviral vectors can be designed with the deletion of genes encoding structural proteins. Such alphavirus vectors are known as "replicons". Alphavirus vectors can potentially target antigen presenting cells, such as dendritic cells, in the draining lymph nodes, which can lead to the efficient generation of antigen-specific immune responses. Also, alphavirus vectors can create a proper environment for the cross-priming of vaccine antigen by inducing apoptosis in some cells. Vaccine immunity can also be further enhanced by the alphavirus vector itself.

[0174] VEE can be pathogenic in humans, but SIN is not. VEE/SIN chimeras can be used to avoid safety concerns when human is the vaccination subject. In a VEE/SIN chimera, VEE can function as the replicon component and SIN as the structural and packaging components.

[0175] Other RNA viruses that can be used as a vector for producing a vaccine as described herein can include, but not limited to, NDV, PIV5, and VSV.

[0176] An adenoviral vector or an adenovirus based vaccine as described herein can be produced by a method provided herein, e.g., in accordance with procedures depicted in FIG. **5**.

[0177] A method of producing an adenovirus based vaccine can comprise preparation and purification of plasmid DNA. The plasmid DNA herein can be a recombinant adenoviral vector DNA that comprises a polynucleotide encoding a polypeptide that comprises one or more epitope sequences as described herein. As discussed above, a recombinant adenoviral vector can lack E region genes, e.g. E1, E3, E5, or a combination thereof. The deletion of the endogenous viral genes can offer genomic space for inser-

tion of gene of interest, e.g., polynucleotide expressing the polypeptide described herein. An E1-deleted Recombinant Adenoviral vector, as an example, can be constructed either by an in vitro ligation method or a homologous recombination method.

[0178] The in vitro ligation method can use whole adenoviral DNA genomes and a plasmid containing the left end of Ad with the right inverted terminal repeat (ITR), the packaging signal and E1A enhancer sequence. After the gene of interest, e.g., the polynucleotide encoding the polypeptide as described herein, can be inserted into the downstream of the viral sequence of the plasmid, the fragment containing viral sequence and gene of interest can be excised and ligated to a restriction site, replacing a portion of the viral E1 region, thereby producing a recombinant adenoviral DNA vector.

[0179] Alternatively, recombinant adenoviral vector can also be made by using homologous recombination method. Two or more plasmids with overlapping fragments that recombine in vivo can be used. An exemplary first plasmid can contain the entire Adenoviral genome with a deletion of the DNA packaging region and E1 region. An exemplary second plasmid (shuttle vector) can contain right ITR, packaging signal, overlapping sequence with the first plasmid. After the gene of interest, e.g. polynucleotide expressing a polypeptide described herein, can be introduced into the second plasmid, the two plasmids can be co-transfected into recombination cells. In the cells, homologous recombination can take place between the first and second plasmids, thereby producing a recombinant adenoviral vector. Non-limiting examples of cells for homologous recombination can include yeast, bacteria, and mammalian cell lines, such as, 293 cells, 293T cells, Hela cells. In some case, the recombinant adenoviral vector can be purified from the recombination cells. Alternatively, the recombination process can be conducted in vitro.

[0180] A method of producing an adenovirus based vaccine can further comprise transfection of host cells with the purified DNA plasmid. In some embodiments, the DNA plasmid, e.g. the recombinant adenoviral vector, is linearized before the transfection. In some cases, the transfection can generate adenoviral plaques.

[0181] In some cases, the recombinant adenoviral vector lack E1 gene, which can mediate the replication of adenovirus. Therefore, in some cases, it is necessary to supplement the E1 gene for the recombinant adenovirus to replicate. In some cases, 293 cell line, which have been generated using adenoviral infection and has E1 gene in the genome, can be used. Other cell lines that are engineered to produce E1 gene product can also be used for this purpose. In some cases, DNA fragments containing other viral genomic elements or one or more helper viruses can also be used for the production of recombinant adenovirus. For example, a helper virus can provide packaging signal for virus packaging, while the recombinant viral vector can lack the packaging signal. Alternatively, DNA fragment that contains the packaging signal can be co-transfected into the host cells for the production of the recombinant adenovirus. One or more plasmid vectors or helper virus used herein that contribute genomic materials for the production of the recombinant adenoviral vector can comprise reporter genes, selection markers, or any other genes that may be useful for the viral production.

[0182] Cell transfection can be performed using any transfection approach available to one skilled in the art. The transfection approach can include, but not limited to, electroporation, microinjection, calcium phosphate precipitation, cationic polymers, dendrimers, liposome, microprojectile bombardment, fugene, direct sonic loading, cell squeezing, optical transfection, protoplast fusion, impalefection, magnetofection, nucleofection, or any combination thereof.

[0183] A method of producing adenovirus based vaccine can further comprise isolation and amplification of viruses. Isolation of viruses can comprise isolating adenoviral plaques, which can be followed by screening of plaques. In some cases, the screening can be conducted by sequencing of the viral nucleic acids. In some cases, the plaques can be screened for the full, unaltered transgene sequence. A correct plaque can be further amplified by infection of successively larger number of cells. A method can further comprise isolating, and optionally lysing, the infected cells, which can be followed by purification of viral particles. The purification can be performed via various approaches, such as, but not limited to, ultracentrifugation and dialysis. A method can further comprise determining infectious titer, by e.g., plaque assay. A method can further comprise determining viral particle concentration, by e.g., ultraviolet absorbance measurement.

[0184] The recombinant virus based vaccine can be delivered to a subject through a parenteral delivery method. A parenteral delivery can include intravenous, transdermal, oral, intrabiliary, intraparenchymal, intra-hepatic artery, intra-portal vein, intratumoral, or transvenous delivery. Sometimes, a parenteral delivery can utilize a needle (e.g., a hypodermic needle) for delivery of the recombinant virus based vaccine. The recombinant virus based vaccine can be formulated in an aqueous solution, e.g., saline. Sometimes, a parenteral delivery can utilize a pneumatic injection as a delivery method. The nucleic acid based vaccine can be formulated as an aqueous solution.

[0185] The recombinant virus based vaccine can also be delivered to a subject through a topical delivery method. The vaccine can be applied directly onto an infected area, e.g., the nasal cavity.

[0186] Antibody Based Vaccine

[0187] Provided herein is an antibody based vaccine that can comprise an entity that binds a peptide or polypeptide sequence described herein. The antibody based vaccine can be used against influenza infection. The entity can be an antibody.

[0188] Antibody-based vaccine can be formulated using any techniques, carriers, and excipients as suitable. The antibody can be a natural antibody, a chimeric antibody, a humanized antibody, or can be an antibody fragment. The antibody can recognize one or more of the epitope sequences described herein. The antibody can recognize one or more sequences selected from SEQ ID NOs: 1-94 or a sequence selected from Table 1, Table 2, or Table 3. The antibody can recognize a sequence that has at most 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% sequence homology or identity to a sequence selected from the group consisting of SEQ ID NOs: 1-94 or a sequence selected from Table 1, Table 2, or Table 3. The antibody can recognize a sequence with at least 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% sequence homology or identity to a sequence selected from the group consisting of SEQ ID NOs: 1-94 or a sequence selected from Table 1, Table 2, or Table 3. The antibody can recognize a sequence length at least 30%, 40%, 50%, 60%, 70%, 80%,

90%, 95%, or 100%, or more of a sequence length of a sequence selected from the group consisting of SEQ ID NOs: 1-94 or a sequence selected from Table 1, Table 2, or Table 3. The antibody can recognize a sequence length at most 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% the sequence length of a sequence selected from the group consisting of SEQ ID NOs: 1-94 or a sequence selected from Table 1, Table 2, or Table 3. In some embodiments, the antibody recognizes epitopes from multiple strains of influenza virus, such as influenza A virus, influenza B virus, influenza C virus.

[0189] An antibody can include fully assembled antibodies, antibody fragments that can bind antigen (e.g., Fab, $F(ab')_2$, Fv, single chain antibodies, diabodies, antibody chimeras, hybrid antibodies, bispecific antibodies, humanized antibodies, and the like), and recombinant peptides comprising the foregoing.

[0190] An antibody can be a monoclonal antibody. The preparation of monoclonal antibodies is known in the art and can be accomplished by fusing spleen cells from a host sensitized to the antigen with myeloma cells in accordance with known techniques or by transforming the spleen cells with an appropriate transforming vector to immortalize the cells. The cells can be cultured in a selective medium, cloned, and screened to select monoclonal antibodies that bind the designated antigens. Numerous references can be found on the preparation of monoclonal and polyclonal antibodies.

[0191] A native antibody (native immunoglobulin) can be heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain can be linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages can vary among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain can also have regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (VH) followed by a number of constant domains. Each light chain can have a variable domain at one end (VL) and a constant domain at its other end; the constant domain of the light chain can be aligned with the first constant domain of the heavy chain, and the light chain variable domain can be aligned with the variable domain of the heavy chain. Particular amino acid residues can form an interface between the light and heavy-chain variable domains.

[0192] Variable regions can confer antigen-binding specificity. In some cases, the variability is not evenly distributed throughout the variable domains of antibodies. Variability can be concentrated in three segments called complementarity determining regions (CDRs) or hypervariable regions, both in the light chain and the heavy-chain variable domains. The more highly conserved portions of variable domains can be located in the framework (FR) regions. The variable domains of native heavy and light chains each can comprise four FR regions, largely adopting a β-pleated-sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the β-pleated-sheet structure. The CDRs in each chain can be held together in close proximity by the FR regions and, with the CDRs from the other chain, contribute to the formation of the antigen-binding site of antibodies. In some cases, the constant domains cannot be involved directly in binding an antibody to an antigen, but can exhibit various effector functions, such as Fc receptor (FcR) binding, participation of the antibody in antibody-dependent cellular toxicity, initiation of complement dependent cytotoxicity, and mast cell degranulation.

[0193] A hypervariable region can refer to the amino acid residues of an antibody that are responsible for antigen-binding. The hypervariable region can comprise amino acid residues from a complementarily determining region or CDR and/or those residues from a "hypervariable loop." Framework or FR residues can be those variable domain residues other than the hypervariable region residues, as herein deemed.

[0194] Antibody fragments can comprise a portion of an intact antibody, e.g., the antigen-binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab, F(ab')2, and Fv fragments; diabodies; minibodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments. Papain digestion of antibodies can produce two identical antigen-binding fragments, called Fab fragments, each with a single antigen-binding site, and a residual Fc fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an F(ab')2 fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

[0195] Fv can be the minimum antibody fragment that contains a complete antigen recognition and binding site. This region can consist of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. It is in this configuration that the three CDRs of each variable domain can interact to define an antigen-binding site on the surface of the VH-VL dimer. Collectively, the six CDRs can confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) can have the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0196] The Fab fragment can contain the constant domain of the light chain and the first constant domain (CHI) of the heavy chain. Fab fragment can differ from Fab' fragments by the addition of a few residues at the carboxy terminus of the heavy chain CHI domain including one or more cysteines from the antibody hinge region. Fab'-SH can be used herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. Fab' fragments can be produced by reducing the F(ab')2 fragment's heavy chain disulfide bridge. Other chemical couplings of antibody fragments are also known.

[0197] The light chains of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

[0198] Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. Five major classes of human immunoglobulins include: IgA, IgD, IgE, IgG, and IgM, and several of these can be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. The heavy-chain constant domains that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known. Different isotypes can have different effector functions. For

example, human IgG1 and IgG3 isotypes have ADCC (antibody dependent cell-mediated cytotoxicity) activity.

[0199] Monoclonal antibodies can be obtained from any suitable species e.g., murine, rabbit, sheep, goat, or human monoclonal antibodies.

[0200] A composition, e.g., vaccine, can comprise at about or at least or at most 5, 10, 25, 50 or 100 different antibodies.

[0201] Antigen Presenting Cell (APC) Based Vaccine

[0202] Provided herein is an APC based vaccine that presents a polypeptide described herein. The APC based vaccine can be used against influenza infection. The APC based vaccine can be formulated using any of the known techniques, carriers, and excipients as suitable and as understood in the art. APCs may include monocytes, monocyte-derived cells, macrophages, and dendritic cells. Sometimes, APC based vaccine can be a dendritic cell-based vaccine.

[0203] A dendritic cell (DC)-based vaccine can be prepared by any methods known in the art. In some cases, dendritic cell-based vaccines can be prepared through an ex vivo or in vivo method. The ex vivo method can comprise the use of autologous DCs pulsed ex vivo with the polypeptides described herein, to activate or load the DCs prior to administration into the patient. The in vivo method can comprise targeting specific DC receptors using antibodies coupled with the peptides described herein. The DC-based vaccine can further comprise DC activators such as TLR3, TLR-7-8, and CD40 agonists. The DC-based vaccine can further comprise adjuvants, and a pharmaceutically acceptable carrier.

[0204] Virus-Based Vaccine

[0205] A virus-based vaccine can be generated based on live virus or on inactivated virus. Viruses can be engineered to express one or more proteins that comprise any of the sequences described herein. Vaccines based on live virus can use an attenuated virus, or a virus that can be cold-adapted. Vaccines based on inactivated virus can comprise whole virion, split virion, or purified surface antigens (e.g., HA and/or N from influenza A virus). Chemical means for inactivating a virus can include treatment with an effective amount of one or more of the following agents: detergents, formaldehyde, β-propiolactone, methylene blue, psoralen, carboxyfullerene (C60), binary ethylamine, acetyl ethyleneimine, or combinations thereof. Non-chemical methods of viral inactivation are known in the art, such as UV light, heat inactivation, or gamma irradiation.

[0206] Virions can be harvested from virus-containing fluids by various methods. For example, a purification process can involve zonal centrifugation using a linear sucrose gradient solution that includes detergent to disrupt the virions. Antigens can be purified, after optional dilution, by diafiltration.

[0207] Split virions can be obtained by treating purified virions with detergents (e.g., ethyl ether, polysorbate 80, deoxycholate, tri-N-butyl phosphate, Triton X-100, Triton N101, cetyltrimethylammonium bromide, Tergitol NP9, etc.) to produce subvirion preparations, including the "Tween-ether" splitting process. Methods of splitting influenza viruses are well known in the art. Splitting of the virus can be carried out by disrupting or fragmenting whole virus, whether infectious or non-infectious with a disrupting concentration of a splitting agent. The disruption can result in a full or partial solubilization of the virus proteins, altering the integrity of the virus. Splitting agents can be non-ionic or ionic (e.g., cationic) surfactants e.g., alkylglycosides, alkylthioglycosides, acyl sugars, sulphobetaines, betains, polyoxyethylenealkylethers, N,N-dialkyl-Glucamides, Hecameg, alkylphenoxy-polyethoxyethanols, quaternary ammonium compounds, sarcosyl, CTABs (cetyl trimethyl ammonium bromides), tri-N-butyl phosphate, Cetavlon, myristyltrimethylammonium salts, lipofectin, lipofectamine, and DOT-MA, the octyl- or nonylphenoxy polyoxyethanols (e.g., the Triton surfactants, such as Triton X-100 or Triton N101), polyoxyethylene sorbitan esters (the Tween surfactants), polyoxyethylene ethers, polyoxyethlene esters, etc. One exemplary splitting procedure can use the consecutive effects of sodium deoxycholate and formaldehyde, and splitting can take place during initial virion purification (e.g., in a sucrose density gradient solution). Thus a splitting process can involve clarification of the virion-containing material (to remove non-virion material), concentration of the harvested virions (e.g., using an adsorption method, such as CaHP04 adsorption), separation of whole virions from non-virion material, splitting of virions using a splitting agent in a density gradient centrifugation step (e.g., using a sucrose gradient that contains a splitting agent such as sodium deoxycholate), and then filtration (e.g., ultrafiltration) to remove undesired materials. Split virions can usefully be resuspended in sodium phosphate-buffered isotonic sodium chloride solution. The BEGRIVAC™, FLUARIX™, FLUZONE™, and FLUSHIELD™ products are split vaccines.

[0208] Purified surface antigen vaccines can comprise the influenza surface antigens haemagglutinin and, typically, also neuraminidase. Processes for preparing these proteins in purified form are well known in the art. The FLUVIRIN™, AGRIPPAL™, and INFLUVAC™ products are examples.

[0209] Vaccine based on inactivated virus can include the virosome (nucleic acid free viral-like liposomal particles). Virosomes can be prepared by solubilization of influenza virus with a detergent followed by removal of the nucleocapsid and reconstitution of the membrane containing the viral glycoproteins. Virosomes can also be prepared by adding viral membrane glycoproteins to excess amounts of phospholipids, to yield liposomes with viral proteins in their membrane.

[0210] Pharmaceutical Composition and Administration

[0211] Provided herein is a pharmaceutical composition that can be used to provide immunity against influenza virus infection. In certain aspects of the disclosure, the pharmaceutical composition can be a vaccine.

[0212] A composition can comprise one or more polypeptides, nucleic acids, proteins (e.g., antibodies or fragments thereof), APCs, or viruses described herein, or a combination thereof, and a pharmaceutically acceptable excipient. In some embodiments, a composition can further comprise carriers for the polypeptide, polynucleotide, or vector. A composition can further comprise a preservative. In some cases, a composition can further comprise other reagents to maintain appropriate physical or chemical properties, such as, but not limited to, salt concentration, osmolality, pH, hydrophobility/hydrophility, and solubility. A composition can further comprise appropriate penetration enhancer for enhanced delivery. A composition can further comprise appropriate adjuvant(s) that can enhance the immunogenicity of the polypeptide, polynucleotide, or vector.

[0213] Formulations

[0214] A composition provided herein, e.g., a vaccine, can be formulated based, in part, on the intended route of administration of the composition. The composition, e.g., vaccine, can comprise one or more active agents, such as one or more peptides, nucleic acids, proteins (e.g., antibodies or fragments thereof), APCs, viruses described herein, or a combination thereof. A composition comprising one or more active agents in combination with one or more adjuvants can be formulated in conventional manner using one or more physiologically acceptable carriers, comprising excipients, diluents, and/or auxiliaries, e.g., which facilitate processing of the one or more active agents into preparations that can be administered. The one or more active agents described herein can be delivered to a subject using a number of routes or modes of administration described herein, e.g., oral, buccal, topical, rectal, transdermal, transmucosal, subcutaneous, intravenous, and intramuscular applications, as well as by inhalation.

[0215] A composition described herein, e.g., a vaccine, can be a liquid preparation such as a suspension, syrup, or elixir. The composition, e.g., vaccine, can also be a preparation for parenteral, subcutaneous, intradermal, intramuscular, or intravenous administration (e.g., injectable administration), such as a sterile suspension or emulsion. In some cases, aqueous solutions can be packaged for use as is, or lyophilized, and the lyophilized preparation being combined with a sterile solution prior to administration. The composition, e.g., vaccine, can be delivered as a solution or as a suspension. In general, formulations such as jellies, creams, lotions, suppositories and ointments can provide an area with more extended exposure to one or more active agents, while formulations in solution, e.g., sprays, can provide more immediate, short-term exposure.

[0216] Formulations for Inhalation (e.g., Nasal Administration or Oral Inhalation)

[0217] A composition described herein, e.g., a vaccine, can be formulated for administration via the nasal passages of a subject. Formulations suitable for nasal administration, wherein the carrier is a solid, can include a coarse powder having a particle size, for example, in the range of about 10 to about 500 microns which can be administered in the manner in which snuff is taken, e.g., by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. The formulation can be a nasal spray, nasal drops, or by aerosol administration by nebulizer. The formulation can include aqueous or oily solutions of the vaccine.

[0218] A composition provided herein, e.g., a vaccine, can be formulated as an aerosol formulation. The aerosol formulation can be, e.g., an aerosol solution, suspension or dry powder. The aerosol can be administered through the respiratory system or nasal passages. For example, the composition can be suspended or dissolved in an appropriate carrier, e.g., a pharmaceutically acceptable propellant, and administered directly into the lungs using a nasal spray or inhalant. For example, an aerosol formulation comprising one or more active agents can be dissolved, suspended or emulsified in a propellant or a mixture of solvent and propellant, e.g., for administration as a nasal spray or inhalant. The aerosol formulation can contain any acceptable propellant under pressure, such as a cosmetically or dermatologically or pharmaceutically acceptable propellant.

[0219] An aerosol formulation for nasal administration can be an aqueous solution designed to be administered to the nasal passages in drops or sprays. Nasal solutions can be similar to nasal secretions in that they can be isotonic and slightly buffered to maintain a pH of about 5.5 to about 6.5. In some cases, pH values outside of this range can be used. Antimicrobial agents or preservatives can also be included in the formulation.

[0220] An aerosol formulation for inhalation can be designed so that one or more active agents are carried into the respiratory system of the subject when administered by the nasal or oral respiratory route. Inhalation solutions can be administered, for example, by a nebulizer. Inhalations or insufflations, comprising finely powdered or liquid drugs, can be delivered to the respiratory system as a pharmaceutical aerosol of a solution or suspension of the agent or combination of agents in a propellant, e.g., to aid in disbursement. Propellants can be liquefied gases, including halocarbons, for example, fluorocarbons such as fluorinated chlorinated hydrocarbons, hydrochlorofluorocarbons, and hydrochlorocarbons, as well as hydrocarbons and hydrocarbon ethers.

[0221] Halocarbon propellants can include fluorocarbon propellants in which all hydrogens are replaced with fluorine, chlorofluorocarbon propellants in which all hydrogens are replaced with chlorine and at least one fluorine, hydrogen-containing fluorocarbon propellants, and hydrogen-containing chlorofluorocarbon propellants. Hydrocarbon propellants can include, for example, propane, isobutane, n-butane, pentane, isopentane, and neopentane. A blend of hydrocarbons can also be used as a propellant. Ether propellants include, for example, dimethyl ether as well as ethers. An aerosol formulation can also comprise more than one propellant. For example, the aerosol formulation can comprise more than one propellant from the same class, such as two or more fluorocarbons; or more than one, more than two, more than three propellants from different classes, such as a fluorohydrocarbon and a hydrocarbon. A composition described herein, e.g., vaccine, can also be dispensed with a compressed gas, e.g., an inert gas such as carbon dioxide, nitrous oxide, or nitrogen.

[0222] The aerosol formulation can also include other components, for example, ethanol, isopropanol, propylene glycol, as well as surfactants or other components, such as oils and detergents. These components can serve to stabilize the formulation and/or lubricate valve components.

[0223] The aerosol formulation can be packaged under pressure and can be formulated as an aerosol using solutions, suspensions, emulsions, powders, and semisolid preparations. For example, a solution aerosol formulation can comprise a solution of an active agent such in (substantially) pure propellant or as a mixture of propellant and solvent. The solvent can be used to dissolve one or more active agents and/or retard the evaporation of the propellant. Solvents can include, for example, water, ethanol, and glycols. Any combination of suitable solvents can be use, optionally combined with preservatives, antioxidants, and/or other aerosol components.

[0224] An aerosol formulation can be a dispersion or suspension. A suspension aerosol formulation can comprise a suspension of one or more active agents, e.g., peptides, and a dispersing agent. Dispersing agents can include, for example, sorbitan trioleate, oleyl alcohol, oleic acid, leci-

thin, and corn oil. A suspension aerosol formulation can also include lubricants, preservatives, antioxidant, and/or other aerosol components.

[0225] An aerosol formulation can similarly be formulated as an emulsion. An emulsion aerosol formulation can include, for example, an alcohol such as ethanol, a surfactant, water, and a propellant, as well as an active agent or combination of active agents, e.g., one or more peptides. The surfactant used can be nonionic, anionic, or cationic. One example of an emulsion aerosol formulation comprises, for example, ethanol, surfactant, water, and propellant. Another example of an emulsion aerosol formulation comprises, for example, vegetable oil, glyceryl monostearate, and propane.

[0226] Formulations for Parenteral Administration

[0227] A composition, e.g., vaccine, comprising one or more active agents can be formulated for parenteral administration and can be presented in unit dose form in ampoules, pre-filled syringes, small volume infusion or in multi-dose containers with an added preservative. The composition can take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, for example solutions in aqueous polyethylene glycol.

[0228] For injectable formulations, a vehicle can be chosen from those known in the art to be suitable, including aqueous solutions or oil suspensions, or emulsions, with sesame oil, corn oil, cottonseed oil, or peanut oil, as well as elixirs, mannitol, dextrose, or a sterile aqueous solution, and similar pharmaceutical vehicles. The formulation can also comprise polymer compositions which are biocompatible, biodegradable, such as poly(lactic-co-glycolic)acid. These materials can be made into micro or nanospheres, loaded with drug and further coated or derivatized to provide superior sustained release performance. Vehicles suitable for periocular or intraocular injection include, for example, suspensions of active agent in injection grade water, liposomes, and vehicles suitable for lipophilic substances and those known in the art.

[0229] Parenteral injection can include subcutaneous, intramuscular, intravenous, intraperitoneal, and intracardiac administration. A subcutaneous administration can be administered as a bolus into the subcutis. Subcutaneous injection sites on a human subject can include the outer area of the upper arm, abdomen, the front of the thigh, the upper back, the upper area of the buttock. Intramuscular administration can be an injection directly into muscle. Intramuscular injection sites can include deltoid, dorsogluteal, rectus femoris, vastus lateralis and ventrogluteal muscles. Intravenous administration can be delivery of a liquid formulation directly into a vein. Intravenous administration can be applied on a peripheral vein (e.g. the veins in the arms, hands, legs, and feet) or a central vein (e.g. superior vena cava, inferior vena cava, and the right atrium of the heart). Intraperitoneal administration can be injection into the peritoneum. Intracardiac administration can be injection directly into heart muscles or ventricles.

[0230] Sometimes, the composition, e.g., vaccine, can be formulated for intravenous administration to mammalian subjects, like human beings. The composition, e.g., vaccine, for intravenous administration can be a solution in sterile isotonic aqueous buffer. In some cases, the composition, e.g., vaccine, can include a solubilizing agent and a local anesthetic such as lidocaine to ease pain at the site of the injection. The ingredients can be supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients can be mixed prior to administration.

[0231] When administration is by injection, a composition, e.g., vaccine, comprising one or more active agents can be formulated in aqueous solutions, specifically in physiologically compatible buffers such as Hanks solution, Ringer's solution, or physiological saline buffer. The solution can contain formulatory agents such as suspending, stabilizing, and/or dispersing agents. Alternatively, the one or more active agents can be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use. In another embodiment, the composition, e.g., vaccine, does not comprise an adjuvant or any other substance added to enhance the immune response stimulated by the active agent. In another embodiment, the composition, e.g., vaccine, can comprise a substance that inhibits an immune response to the one or more active agents.

[0232] In some embodiments, one or more active agents are formulated as a depot preparation. Such long acting formulations can be administered by implantation or transcutaneous delivery (e.g., subcutaneously or intramuscularly), intramuscular injection or use of a transdermal patch. Thus, for example, one or more active agents can be formulated with suitable polymeric or hydrophobic materials (e.g., as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

[0233] Formulations for Topical Administration

[0234] In certain aspects of the disclosure, a composition provided herein, e.g., a vaccine, can comprise one or more agents that exert local and regional effects when administered topically or injected at or near particular sites of infection. Direct topical application, e.g., of a viscous liquid, solution, suspension, dimethylsulfoxide (DMSO)-based solutions, liposomal formulations, gel, jelly, cream, lotion, ointment, suppository, foam, or aerosol spray, can be used for local administration, to produce for example local and/or regional effects. Pharmaceutically appropriate vehicles for such formulation include, for example, lower aliphatic alcohols, polyglycols (e.g., glycerol or polyethylene glycol), esters of fatty acids, oils, fats, silicones, and the like. Such preparations can also include preservatives (e.g., p-hydroxybenzoic acid esters) and/or antioxidants (e.g., ascorbic acid and tocopherol). In some embodiments, local/topical formulations comprising one or more active agents are used to treat epidermal or mucosal viral infections.

[0235] A composition provided herein, e.g., a vaccine, can contain a dermatologically acceptable carrier. Such carriers are compatible with skin, nails, mucous membranes, tissues, and/or hair, and can include any dermatological carrier meeting these requirements. Such carriers can be readily selected by one of ordinary skill in the art. In formulating skin ointments, one or more agents can be formulated in an oleaginous hydrocarbon base, an anhydrous absorption base, a water-in-oil absorption base, an oil-in-water water-removable base and/or a water-soluble base. Examples of such carriers and excipients include humectants (e.g., urea), gly-

cols (e.g., propylene glycol), alcohols (e.g., ethanol), fatty acids (e.g., oleic acid), surfactants (e.g., isopropyl myristate and sodium lauryl sulfate), pyrrolidones, glycerol monolaurate, sulfoxides, terpenes (e.g., menthol), amines, amides, alkanes, alkanols, water, calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

[0236] Ointments and creams can, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Lotions can be formulated with an aqueous or oily base and will in general also containing one or more emulsifying agents, stabilizing agents, dispersing agents, suspending agents, thickening agents, or coloring agents. The construction and use of transdermal patches for the delivery of pharmaceutical agents is known in the art. Such patches can be constructed for continuous, pulsatile, or on demand delivery of pharmaceutical agents.

[0237] Lubricants which can be used to form compositions and dosage forms can include calcium stearate, magnesium stearate, mineral oil, light mineral oil, glycerin, sorbitol, mannitol, polyethylene glycol, other glycols, stearic acid, sodium lauryl sulfate, tale, hydrogenated vegetable oil (e.g., peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil, and soybean oil), zinc stearate, ethyl oleate, ethyl laureate, agar, or mixtures thereof. Additional lubricants include, for example, a syloid silica gel, a coagulated aerosol of synthetic silica, or mixtures thereof. A lubricant can optionally be added, in an amount of less than about 1 weight percent of the composition.

[0238] A composition provided herein, e.g., a vaccine, can be in any form suitable for topical application, including aqueous, aqueous-alcoholic or oily solutions, lotion or serum dispersions, aqueous, anhydrous or oily gels, emulsions obtained by dispersion of a fatty phase in an aqueous phase (O/W or oil in water) or, conversely, (W/O or water in oil), microemulsions or alternatively microcapsules, microparticles or lipid vesicle dispersions of ionic and/or nonionic type. Other than the one or more active agents, the amounts of the various constituents of the compositions provided herein can be those used in the art. These compositions can constitute protection, treatment or care creams, milks, lotions, gels or foams for the face, for the hands, for the body and/or for the mucous membranes, or for cleansing the skin. The compositions can also consist of solid preparations constituting soaps or cleansing bars.

[0239] A composition provided herein, e.g., a vaccine, for local/topical application can include one or more antimicrobial preservatives such as quaternary ammonium compounds, organic mercurials, p-hydroxy benzoates, aromatic alcohols, chlorobutanol, and the like.

[0240] Formulations for Oral Administration

[0241] Sometimes, a composition provided herein, e.g., a vaccine, can be formulated for oral administration.

[0242] For oral administration, a composition as provided herein can be formulated readily by combining the one or more active agents with pharmaceutically acceptable carriers known in the art. Such carriers enable active agents to be formulated as tablets, including chewable tablets, pills, dragees, capsules, lozenges, hard candy, liquids, gels, syrups, slurries, powders, suspensions, elixirs, wafers, and the like, for oral ingestion by a patient to be treated. Such formulations can comprise pharmaceutically acceptable carriers including solid diluents or fillers, sterile aqueous media and various non-toxic organic solvents. A solid carrier can be one or more substances which can also act as diluents, flavoring agents, solubilizers, lubricants, suspending agents, binders, preservatives, tablet disintegrating agents, or an encapsulating material. In powders, the carrier can be a finely divided solid which is a mixture with the finely divided active component. In tablets, the active component generally is mixed with the carrier having the desired binding capacity in suitable proportions and compacted in the shape and size desired. The powders and tablets can contain from about one (1) to about seventy (70) percent of the one or more active agents. Suitable carriers include but are not limited to magnesium carbonate, magnesium stearate, tale, sugar, lactose, pectin, dextrin, starch, gelatin, tragacanth, methylcellulose, sodium carboxymethylcellulose, a low melting wax, cocoa butter, and the like. Generally, the one or more active agents can be included at concentration levels ranging from about 0.5%, about 5%, about 10%, about 20%, or about 30% to about 50%, about 60%, about 70%, about 80%, or about 90% by weight of the total composition of oral dosage forms, in an amount sufficient to provide a desired unit of dosage.

[0243] Aqueous suspensions for oral use can contain one or more active agents with pharmaceutically acceptable excipients, such as a suspending agent (e.g., methyl cellulose), a wetting agent (e.g., lecithin, lysolecithin and/or a long-chain fatty alcohol), as well as coloring agents, preservatives, flavoring agents, and the like.

[0244] Oils or non-aqueous solvents can be required to bring the one or more active agents into solution, due to, for example, the presence of large lipophilic moieties. Alternatively, emulsions, suspensions, or other preparations, for example, liposomal preparations, can be used. With respect to liposomal preparations, any known methods for preparing liposomes for treatment of a condition can be used. Ligands can also be attached to the liposomes to direct these compositions to particular sites of action.

[0245] Pharmaceutical preparations for oral use can be obtained as a solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; flavoring elements, cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinyl pyrrolidone (PVP). If desired, disintegrating agents can be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. The agents can also be formulated as a sustained release preparation.

[0246] Dragee cores can be provided with suitable coatings. For this purpose, concentrated sugar solutions can be used, which can optionally contain gum arabic, tale, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments can be added to the tablets or dragee coatings for identification or to characterize different combinations of active agents.

[0247] Pharmaceutical preparations that can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the

active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as tale or magnesium stearate and, optionally, stabilizers. In soft capsules, the active agents can be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers can be added. All formulations for oral administration can be in dosages suitable for administration.

[0248] Other forms suitable for oral administration include liquid form preparations including emulsions, syrups, elixirs, aqueous solutions, aqueous suspensions, or solid form preparations which are intended to be converted shortly before use to liquid form preparations. Emulsions can be prepared in solutions, for example, in aqueous propylene glycol solutions or can contain emulsifying agents, for example, such as lecithin, sorbitan monooleate, or acacia. Aqueous solutions can be prepared by dissolving the active component in water and adding suitable colorants, flavors, stabilizers, and thickening agents. Aqueous suspensions can be prepared by dispersing the finely divided active component in water with viscous material, such as natural or synthetic gums, resins, methylcellulose, sodium carboxymethylcellulose, and other well-known suspending agents. Suitable fillers or carriers with which the compositions can be administered include agar, alcohol, fats, lactose, starch, cellulose derivatives, polysaccharides, polyvinylpyrrolidone, silica, sterile saline and the like, or mixtures thereof used in suitable amounts. Solid form preparations include solutions, suspensions, and emulsions, and can contain, in addition to the active component, colorants, flavors, stabilizers, buffers, artificial and natural sweeteners, dispersants, thickeners, solubilizing agents, and the like.

[0249] A syrup or suspension can be made by adding the active compound to a concentrated, aqueous solution of a sugar, e.g., sucrose, to which can also be added any accessory ingredients. Such accessory ingredients can include flavoring, an agent to retard crystallization of the sugar or an agent to increase the solubility of any other ingredient, e.g., as a polyhydric alcohol, for example, glycerol or sorbitol.

[0250] When formulating compounds for oral administration, it can be desirable to utilize gastroretentive formulations to enhance absorption from the gastrointestinal (GI) tract. A formulation which is retained in the stomach for several hours can release an active agent slowly and provide a sustained release that can be used herein. Expandable, floating and bioadhesive techniques can be utilized to maximize absorption an active agent.

[0251] Formulations for Ophthalmic Administration

[0252] In some instances, a composition provided herein can be administered through eyes, e.g. delivered in eye drops. Eye drops can be prepared by dissolving the one or more active agents in a sterile aqueous solution such as physiological saline, buffering solution, etc., or by combining powder compositions to be dissolved before use. Other vehicles can be chosen, as is known in the art, including but not limited to: balance salt solution, saline solution, water soluble poly ethers such as polyethyene glycol, polyvinyls, such as polyvinyl alcohol and povidone, cellulose derivatives such as methylcellulose and hydroxypropyl methylcellulose, petroleum derivatives such as mineral oil and white petrolatum, animal fats such as lanolin, polymers of acrylic acid such as carboxypolymethylene gel, vegetable fats such as peanut oil and polysaccharides such as dextrans, and glycosaminoglycans such as sodium hyaluronate. If desired, additives ordinarily used in the eye drops can be added. Such additives include isotonizing agents (e.g., sodium chloride, etc.), buffer agent (e.g., boric acid, sodium monohydrogen phosphate, sodium dihydrogen phosphate, etc.), preservatives (e.g., benzalkonium chloride, benzethonium chloride, chlorobutanol, etc.), thickeners (e.g., saccharide such as lactose, mannitol, maltose, etc.; e.g., hyaluronic acid or its salt such as sodium hyaluronate, potassium hyaluronate, etc.; e.g., mucopolysaccharide such as chondroitin sulfate, etc.; e.g., sodium polyacrylate, carboxyvinyl polymer, crosslinked polyacrylate, polyvinyl alcohol, polyvinyl pyrrolidone, methyl cellulose, hydroxy propyl methylcellulose, hydroxyethyl cellulose, carboxymethyl cellulose, hydroxy propyl cellulose, or other agents known to those skilled in the art).

[0253] Other Formulations

[0254] In some embodiments, a composition provided herein is administered in otic solutions, suspensions, ointments, or inserts. In some embodiments, a composition described herein, e.g., a vaccine, is formulated for administration as a suppository. For example, a low melting wax, such as a mixture of triglycerides, fatty acid glycerides, Witepsol S55 (trademark of Dynamite Nobel Chemical, Germany), or cocoa butter can be first melted and the active component can be dispersed homogeneously, for example, by stirring. The molten homogeneous mixture can then be poured into convenient sized molds, allowed to cool, and to solidify. In some embodiments, a composition described herein, e.g., a vaccine, is formulated for vaginal administration. In some cases, pessaries, tampons, creams, gels, pastes, foams, or sprays contain one or compositions, e.g., vaccines described herein.

[0255] Ingredients, e.g., Carriers, Excipients

[0256] A composition provided herein, e.g., a vaccine, can include one or more carriers and excipients (including but not limited to buffers, carbohydrates, mannitol, proteins, peptides or amino acids such as glycine, antioxidants, bacteriostats, chelating agents, suspending agents, thickening agents and/or preservatives), water, oils including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like, saline solutions, aqueous dextrose and glycerol solutions, flavoring agents, coloring agents, detackifiers and other acceptable additives, adjuvants, or binders, other pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH buffering agents, tonicity adjusting agents, emulsifying agents, wetting agents and the like. Examples of excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, tale, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol, and the like. In another instance, the composition is substantially free of preservatives. In other embodiments, the composition, e.g., vaccine, contains at least one preservative. General methodology on pharmaceutical dosage forms can be found in Ansel et ah, Pharmaceutical Dosage Forms and Drug Delivery Systems (Lippencott Williams & Wilkins, Baltimore Md. (1999)). It will be recognized that, while any suitable carrier known to those of ordinary skill in the art can be employed to administer the pharmaceutical compositions described herein, the type of carrier can vary depending on the mode of administration. Suitable formulations and additional carriers are described in Remington "The Science and Practice of Pharmacy" (20th

Ed., Lippincott Williams & Wilkins, Baltimore Md.), the teachings of which are incorporated by reference in their entirety herein.

[0257] Liposomes and Microspheres

[0258] A composition provided herein, e.g., a vaccine, can be encapsulated within liposomes. Biodegradable microspheres can also be employed as carriers for the composition.

[0259] A composition provided herein, e.g., a vaccine, can be administered in liposomes or microspheres (or microparticles). Methods for preparing liposomes and microspheres for administration to a patient are known to those of skill in the art. For example, U.S. Pat. No. 4,789,734, the contents of which are hereby incorporated by reference, describes methods for encapsulating biological materials in liposomes. The material can be dissolved in an aqueous solution, the appropriate phospholipids and lipids added, along with surfactants if required, and the material dialyzed or sonicated, as desired. Microspheres formed of polymers or proteins are known to those skilled in the art, and can be tailored for passage through the gastrointestinal tract directly into the blood stream. Alternatively, the compound can be incorporated and the microspheres, or composite of microspheres, implanted for slow release over a period of time ranging from days to months.

[0260] Preservatives/Sterility

[0261] A composition provided herein, e.g., a vaccine, can include material for a single administration (e.g., immunization), or can include material for multiple administrations (e.g., immunizations) (e.g., a "multidose" kit). The composition, e.g., vaccine, can include one or more preservatives such as thiomersal or 2-phenoxyethanol. In some embodiments, the vaccine is substantially free from (e.g., <10 μg/ml) mercurial material e.g., thiomersal-free. In some embodiments, a-Tocopherol succinate is used as an alternative to mercurial compounds. Preservatives can be used to prevent microbial contamination during use. Suitable preservatives include: benzalkonium chloride, thimerosal, chlorobutanol, methyl paraben, propyl paraben, phenylethyl alcohol, edetate disodium, sorbic acid, Onamer M, or other agents known to those skilled in the art. In ophthalmic products, e.g., such preservatives can be employed at a level of from 0.004% to 0.02%. In the compositions of the present application the preservative, e.g., benzalkonium chloride, can be employed at a level of from 0.001% to less than 0.01%, e.g., from 0.001% to 0.008%, preferably about 0.005% by weight. A concentration of benzalkonium chloride of 0.005% can be sufficient to preserve a composition provided herein from microbial attack.

[0262] As an alternative (or in addition) to including a preservative in multidose compositions, the composition, e.g., vaccine, can be contained in a container having an aseptic adaptor for removal of material.

[0263] In some cases, a composition provided herein, e.g., a vaccine, can be sterile. The composition, e.g., vaccine, can be non-pyrogenic e.g., containing <1 EU (endotoxin unit, a standard measure) per dose, and can be <0.1 EU per dose. The composition, e.g., vaccine, can be formulated as a sterile solution or suspension, in suitable vehicles, known in the art. The composition, e.g., vaccine, can be sterilized by conventional, known sterilization techniques, e.g., the composition can be sterile filtered.

[0264] Salts/Osmolality

[0265] In some embodiments, a composition provided herein, e.g., vaccine, comprises one or more salts. For controlling the tonicity, a physiological salt such as sodium salt can be included a composition provided herein, e.g., vaccine. Other salts can include potassium chloride, potassium dihydrogen phosphate, disodium phosphate, and/or magnesium chloride, or the like. In some embodiments, the composition, e.g., vaccine, is formulated with one or more pharmaceutically acceptable salts. The one or more pharmaceutically acceptable salts can include those of the inorganic ions, such as, for example, sodium, potassium, calcium, magnesium ions, and the like. Such salts can include salts with inorganic or organic acids, such as hydrochloric acid, hydrobromic acid, phosphoric acid, nitric acid, sulfuric acid, methanesulfonic acid, p-toluenesulfonic acid, acetic acid, fumaric acid, succinic acid, lactic acid, mandelic acid, malic acid, citric acid, tartaric acid, or maleic acid. If an active agent (e.g., polypeptide) contains a carboxy group or other acidic group, it can be converted into a pharmaceutically acceptable addition salt with inorganic or organic bases. Examples of suitable bases include sodium hydroxide, potassium hydroxide, ammonia, cyclohexylamine, dicyclohexyl-amine, ethanolamine, diethanolamine, triethanolamine, and the like.

[0266] A composition, e.g., vaccine, can have an osmolality of between 200 mOsm/kg and 400 mOsm/kg, between 240-360 mOsm/kg, or within the range of 290-310 mOsm/kg.

[0267] Buffers/pH

[0268] A composition provided herein, e.g., vaccine, can comprise one or more buffers, such as a Tris buffer; a borate buffer; a succinate buffer; a histidine buffer (e.g., with an aluminum hydroxide adjuvant); or a citrate buffer. Buffers, in some cases, are included in the 5-20 mM range.

[0269] A composition provided herein, e.g., vaccine, has a pH between about 5.0 and about 8.5, between about 6.0 and about 8.0, between about 6.5 and about 7.5, or between about 7.0 and about 7.8.

[0270] Detergents/Surfactants

[0271] A composition provided herein, e.g., vaccine, includes one or more detergents and/or surfactants, e.g., polyoxyethylene sorbitan esters surfactants (commonly referred to as "Tweens"), e.g., polysorbate 20 and polysorbate 80; copolymers of ethylene oxide (EO), propylene oxide (PO), and/or butylene oxide (BO), sold under the DOWFAX™ tradename, such as linear EO/PO block copolymers; octoxynols, which can vary in the number of repeating ethoxy (oxy-1,2-ethanediyl) groups, e.g., octoxynol-9 (Triton X-100, or t-octylphenoxypolyethoxyethanol); (octylphenoxy)polyethoxyethanol (IGEPAL CA-630/NP-40); phospholipids such as phosphatidylcholine (lecithin); nonylphenol ethoxylates, such as the Tergitol™ NP series; polyoxyethylene fatty ethers derived from lauryl, cetyl, stearyl and oleyl alcohols (known as Brij surfactants), such as triethyleneglycol monolauryl ether (Brij 30); and sorbitan esters (commonly known as "SPANs"), such as sorbitan trioleate (Span 85) and sorbitan monolaurate, an octoxynol (such as octoxynol-9 (Triton X-100) or t-octylphenoxypolyethoxyethanol), a cetyl trimethyl ammonium bromide ("CTAB"), or sodium deoxycholate, particularly for a split or surface antigen vaccine. The one or more detergents and/or surfactants can be present only at trace amounts. In some cases, the composition, e.g., vaccine, can include less than 1 mg/ml of each of octoxynol-10 and polysorbate 80.

Non-ionic surfactants can be used herein. Surfactants can be classified by their "HLB" (hydrophile/lipophile balance). In some cases, surfactants have a HLB of at least 10, at least 15, and/or at least 16.

[0272] In some embodiments, mixtures of surfactants is used in a composition e.g., vaccine, e.g., Tween 80/Span 85 mixtures. A combination of a polyoxyethylene sorbitan ester and an octoxynol can also be suitable. Another combination can comprise laureth 9 plus a polyoxyethylene sorbitan ester and/or an octoxynol. The amounts of surfactants (% by weight) can be: polyoxyethylene sorbitan esters (such as Tween 80) 0.01 to 1%, in particular about 0.1%; octyl- or nonylphenoxy polyoxyethanols (such as Triton X-100, or other detergents in the Triton series) 0.001 to 0.1%, in particular 0.005 to 0.02%; polyoxyethylene ethers (such as laureth 9) 0.1 to 20%, preferably 0.1 to 10% and in particular 0.1 to 1% or about 0.5%.

[0273] Adjuvants

[0274] A composition provided herein, e.g., vaccine, can comprise one or more adjuvants. An adjuvant can be used to enhance the immune response (humoral and/or cellular) elicited in a subject receiving the vaccine. Sometimes, an adjuvant can elicit a Th1-type response. In some cases, an adjuvant can elicit a Th2-type response. A Th1-type response can be characterized by the production of cytokines such as IFN-γ as opposed to a Th2-type response which can be characterized by the production of cytokines such as IL-4, IL-5, and IL-10.

[0275] Lipid-based adjuvants, such as MPL and MDP, can be used with a composition, e.g., vaccine, disclosed herein. Monophosphoryl lipid A (MPL), for example, is an adjuvant that can cause increased presentation of liposomal antigen to specific T Lymphocytes. In addition, a muramyl dipeptide (MDP) can also be used as a suitable adjuvant in conjunction with a composition, e.g., vaccine, described herein.

[0276] Adjuvant can also comprise stimulatory molecules such as cytokines. Non-limiting examples of cytokines include: CCL20, a-interferon (IFN-a), β-interferon (IFN-β), γ-interferon, platelet derived growth factor (PDGF), TNFa, TNFp, GM-CSF, epidermal growth factor (EGF), cutaneous T cell-attracting chemokine (CTACK), epithelial thymus-expressed chemokine (TECK), mucosae-associated epithelial chemokine (MEC), IL-12, IL-15, IL-28, MHC, CD80, CD86, IL-1, IL-2, IL-4, IL-5, IL-6, IL-10, IL-18, MCP-1, MIP-la, MIP-1-, IL-8, L-selectin, P-selectin, E-selectin, CD34, GlyCAM-1, MadCAM-1, LFA-1, VLA-1, Mac-1, p150.95, PECAM, ICAM-1, ICAM-2, ICAM-3, CD2, LFA-3, M-CSF, G-CSF, mutant forms of IL-18, CD40, CD40L, vascular growth factor, fibroblast growth factor, IL-7, nerve growth factor, vascular endothelial growth factor, Fas, TNF receptor, Fit, Apo-1, p55, WSL-1, DR3, TRAMP, Apo-3, AIR, LARD, NGRF, DR4, DRS, KILLER, TRAIL-R2, TRICK2, DR6, Caspase ICE, Fos, c-jun, Sp-1, Ap-1, Ap-2, p38, p65Rel, MyD88, IRAK, TRAF6, IkB, Inactive NIK, SAP K, SAP-I, JNK, interferon response genes, NFkB, Bax, TRAIL, TRAILrec, TRAILrecDRC 5, TRAIL-R3, TRAIL-R4, RANK, RANK LIGAND, Ox40, Ox40 LIGAND, NKG2D, MICA, MICB, NKG2A, NKG2B, NKG2C, NKG2E, NKG2F, TAPI, and TAP2.

[0277] Additional adjuvants can include: MCP-1, MIP-la, MIP-1p, IL-8, RANTES, L-selectin, P-selectin, E-selectin, CD34, GlyCAM-1, MadCAM-1, LFA-1, VLA-1, Mac-1, p150.95, PECAM, ICAM-1, ICAM-2, ICAM-3, CD2, LFA-3, M-CSF, G-CSF, IL-4, mutant forms of IL-18, CD40, CD40L, vascular growth factor, fibroblast growth factor, IL-7, IL-22, nerve growth factor, vascular endothelial growth factor, Fas, TNF receptor, Fit, Apo-1, p55, WSL-1, DR3, TRAMP, Apo-3, AIR, LARD, NGRF, DR4, DR5, KILLER, TRAIL-R2, TRICK2, DR6, Caspase ICE, Fos, c-jun, Sp-1, Ap-1, Ap-2, p38, p65Rel, MyD88, IRAK, TRAF6, IkB, Inactive NIK, SAP K, SAP-1, JNK, interferon response genes, NFkB, Bax, TRAIL, TRAILrec, TRAILrecDRC5, TRAIL-R3, TRAIL-R4, RANK, RANK LIGAND, Ox40, Ox40 LIGAND, NKG2D, MICA, MICB, NKG2A, NKG2B, NKG2C, NKG2E, NKG2F, TAPI, TAP2 and functional fragments thereof.

[0278] In some cases, the one or more adjuvants can be a modulator of a toll like receptor. Examples of modulators of toll-like receptors include TLR-9 agonists and TLR-2 agonists and are not limited to small molecule modulators of toll-like receptors such as Imiquimod (R837). Other examples of adjuvants that can be used a composition described herein, e.g., a vaccine, include saponin, CpG ODN and the like.

[0279] In some cases, the one or more adjuvants is selected from bacteria toxoids, polyoxypropylene-polyoxyethylene block polymers, aluminum salts, liposomes, CpG polymers, oil-in-water emulsions, or a combination thereof.

[0280] Sometimes, the one or more adjuvants can be based on aluminum salts (alum) or derivatives thereof. Exemplary Alums can comprise aluminum hydroxide, aluminum phosphate, potassium aluminum sulfate, sodium aluminum sulfate, ammonium aluminum sulfate, cesium aluminum sulfate, or a mixture of aluminum and magnesium hydroxide. Alum can also comprise crystalline aluminum oxyhydroxide (AlOOH). Sometimes, AlOOH adjuvants can compose of nanolength scale plate-like primary particles that form aggregates, representing the functional subunits in the material. These aggregates can be porous and can have irregular shapes that range from about 1 to about 20 μm in diameter. Upon mixing with antigen, the aggregates can be broken into smaller fragments that can reaggregate to distribute the absorbed antigen throughout the vaccine. In some embodiments, the adjuvant comprises ordered rod-like AlO(OH) nanoparticles.

[0281] In some embodiments, the one or more adjuvants are an oil-in-water emulsion. The oil-in-water emulsion can include at least one oil and at least one surfactant, with the oil(s) and surfactant(s) being biodegradable and biocompatible. The oil droplets in the emulsion can be less than 5 μm in diameter, and can even have a sub-micron diameter, with these small sizes being achieved with a microfluidiser to provide stable emulsions. Droplets with a size less than 220 nm can be preferred as they can be subjected to filter sterilization.

[0282] The oils used can include such as those from an animal (such as fish) or vegetable source. Sources for vegetable oils can include nuts, seeds, and grains. Peanut oil, soybean oil, coconut oil, and olive oil, the most commonly available, exemplify the nut oils. Jojoba oil can be used e.g., obtained from the jojoba bean. Seed oils include safflower oil, cottonseed oil, sunflower seed oil, sesame seed oil, etc. The grain group can include: corn oil and oils of other cereal grains such as wheat, oats, rye, rice, teff, triticale, and the like. 6-10 carbon fatty acid esters of glycerol and 1,2-propanediol, while not occurring naturally in seed oils, can be prepared by hydrolysis, separation and esterification of the appropriate materials starting from the nut and seed oils.

Fats and oils from mammalian milk can be metabolizable and can be used in with the compositions, e.g., vaccines described herein. The procedures for separation, purification, saponification, and other means for obtaining pure oils from animal sources are known in the art. Fish can contain metabolizable oils which can be readily recovered. For example, cod liver oil, shark liver oils, and whale oil such as spermaceti can exemplify several of the fish oils which can be used herein. A number of branched chain oils can be synthesized biochemically in 5-carbon isoprene units and can be generally referred to as terpenoids. Shark liver oil contains a branched, unsaturated terpenoid known as squalene, 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene. Squalane, the saturated analog to squalene, can also be used. Fish oils, including squalene and squalane, can be readily available from commercial sources or can be obtained by methods known in the art.

[0283] Other useful oils include tocopherols, which can be included in a composition described herein, e.g., a vaccine, for use in elderly patients (e.g., aged 60 years or older), as vitamin E can have a positive effect on the immune response in this subject group. Further, tocopherols can have antioxidant properties that can help to stabilize the emulsions. Various tocopherols exist (α, β, γ, δ, ε or ξ); in some cases, a is used. An example of a-tocopherol is DL-a-tocopherol. a-tocopherol succinate can be compatible with compositions provided herein, e.g., influenza vaccines, and can be a useful preservative as an alternative to mercurial compounds. In some embodiments, mixtures of oils can be used e.g., squalene and a-tocopherol. An oil content in the range of 2-20% (by volume) can be used.

[0284] Specific oil-in-water emulsion adjuvants include, e.g., a submicron emulsion of squalene, polysorbate 80, and sorbitan trioleate. The composition of the emulsion by volume can be about 5% squalene, about 0.5% polysorbate 80 and about 0.5% Span 85. In weight terms, these ratios become 4.3% squalene, 0.5% polysorbate 80 and 0.48% Span 85. This adjuvant is known as "MF59". The MF59 emulsion advantageously includes citrate ions e.g., 10 mM sodium citrate buffer.

[0285] An oil-in water emulsion can be a submicron emulsion of squalene, a tocopherol, and polysorbate 80. These emulsions can have from 2 to 10% squalene, from 2 to 10% tocopherol and from 0.3 to 3% polysorbate 80, and the weight ratio of squalene:tocopherol can be preferably ≤1 (e.g., 0.90) as this can provide a more stable emulsion. Squalene and polysorbate 80 can be present at a volume ratio of about 5:2 or at a weight ratio of about 11:5. One such emulsion can be made by dissolving Tween 80 in PBS to give a 2% solution, then mixing 90 ml of this solution with a mixture of (5 g of DL-a-tocopherol and 5 ml squalene), then microfluidising the mixture. The resulting emulsion has submicron oil droplets e.g., with an average diameter of between 100 and 250 nm, preferably about 180 nm. The emulsion may also include a 3-de-O-acylated monophosphoryl lipid A (3d-MPL). Another useful emulsion of this type can comprise, per human dose, 0.5-10 mg squalene, 0.5-11 mg tocopherol, and 0.1-4 mg polysorbate 80.

[0286] An oil-in water emulsion can be an emulsion of squalene, a tocopherol, and a Triton detergent (e.g., Triton X-100). The emulsion can also include a 3d-MPL (see below). The emulsion can contain a phosphate buffer.

[0287] An oil-in water emulsion can be an emulsion comprising a polysorbate (e.g., polysorbate 80), a Triton detergent (e.g., Triton X-100) and a tocopherol (e.g., an a-tocopherol succinate). The emulsion can include these three components at a mass ratio of about 75:11:10 (e.g., 750 μ/ml polysorbate 80, 110 u/ml Triton X-100 and 100 μ/ml α-tocopherol succinate), and these concentrations should include any contribution of these components from antigens. The emulsion can also include squalene. The emulsion can also include a 3d-MPL. The aqueous phase can contain a phosphate buffer.

[0288] An oil-in water emulsion can be an emulsion of squalane, polysorbate 80, and poloxamer 401 ("Pluronic™ L121"). The emulsion can be formulated in phosphate buffered saline, pH 7.4. This emulsion can be a useful delivery vehicle for muramyl dipeptides, and can be used with threonyl-MDP in the "SAF-1" adjuvant (0.05-1% Thr-MDP, 5% squalane, 2.5% Pluronic L121 and 0.2% polysorbate 80). It can also be used without the Thr-MDP, as in the "AF" adjuvant (5% squalane, 1.25% Pluronic L121 and 0.2% polysorbate 80).

[0289] An oil-in water emulsion can be an emulsion comprising squalene, an aqueous solvent, a polyoxyethylene alkyl ether hydrophilic nonionic surfactant (e.g., polyoxyethylene (12) cetostearyl ether) and a hydrophobic nonionic surfactant (e.g., a sorbitan ester or mannide ester, such as sorbitan monoleate or "Span 80"). The emulsion can be thermoreversible and/or has at least 90% of the oil droplets (by volume) with a size less than 200 nm. The emulsion can also include one or more of alditol; a cryoprotective agent (e.g., a sugar, such as dodecylmaltoside and/or sucrose); and/or an alkylpolyglycoside. The emulsion can include a TLR4 agonist. Such emulsions can be lyophilized.

[0290] An oil-in water emulsion can be an emulsion of squalene, poloxamer 105 and Abil-Care. The final concentration (weight) of these components in adjuvanted vaccines can be 5% squalene, 4% poloxamer 105 (pluronic polyol) and 2% Abil-Care 85 (Bis-PEG/PPG-16/16 PEG/PPG-16/16 dimethicone; caprylic/capric triglyceride).

[0291] An oil-in water emulsion can be an emulsion having from 0.5-50% of an oil, 0.1-10% of a phospholipid, and 0.05-5% of a non-ionic surfactant. Phospholipid components can include phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidylglycerol, phosphatidic acid, sphingomyelin, and cardiolipin. Submicron droplet sizes can be advantageous.

[0292] An oil-in water emulsion can be a submicron oil-in-water emulsion of a non-metabolisable oil (such as light mineral oil) and at least one surfactant (such as lecithin, Tween 80 or Span 80). Additives can include, QuilA saponin, cholesterol, a saponin-lipophile conjugate (such as GPI-OlOO, produced by addition of aliphatic amine to desacylsaponin via the carboxyl group of glucuronic acid), dimethyldioctadecylammonium bromide, and/or N,N-dioctadecyl-N,N-bis (2-hydroxyethyl) propanediamine.

[0293] In some embodiments, a composition provided herein, e.g., vaccine, contains adjuvants such as hydrophilic or lipophilic gelling agents, hydrophilic or lipophilic active agents, preserving agents, antioxidants, solvents, fragrances, fillers, sunscreens, odor-absorbers, and dyestuffs. The amounts of these various adjuvants can be those used in the fields considered and, for example, are from about 0.01% to about 20% of the total weight of the composition. Depending on their nature, these adjuvants can be introduced into a fatty phase, into an aqueous phase and/or into lipid vesicles.

[0294] A composition provided herein, e.g., a vaccine, comprising one or more active agent such as a peptide, a nucleic acid molecule, an antibody or fragments thereof, an APC, and/or virus described herein, in combination with one or more adjuvants, can be formulated to comprise certain molar ratios. For example, molar ratios of about 99:1 to about 1:99 of an active agent in combination with one or more adjuvants can be used. In some embodiments, the range of molar ratios of an active agent in combination with one or more adjuvants can be selected from about 80:20 to about 20:80; about 75:25 to about 25:75, about 70:30 to about 30:70, about 66:33 to about 33:66, about 60:40 to about 40:60; about 50:50; and about 90:10 to about 10:90. The molar ratio of an active agent in combination with one or more adjuvants can be about 1:9, and in some cases can be about 1:1. The active agent such as a peptide or polypeptide, a nucleic acid molecule, an antibody or fragments thereof, an APC, and/or virus described herein, in combination with one or more adjuvants can be formulated together, in the same dosage unit e.g., in one vial, suppository, tablet, capsule, an aerosol spray; or each agent, form, and/or compound can be formulated in separate units, e.g., two vials, suppositories, tablets, two capsules, a tablet and a vial, an aerosol spray, and the like.

[0295] A composition provided herein, e.g., vaccine, can comprise one or more adjuvants selected from the list Alum, monophosphoryl lipid A (MPL), imiquimod (R837) (a small synthetic antiviral molecule-TLR7 ligand), Pam2Cys, and ordered rod-like AlO(OH) nanoparticles (Rod). In some embodiments, a composition provided herein, e.g., vaccine, comprises Alum. In some embodiments, a composition, e.g., vaccine, provided herein comprises Rod. In some embodiments, a composition provided herein, e.g., vaccine, comprises Rod, MPL, and R837. In some embodiments, a composition provided herein, e.g., vaccine, comprises MPL and R837. In some embodiments, a composition provided herein, e.g., vaccine, comprises Alum, MPL, and R837. In some embodiments, a composition provided herein, e.g., vaccine, comprises Pam2Cys.

[0296] Additional Agents

[0297] A composition provided herein, e.g., a vaccine, can be administered with an additional active agent. The choice of the additional active agent can depend, at least in part, on the condition being treated. The additional active agent can include, for example, any active agent having a therapeutic effect for a pathogen infection (e.g., viral infection), including, e.g., drugs used to treat inflammatory conditions such as an NSAID, e.g., ibuprofen, naproxen, acetaminophen, ketoprofen, or aspirin. In some embodiments, a formulation for treating or preventing an influenza infection can contain one or more conventional influenza antiviral agents, such as Vitamin D, amantadine, arbidol, laninamivir, rimantadine, zanamivir, peramivir, and oseltamivir. In treatments for retroviral infections, such as HIV, formulations can contain one or more conventional antiviral drugs, such as protease inhibitors (lopinavir/ritonavir (Kaletra®), indinavir (Crixivan®), ritonavir (Norvir®), nelfmavir (Viracept®), saquinavir hard gel capsules (Invirase®), atazanavir (Reyataz®), amprenavir (Agenerase®), fosamprenavir (TelzirR), tipranavir (Aptivus®)), reverse transcriptase inhibitors, including non-Nucleoside and Nucleoside/nucleotide inhibitors (AZT (zidovudine, RetrovirR), ddl (didanosine, VidexR), 3TC (lamivudine, Epivir®), d4T (stavudine, ZeritR), abacavir (ZiagenR), FTC (emtricitabine, Emtriva®), tenofovir (Viread®), efavirenz (Sustiva®) and nevirapine (Viramune®)), fusion inhibitors T20 (enfuvirtide, FuzeonR), integrase inhibitors (MK-0518 and GS-9137), and maturation inhibitors (PA-457 (Bevirimat®)). As another example, formulations can additionally contain one or more supplements, such as vitamin C, vitamin E, and other vitamins and anti-oxidants.

[0298] A composition provided herein, e.g., a vaccine, can include one or more antibiotics (e.g., neomycin, kanamycin, polymyxin B).

[0299] In some embodiments, the composition, e.g., a vaccine, can be gluten free.

[0300] Co-Solvents

[0301] The solubility of the components of a composition provided herein can be enhanced by a co-solvent in the composition. Such co-solvents include polysorbate 20, 60, and 80, Pluronic F68, F-84 and P-103, cyclodextrin, or other agents known to those skilled in the art. Such co-solvents can be employed at a level of from about 0.01% to 2% by weight.

[0302] Penetration Enhancers

[0303] In some embodiments, a composition provided herein, e.g., a vaccine, can include one or more penetration enhancers. For example, the composition can comprise suitable solid or gel phase carriers or excipients that increase penetration or help delivery of agents or combinations of agents across a permeability barrier, e.g., the skin. Examples of penetration-enhancing compounds include, e.g., water, alcohols (e.g., terpenes like methanol, ethanol, 2-propanol), sulfoxides (e.g., dimethyl sulfoxide, decylmethyl sulfoxide, tetradecylmethyl sulfoxide), pyrrolidones (e.g., 2-pyrrolidone, N-methyl-2-pyrrolidone, N-(2-hydroxyethyl)pyrrolidone), laurocapram, acetone, dimethylacetamide, dimethylformamide, tetrahydrofurfuryl alcohol, L-α-amino acids, anionic, cationic, amphoteric or nonionic surfactants (e.g., isopropyl myristate and sodium lauryl sulfate), fatty acids, fatty alcohols (e.g., oleic acid), amines, amides, clofibric acid amides, hexamethylene lauramide, proteolytic enzymes, a-bisabolol, d-limonene, urea and N,N-diethyl-m-toluamide, and the like. Additional examples include humectants (e.g., urea), glycols (e.g., propylene glycol and polyethylene glycol), glycerol monolaurate, alkanes, alkanols, ORGELASE, calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and/or other polymers. In some embodiments, the compositions will include one or more such penetration enhancers.

[0304] Additives for Sustained Release Formulations

[0305] In some embodiments, one or more active agents can be attached releasably to biocompatible polymers for use in sustained release formulations on, in or attached to inserts for topical, intraocular, periocular, or systemic administration. The controlled release from a biocompatible polymer can be utilized with a water soluble polymer to form an instillable formulation. The controlled release from a biocompatible polymer, such as PLGA microspheres or nanospheres, can be utilized in a formulation suitable for intra ocular implantation or injection for sustained release administration. Any suitable biodegradable and biocompatible polymer can be used.

[0306] Administration Routes

[0307] A composition described herein, e.g., a vaccine, can be delivered via a variety of routes to a subject, e.g., a human. Delivery routes can include oral (including buccal and sublingual), rectal, nasal, topical, transdermal, transmu-

cosal, pulmonary, vaginal, suppository, or parenteral (including intramuscular, intra-arterial, intrathecal, intradermal, intraperitoneal, subcutaneous and intravenous) administration or in a form suitable for administration by aerosolization, inhalation or insufflation. The composition, e.g., vaccine, can be administered to muscle, or can be administered via intradermal or subcutaneous injections, or transdermally, such as by iontophoresis. The composition, e.g., vaccine, can be delivered to a subject by epidermal administration.

[0308] Therapeutic Regimens

[0309] A composition provided herein, e.g., a vaccine, can be administered to a subject in a dosage volume of about 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.55, 0.6, 0.7, 0.8, 0.9, 1.0 mL, or more. A half dose, e.g., about 0.25 mL, can be administered to a child. Sometimes the vaccine can be administered in a higher dose, e.g., about 1 mL.

[0310] The composition, e.g., vaccine, can be administered as a 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more dose-course regimen. Sometimes, the vaccine can be administered as a 2, 3, or 4 dose-course regimen. Sometimes the vaccine can be administered as a 2 dose-course regimen.

[0311] The administration of the first dose and second dose of the 2 dose-course regimen can be separated by about 0 day, 1 day, 2 days, 5 days, 7 days, 14 days, 21 days, 30 days, 2 months, 4 months, 6 months, 9 months, 1 year, 1.5 years, 2 years, 3 years, 4 years, 5 years, 10 years, 20 years, or more. A composition described herein, e.g., vaccine, can be administered to a subject once a year, twice a year, three times a year, every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more years. Sometimes, the composition, e.g., vaccine, can be administered to a subject every 2, 3, 4, 5, 6, 7, or more years. Sometimes, the composition, e.g., vaccine, can be administered every 4, 5, 6, 7, or more years. Sometimes, the composition, e.g., vaccine, can be administered to a subject once. Sometimes, the composition, e.g., vaccine, can be taken by a subject as a multiple dose vaccine over a period of time, e.g., a 2-dose vaccine wherein the second dose can be taken 4-5 years after the first dose. In some cases, a composition, e.g., vaccine, is administered at a time from August to March, from September to February, from October to January, from November to December, e.g. in a region in the North Hemisphere. In some cases, a composition, e.g., vaccine, is administered at a time from March to October, from April to September, from May to August, from June to July, e.g. in a region in the South Hemisphere.

[0312] The dosage examples are not limiting and are only used to exemplify particular dosing regiments for administering a composition, e.g., vaccine, described herein. In some embodiments, a "therapeutically effective amount" for use in a human can be determined from an animal model. For example, a dose for a human can be formulated to achieve circulating, liver, topical, and/or gastrointestinal concentrations that have been found to be therapeutically effective in an animal. Based on animal data, and other types of similar data, those skilled in the art can determine a therapeutically effective amount of a composition, e.g., vaccine, appropriate for administration to a human.

[0313] A composition described herein, e.g., a vaccine, can be used to treat or prevent seasonal influenza or pandemic influenza. In some embodiments, the methods and compositions described herein can target an influenza virus subtype. Influenza A virus can be subtyped based on hemagglutinin (HA) and neuraminidase (N), two proteins expressed on the surface of the viral envelope. Influenza A virus can display about 18 HA subtypes: H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, H17, and H18; and about eleven N subtypes: N1, N2, N3, N4, N5, N6, N7, N8, N9, N10, and N11. Together, the HA and N subtypes can be combined in any combination. Non-limiting examples of the HA and N subtype combinations that have been observed include: H1N1, H1N2, H1N7, H2N2, H3N2, H3N8, H4N8, H5N1, H5N2, H5N8, H5N9, H6N5, H7N1, H7N2, H7N3, H7N4, H7N7, H7N9, H8N4, H9N2, H10N7, H11N6, H12N5, H13N6, and H14N5. In some embodiments, the vaccines described herein can target an influenza A virus that has a combination of the HA and N subtypes disclosed herein. In some cases, the combination can be represented by HxNy, wherein x represents any HI-HI 8 subtypes, and y represents any Nl-Nl 1 subtypes. For example, in some embodiments, vaccines disclosed herein can target a subtype represented as H1Ny, which is HI in combination with any N subtype described herein, or a subtype represented as H2Ny, and the like. In some embodiments, a vaccine described herein can target an influenza A virus that has the HA and N subtype combinations H1N1, H1N2, H1N7, H2N2, H3N2, H3N8, H4N8, H5N1, H5N2, H5N8, H5N9, H6N5, H7N1, H7N2, H7N3, H7N4, H7N7, H7N9, H8N4, H9N2, H10N7, H11N6, H12N5, H13N6, or H14N5.

[0314] In some embodiments, a composition, e.g., vaccine, described herein can target an influenza B virus. Influenza B viruses can be classified into lineages and strains. An influenza B virus can belong to either the B/Yamagata or the B/Victoria lineage. Exemplary influenza B virus strains include Brisbane/60/2008, Massachusetts/2/2012, and Wisconsin/l/2010.

[0315] In some embodiments, a composition, e.g., vaccine, described herein can target an influenza A virus, influenza B virus, and/or an influenza C virus. In some embodiments, a composition, e.g., vaccine, described herein can target strains of influenza A virus, influenza B virus, influenza C virus, or a combination thereof.

[0316] In some embodiments, a composition, e.g., vaccine, described herein can be used to treat a patient who has an influenza infection, such as an influenza A virus infection, an influenza B virus infection, or an influenza C virus infection. Sometimes, a composition, e.g., vaccine, described herein can be used as a vaccination method against the infection of influenza A virus, influenza B virus, or influenza C virus. Sometimes, a composition, e.g., vaccine, described herein offers cross-protection against the different strains associated with the influenza A virus, the influenza B virus, and/or the influenza C virus.

[0317] The term "therapeutically effective amount" as used herein can mean an amount which is effective to alleviate, ameliorate, or prevent a symptom or sign of a disease or condition to be treated. For example, in some embodiments, a therapeutically effective amount can be an amount which has a beneficial effect in a subject having signs and/or symptoms of a viral infection, e.g., an influenza infection, e.g., an influenza A infection. In some embodiments, a therapeutically effective amount can be an amount which inhibits or reduces signs and/or symptoms of a viral infection, e.g., an influenza infection, e.g., an influenza A infection, as compared to a control. Signs and symptoms of an influenza infection, e.g., an influenza A infection, are well-known in the art and can include fever, cough, sore

throat, runny nose, stuffy nose, headache, muscle aches, chills, fatigue (tiredness), nausea, vomiting, diarrhea, pain (e.g., abdominal pain), conjunctivitis, shortness of breath, difficulty breathing, pneumonia, acute respiratory distress, viral pneumonia, respiratory failure, neurologic change (e.g., altered mental status, seizure), or a combination thereof. In some embodiments, the therapeutically effective amount can be one which is sufficient to reduce any of the signs and/or symptoms by about, or at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% in a subject as compared to a control.

[0318] A therapeutically effective amount, when referring to one or more active agents, can be a dose range, mode of administration, formulation, etc., that has been recommended or approved by any of the various regulatory or advisory organizations in the medical or pharmaceutical arts (e.g., FDA, AMA) or by the manufacturer or supplier.

[0319] In some aspects of the present disclosure, the composition can comprise a peptide based formulation. The composition comprising a polypeptide described herein can be administered to a subject between about 1 nmol/dose and about 1000 μmol/dose. In some embodiments, the composition can be administered to a subject at a dose about 1 nmol/dose, about 5 nmol/dose, about 10 nmol/dose, about 20 nmol/dose, about 30 nmol/dose, about 40 nmol/dose, about 50 nmol/dose, about 60 nmol/dose, about 70 nmol/dose, about 80 nmol/dose, about 90 nmol/dose, about 100 nmol/dose, about 200 nmol/dose, about 300 nmol/dose, about 400 nmol/dose, about 500 nmol/dose, about 600 nmol/dose, about 700 nmol/dose, about 800 nmol/dose, about 900 nmol/dose, about 1 μmol/dose, about 1 μmol/dose, about 2 μmol/dose, about 3 μmol/dose, about 4 μmol/dose, about 5 μmol/dose, about 6 μmol/dose, about 7 μmol/dose, about 8 μmol/dose, about 9 μmol/dose, about 10 μmol/dose, about 20 μmol/dose, about 50 μmol/dose, about 100 μmol/dose, about 200 μmol/dose, about 300 μmol/dose, about 400 μmol/dose, about 500 μmol/dose, about 750 μmol/dose, about 1000 μmol/dose, or any dose between any two thereof. In some cases, the composition can be administered to a subject at a dose about 50 nmol/dose.

[0320] In some aspects of the present disclosure, the composition can comprise a polynucleotide encoding a polypeptide described herein. The composition administered to a subject can comprise the polynucleotide at a dose between about 1 pmol/dose and about 1000 μmol/dose. In some embodiments, the composition administered to a subject can comprise the polynucleotide at a dose between about 1 pmol/dose, about 5 pmol/dose, about 10 pmol/dose, about 20 pmol/dose, about 30 pmol/dose, about 40 pmol/dose, about 50 pmol/dose, about 60 pmol/dose, about 70 pmol/dose, about 80 pmol/dose, about 90 pmol/dose, about 100 pmol/dose, about 200 pmol/dose, about 300 pmol/dose, about 400 pmol/dose, about 500 pmol/dose, about 600 pmol/dose, about 700 pmol/dose, about 800 pmol/dose, about 900 pmol/dose, 1 nmol/dose, about 5 nmol/dose, about 10 nmol/dose, about 20 nmol/dose, about 30 nmol/dose, about 40 nmol/dose, about 50 nmol/dose, about 60 nmol/dose, about 70 nmol/dose, about 80 nmol/dose, about 90 nmol/dose, about 100 nmol/dose, about 200 nmol/dose, about 300 nmol/dose, about 400 nmol/dose, about 500 nmol/dose, about 600 nmol/dose, about 700 nmol/dose, about 800 nmol/dose, about 900 nmol/dose, about 1 μmol/dose, about 1 μmol/dose, about 2 μmol/dose, about 3 μmol/dose, about 4 μmol/dose, about mol/dose, about 6 μmol/dose, about 7 μmol/dose, about 8 μmol/dose, about 9 μmol/dose, about 10 μmol/dose, about 20 μmol/dose, about 50 μmol/dose, about 100 μmol/dose, about 200 μmol/dose, about 300 μmol/dose, about 400 μmol/dose, about 500 μmol/dose, about 750 μmol/dose, about 1000 μmol/dose, or any dose between any two thereof.

[0321] In some aspects of the present disclosure, the composition can comprise a recombinant virus containing a polynucleotide encoding a polypeptide as described herein. The composition comprising recombinant virus can be administered to a subject between about 10^3 and 10^{12} viral particles or plaque forming units (PFU), or between about 10^5 and 10^{10} PFU, or between about 10^5 and 10^8 PFU, or between about 10^8 and 10^{10} PFU. In some embodiments, the amount of a virus vaccine of this disclosure administered to a subject can be between about 10^3 and 10^{12} viral particles or plaque forming units (PFU), or between about 10^5 and 10^{10} PFU, or between about 10^5 and 10^8 PFU, or between about 10^8 and 10^{10} PFU. Sometimes, a virus vaccine of this disclosure administered to a subject can be administered at a dose about 10^3 PFU/dose to about 10^4 PFU/dose, about 10^4 PFU/dose to about 10^5 PFU/dose, about 10^5 PFU/dose to about 10^6 PFU/dose, about 10^7 PFU/dose to about 10^8 PFU/dose, about 10^9 PFU/dose to about 10^{10} PFU/dose, about 10^{10} PFU/dose to about 10^{11} PFU/dose, about 10^{11} PFU/dose to about 10^{12} PFU/dose, about 10^{12} PFU/dose to about 10^{13} PFU/dose, about 10^{13} PFU/dose to about 10^{14} PFU/dose, or about 10^{14} PFU/dose to about 10^{15} PFU/dose. A virus vaccine of this disclosure administered to a subject can comprise about 2×10^3 PFU/dose, 3×10^4 PFU/dose, 4×10^3 PFU/dose, 5×10^3 PFU/dose, 6×10^3 PFU/dose, 7×10^3 PFU/dose, 8×10^3 PFU/dose, 9×10^3 PFU/dose, about 10^4 PFU/dose, about 2×10^4 PFU/dose, about 3×10^4 PFU/dose, about 4×10^4 PFU/dose, about 5×10^4 PFU/dose, about 6×10^4 PFU/dose, about 7×10^4 PFU/dose, about 8×10^4 PFU/dose, about 9×10^4 PFU/dose, about 10^5 PFU/dose, 2×10^5 PFU/dose, 3×10^5 PFU/dose, 4×10^5 PFU/dose, 5×10^5 PFU/dose, 6×10^5 PFU/dose, 7×10^5 PFU/dose, 8×10^5 PFU/dose, 9×10^5 PFU/dose, about 10^6 PFU/dose, about 2×10^6 PFU/dose, about 3×10^6 PFU/dose, about 4×10^6 PFU/dose, about 5×10^6 PFU/dose, about 6×10^6 PFU/dose, about 7×10^6 PFU/dose, about 8×10^6 PFU/dose, about 9×10^6 PFU/dose, about 10^7 PFU/dose, about 2×10^7 PFU/dose, about 3×10^7 PFU/dose, about 4×10^7 PFU/dose, about 5×10^7 PFU/dose, about 6×10^7 PFU/dose, about 7×10^7 PFU/dose, about 8×10^7 PFU/dose, about 9×10^7 PFU/dose, about 10^8 PFU/dose, about 2×10^8 PFU/dose, about 3×10^8 PFU/dose, about 4×10^8 PFU/dose, about 5×10^8 PFU/dose, about 6×10^8 PFU/dose, about 7×10^8 PFU/dose, about 8×10^8 PFU/dose, about 9×10^8 PFU/dose, about 10^9 PFU/dose, about 2×10^9 PFU/dose, about 3×10^9 PFU/dose, about 4×10^9 PFU/dose, about 5×10^9 PFU/dose, about 6×10^9 PFU/dose, about 7×10^9 PFU/dose, about 8×10^9 PFU/dose, about 9×10^9 PFU/dose, about 10^{10} PFU/dose, about 2×10^{10} PFU/dose, about 3×10^{10} PFU/dose, about 4×10^{10} PFU/dose, about 5×10^{10} PFU/dose, about 6×10^{10} PFU/dose, about 7×10^{10} PFU/dose, about 8×10^{10} PFU/dose, about 9×10^{10} PFU/dose, about 10^{10} PFU/dose, about 2×10^{10} PFU/dose, about 3×10^{10} PFU/dose, about 4×10^{10} PFU/dose, about 5×10^{10} PFU/dose, about 6×10^{10} PFU/dose, about 7×10^{10} PFU/dose, about 8×10^{10} PFU/dose, about 9×10^{10} PFU/dose, about 10^{11} PFU/dose, about 2×10^{11} PFU/dose, about 3×10^{11} PFU/dose, about 4×10^{11} PFU/dose, about 5×10^{11} PFU/dose, about 6×10^{11} PFU/dose, about 7×10^{11} PFU/dose, about 8×10^{11} PFU/dose, about 9×10^{11} PFU/dose,

or about 10^{12} PFU/dose, about 10^{12} PFU/dose to about 10^{13} PFU/dose, about 10^{13} PFU/dose to about 10^{14} PFU/dose, or about 10^{14} PFU/dose to about 10^{15} PFU/dose, or any dose between any two thereof.

[0322] Sometimes, a virus vaccine of this disclosure administered to a subject can be administered at a dose about 10^4 viral particles/dose, about 10^4 viral particles/dose to about 10^5 viral particles/dose, about 10^5 viral particles/dose to about 10^6 viral particles/dose, about 10^7 viral particles/dose to about 10^8 viral particles/dose, about 10^9 viral particles/dose to about 10^{10} viral particles/dose, about 10^{10} viral particles/dose to about 10^{11} viral particles/dose, about 10^{11} viral particles/dose to about 10^{12} viral particles/dose, about 10^{12} viral particles/dose to about 10^{13} viral particles/dose, about 10^{13} viral particles/dose to about 10^{14} viral particles/dose, or about 10^{14} viral particles/dose to about 10^{15} viral particles/dose. In some embodiments, a virus vaccine of this disclosure administered to a subject can be administered at a dose about 1×10^9 viral particles/dose, about 1.5×10^9 viral particles/dose, about 2×10^9 viral particles/dose, about 2.5×10^9 viral particles/dose, about 3×10^9 viral particles/dose, about 3.5×10^9 viral particles/dose, about 4×10^9 viral particles/dose, about 4.5×10^9 viral particles/dose, about 5×10^9 viral particles/dose, about 5.5×10^9 viral particles/dose, about 6×10^9 viral particles/dose, about 6.5×10^9 viral particles/dose, about 7×10^9 viral particles/dose, about 7.5×10^9 viral particles/dose, about 8×10^9 viral particles/dose, about 8.5×10^9 viral particles/dose, about 9×10^9 viral particles/dose, about 1×10^{10} viral particles/dose, about 2×10^{10} viral particles/dose, about 3×10^{10} viral particles/dose, about 4×10^{10} viral particles/dose, about 5×10^{10} viral particles/dose, about 6×10^{10} viral particles/dose, about 7×10^{10} viral particles/dose, about 8×10^{10} viral particles/dose, about 9×10^{10} viral particles/dose, or any dose in between any two thereof. In some embodiments, a virus vaccine of this disclosure administered to a subject can be administered at a dose about 7.5×10^9 viral particles/dose.

[0323] A composition provided herein, e.g., a vaccine can be administered before, during, or after the onset of a symptom associated with a pathogen infection, e.g., an influenza A infection. Exemplary symptoms can include fever, cough, sore throat, runny nose, stuffy nose, headache, muscle aches, chills, fatigue, nausea, vomiting, diarrhea, pain (e.g., abdominal pain), conjunctivitis, shortness of breath, difficulty breathing, pneumonia, acute respiratory distress, viral pneumonia, respiratory failure, neurologic change (e.g., altered mental status, seizure), or a combination thereof. In some cases, the composition, e.g., vaccine, can be administered to a subject in order to treat a pathogen infection, e.g., influenza infection, e.g., influenza A infection. Sometimes, the composition, e.g., vaccine, can be administered to a subject for a preventive purpose, such as a prophylactic treatment of a pathogen infection, e.g., influenza infection, e.g., influenza A infection. The composition, e.g., vaccine, can be administered to a subject to illicit an immune response from a subject. The composition, e.g., vaccine, can be administered to a subject to illicit an immune response from the subject prior to a pathogen infection, during a pathogen infection, or as a prophylactic measure against a pathogen infection. Following administration of a composition provided herein, e.g., a vaccine, a symptom associated with a pathogen can be reduced about, at least, or at most 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%, with about, at least, or at most 1 day, 5 days, 1 week, 2 weeks, 3 weeks, or 1 month.

[0324] A complication from an influenza infection, e.g. an influenza A infection, can be, e.g., pneumonia, bronchitis, sinus infection, or an ear infection. In some cases, an influenza infection, e.g., an influenza A infection, can make a chronic health problem worse, e.g., a person with asthma may experience an asthma attack while the person has an influenza infection, or a person with chronic congestive heart failure can experience worsening of this condition while the person has an influenza infection. In some embodiments, a therapeutically effective amount of a composition, e.g., a vaccine described herein, can be administered to a subject with a complication from an influenza infection. In some embodiments, a therapeutically effective amount of a composition, e.g., a vaccine described herein, can be administered to a subject with an influenza infection (e.g., an influenza A infection) and one or more chronic health problems.

[0325] A composition provided herein, e.g., a vaccine, or a kit described herein can be stored at between 2° C. and 8° C. Sometimes, the composition, e.g., vaccine, can be stored at room temperature. In some embodiments, the composition, e.g., vaccine, may not be stored frozen. In some embodiments, the composition, e.g., vaccine, can be stored in a temperature such as at −20° C. or −80° C. In some embodiments, the composition, e.g., vaccine, can be stored away from sunlight.

EXAMPLE

[0326] The following examples are offered by way of illustration, not by way of limitation.

Example 1

[0327] The peptide sequence TYQRTRALV (SEQ ID NO: 37) was selected based on 1) being an experimentally validated BALB/C influenza A virus CD8 T-cell epitope in worldwideweb.fludb.org and 2) having an average invariance ratio (frequency of mutant in final population/frequency in initial population) of <0.08 among all possible mutations in the stretch (generally each residue has 6-7 possible mutations from single nucleotide changes within the codon). Peptide was conjugated to Pam2Cys (a liposomal adjuvant that activates TLR2 and is covalently linked to the peptide) and a CD4 T cell epitope from HA (GALNNRFQIKGVELKS (SEQ ID NO: 115)) (epitopes connected via a central lysine, and Pam2Cys conjugated to central lysine via two serines). (CS Bio, Menlo Park, CA synthesized the lipopeptides) A formulation was made with 50 nmol of Pam2Cys-peptide having the sequence TYQRTRALV (SEQ ID NO: 37) per 20 μL of phosphate buffered saline (PBS) (2.5 μM). A volume of 20 μL of the formulation was administered intranasally to each of 10 BALB/C mice ("Group 2"). A volume of 20 μl of PBS was administered intranasally to each of 10 control BALB/C mice ("Group 1"). Four weeks after the administrations, the mice in both groups were challenged with 100 $TCID_{50}$ PR8 influenza A virus. 60% (6 of 10) of mice in the vaccine group (Group 2) compared to 0% (0 of 10) in the control group (Group 1) survived after 9 days from the lethal challenge of influenza A virus, showing statistically significant protection by vaccine (see FIG. **2**).

Example 2

[0328] This example describes vaccination with a vaccine comprising 4 influenza virus epitopes. A recombinant adenovirus carrying 4 influenza virus epitopes: ELRSRYWAIRTRSG (NP) (SEQ ID NO: 17), FYIQMCTEL (NP) (SEQ ID NO: 79), TYNAELLVLL (HA) (SEQ ID NO: 80), and TYQRTRALV (NP) (SEQ ID NO: 37) was generated. The recombinant adenovirus contained a single transgene with the four peptides encoded in tandem and separated by the linker RVKR (SEQ ID NO: 110). The sequence of the transgene product is GALNNRFQIKGVELKSKTYQRTRALVRVKRELRSRYWAIRTRSGRVKRFYIQMCTELRVKRTYN AELLVLL (SEQ ID NO: 81). For generating the recombinant adenoviral vector, the transgene was codon optimized for expression in mouse cells. The recombinant adenovirus was administered subcutaneously and intranasally simultaneously to Group 1 mice (10 BALB/C mice) at a dose of 2×10^7 pfu (plaque-forming units) in 20 d (7.5×10^9 viral particles to each s.c. and i.n). The mice were challenged 28 days later with a lethal dose of 5-10 LD50 (lethal dose 50) (~100 TCID50 or tissue culture infectious dose 50) of H1N1 flu. All 10 mice in this group survived. 9 out of the 10 mice initially lost weight but regained most of their weight by day 12 post-challenge. See FIG. **3**A and FIG. **4**.

[0329] In a control group (Group 2, unvaccinated, injected with saline s.c. and i.n.), 10 BALB/C mice were challenged with a lethal dose of 5-10 LD50 (lethal dose 50) (~100 TCID50 or tissue culture infectious dose 50) of H1N1 flu. 0 of 10 mice survived, all of the mice died by day 9 post-challenge. See FIG. **3**B and FIG. **4**.

[0330] In another set of experiments, AdCre-injected mice were used as control group (Group 1 in FIG. **3**C) for the Ad vector. AdCre is an adenovirus produced using the same adenoviral vector as the recombinant adenovirus vaccine, but inserted with a Cre recombinase gene instead of the nucleotide sequence expressing the 4 epitopes. As shown in FIG. **3**C, **3** of 5 mice in the control group perished, and the other 2 5 mice lost substantial weight. In contrast, in the vaccinated group (Group 2 in FIG. **3**D), 0 of 5 mice lost >10% body weight. All 5 mice in AdCre had symptom scores of 3 or greater (not shown), whereas all 5 mice in the vaccine group had no higher than 1.

Example 3

[0331] This example describes vaccination with a vaccine comprising 9 influenza virus epitopes. A recombinant adenovirus carrying 9 influenza virus epitopes is generated. A vaccine cocktail of peptides comprising 9 influenza virus epitopes can also be used. Influenza virus epitopes can comprise any combination of the epitopes listed in Table 1.

[0332] Vaccination can be performed utilizing HLA-B44-transgenic mice. A prime-boost regimen can be employed. Priming can be with 9 Pam2Cys-adjuvanted peptides. Boosting can be with a recombinant adenovirus carrying 9 influenza virus epitopes or with a universal helper T cell epitope. Following vaccination, mice are challenged with the PR8 strain of influenza virus. Mice are monitored for health, weight, and survival to assess protection conferred by vaccination.

Example 4

[0333] This example describes experimental procedures and materials for heterosubtypic protection test of influenza virus.

[0334] Kill Curve

[0335] A kill curve experiment (a lethal dose curve) can be performed to determine the LD50 (a dose at which 50% of subject animals die), so that a challenge dose of 5-10LD50 can be used for the protection experiment.

[0336] Day 1: 3 mice in each Group, i.n. in 25 ul

[0337] Vict Id is H3N2, and is 3.2×10^7 TCID50/ml

[0338] 1) Group A: 10 TCID50 Vict Id (H3N2) (Make this 5th: Dilution #5: 50 ul Dilution #4+450 ul PBS)

[0339] 2) Group B: 100 TCID50 Vict Id (Make this 4th: Dilution #4: 50 ul Dilution #3+450 ul PBS)

[0340] 3) Group C: 1,000 TCID50 Vict Id (Make this 3rd: Dilution #3: 50 ul Dilution #2+450 ul PBS)

[0341] 4) Group D: 10,000 TCID50 Vict Id (Make this 2nd: Dilution #2: 50 ul Dilution #1+450 ul PBS)

[0342] 5) Group E: 100,000 TCID50 Vict Id (Make this 1st: Dilution #1: 100 ul virus stock+700 ul PBS)

[0343] Heterosubtypic Protection

[0344] Day 1: Immunize mice:

[0345] 1) Group 1: 10 mice with 20 ul saline i.n. (intranasal) and 20 ul saline s.c. (subcutaneous)

[0346] 2) Group 2: 10 mice with 2×10^7 pfu in 20 ul AdBALB i.n. and same s.c.

[0347] For 2×10^7 pfu AdBALAB: To 3 vials AdBALB, add 115 ul PBS each. Mix vials together (should be 420 ul total, just enough; can also use some from vial in Group 6) and administer above.

[0348] 3) Group 3: 5 mice with 2×10^7 pfu in 20 ul AdBALB5-pep i.n. and same s.c.

[0349] For 2×10^7 pfu AdBALABS-pep: To 1 vial AdBALB5-pep, add 230 ul PBS and administer above.

[0350] 4) Group 4: 5 mice with 7.5×10^9 viral particles in 20 ul i.n. and same s.c.

[0351] For 10×10^9 pfu AdBALABS-pep: To 1 vial AdBALB5-pep, add 722 ul PBS and administer above.

[0352] 5) Group 5: 5 mice with 20 ul AdCre i.m. (intramuscular)

[0353] For 20 ul AdCre: To 1 vial AdCre, add 653 ul PBS and administer above.

[0354] 6) Group 6: 5 mice with 20 ul AdBALB i.m.

[0355] For 20 ul AdBALB: To 1 vial AdBALB, add 115 ul PBS and administer above.

[0356] Days 2-15: Monitor body weight, survival, and clinical scores

[0357] Day 29: Challenge mice: All i.n. in 20 ul

[0358] 1) Groups 1-4: 5-10 LD50 (selected on the basis of kill curve) Vict id

[0359] 2) Groups 5-6: 100 TCID50 PR8 (Dilute virus stock 1/10,000: 10 ul virus stock+990 ul PBS; 10 ul of this dilution+990 ul PBS for working stock)

[0360] Days 30-43: Monitor body weight, survival, and clinical scores

[0361] Reagents

[0362] Total 55 BALB/C 6-12 wk-old female mice.

[0363] Adenoviruses: AdBALB (control virus with no epitope sequences); AdBALB5-pep (vaccine carrying 5 different influenza epitope sequences); AdCre (control virus

with no epitope sequences but Cre transgene). Influenza viruses: PR8; Vict id (H3N2).

Example 5

[0364] This example describes a recombinant adenovirus based vaccine (AdFlu51pep) having 51 different influenza virus epitopes. The recombinant adenovirus is engineered with a single transgene that expresses all 51 epitope sequences in Table 3, which are encoded in tandem and separated by a linker RVKR (SEQ ID NO: 110). The order of the 51 epitope sequences can be random, thereby generating a variety of different transgene sequences. The sequence of one example of the transgene products, or a portion of the transgene product, is as listed in Table 4.

TABLE 4

SEQ ID NO: 106
ELRSRYWAIRTRSGRVKRELRSRHWAIRTRSGRVKRELRSRYWASRTRSGRVKRFMYSDFHFIRVKREMY SDLHFIRVKRFMYTDFHFIRVKREMFSDFHFIRVKRGTFEFTSFFYRVKRGTFEFTSYFYRVKRILKGKF QTARVKRIIKGKFQTARVKRILKGKFQIARVKRILRGSIAHKRVKRILRGSVAHKRVKRVLRGSIAHKRV KRLIFLARSALRVKRLVELARSALRVKRLTFLARSALRVKRYSHGTGTGYRVKRYSHWTGTGYRVKRYSH GSGTGYRVKRFLARSALILRGSVAHKRVKRFLARSALVLRGSVAHKRVKRIAYERMCNILKGKFQTAARV KRVAYERMCNILKGKFQTAARVKRVAYERMCNIIKGKFQTAARVKRVAYERMCNILKGKFKTAARVKRVA YERMCNILKGKFQIAARVKRVAYERMCNILKGKFQTAVRVKRDVVNFVSMEFSLTDPRLRVKRDVVNFVS MEFSLTYPRLRVKRDVVNFVSMEFSLTDQRLRVKRFLARSALILRGSVAHKSRVKRFLARSALVLRGSVA HKSRVKRKWGMEMRRCLLQSLQQIRVKRKLGMEMRRCLLQSLQQIRVKRKWGMEMRRCLLQSLQQVRVKR KWGMELRRCLLQSLQQIRVKRFQGRGVFELRVKRGQISIQPTFSRVKRSQISVQPTFSRVKRGQVSVQPT FSRVKRGQISVQPTFSRVKRWHSNLNDATYQRTRALVRTGMDPRMRVKRWHSNLNDTTYQRTRALVRTGM DPRMRVKRWHSNLNDSTYQRTRALVRTGMDPRMRVKRWHSNLNDATYQRKRALVRTGMDPRMRVKRWHSN LNDATYQRTRSLVRTGMDPRMRVKRWHSNLNDATYQRTRAIVRIGMDPRMRVKRWHSNLNDATYQRTRAL VRSGMDPRMRVKRWHSNLNDATYQRTRALVRTGRDPRM

[0365] The transgene can express the polypeptide linked to one or more copies (e.g., 2, 3, 4, or 5) of influenza B virus NP protein, e.g., SEQ ID NO: 116, 117 or 118, or fragments of the influenza B virus NP protein (e.g., amino acids 2-560 of SEQ ID NO: 116, 117 or 118). In some cases, the vaccine comprises a second adenovirus with a second transgene that can expresses a polypeptide comprising one or more copies (e.g., 2, 3, 4, 5) of influenza B virus NP protein, e.g., SEQ ID NO: 116, 117 or 118, or fragments of influenza B virus NP protein. In some cases, an adenovirus can comprise a transgene that can express a polypeptide comprising SEQ ID NO. 106 and a second polypeptide comprising one or more copies (e.g., 2, 3, 4, or 5) of influenza B virus NP protein, e.g., SEQ ID NO: 116, 117 or 118, or fragments of the influenza B virus NP protein.

Example 6

[0366] This example describes production of an adenovirus based influenza vaccine as described in Example 5.

[0367] First, shuttle vector (Add2) containing the transgene that expresses SEQ ID NO: 106 can be constructed using conventional molecular cloning techniques. The transgene is driven by CMV promoter. Expression of the transgene can be checked at this point by transfection into 293T cells. Vector construct will be optimized if no polypeptide expression can be observed in transfected 293T cells.

[0368] Maxiprep pAdFlu51pep will then be linearized and transfected into 293 cells for generation of adenoviral plaques. A replication defective C68 helper virus or C6 virus will be supplemented to the transfected 293 cells for adenovirus packaging. Viral plaques will form 7-10 days after plating. Plaques can then be picked, frozen for later use, or subject to expansion of the viruses. Laboratory scale expansion of the pAdFlu51pep virus can be performed by serial passage of subconfluent 293 cells through different sizes of cell culture flasks, like T25, T75, or even T150.

[0369] Cell culture media and lysed cells will be subject to centrifugation for viral stock. Titering will be performed according to routine virus tittering procedures.

Example 7

[0370] This example describes clinical studies for the adenovirus based vaccine as described in Example 5 in human subject.

[0371] An open-label, uncontrolled Phase 1 study is carried out to evaluate the adenovirus based vaccine (AdFlu51pep vaccine) containing the 51 different epitope sequences in Table 3 in healthy adults.

[0372] Objectives: Evaluate the safety, tolerability and immunogenicity of the AdFlu51pep vaccine in healthy adult volunteers of both sexes between the ages of 18 and 55 years.

[0373] Dose

[0374] The starting dose for AdFlu51pep vaccine ranges from 3×10^5 to 1×10^9 PFU by intramuscular injection into deltoid once, including dose levels at 3×10^5 PFU, 3×10^6 PFU, 3×10^7 PFU, 3×10^8 PFU, and 1×10^9 PFU.

[0375] Each dose level enrolls between 6 and 12 evaluable healthy adults.

[0376] Safety Monitoring

[0377] Injection-site and systemic reactogenicity and medication use can be recorded for 7 days after injection and at follow-up (days 14 and 28). Clinical and laboratory evaluations can be performed during each study visit. Laboratory analyses can include a complete blood count and measurements of creatinine, C-reactive protein, and liver function. Adverse events will be listed for each participant.

[0378] Immunogenicity

[0379] Immunogenicity can be assessed by assaying serum samples of the participants. IFN-γ ELISPOT to assay T cell immunogenicity will be conducted with participants' serum samples collected at baseline (before vaccination) and at 28 and 180 days after injection.

[0380] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations,

changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein can be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

SEQUENCE LISTING

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Sequence total quantity: 118
SEQ ID NO: 1          moltype = AA  length = 9
FEATURE               Location/Qualifiers
REGION                1..9
                      note = Description of Artificial Sequence: Synthetic peptide
source                1..9
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 1
GPATAQMAL                                                          9

SEQ ID NO: 2          moltype = AA  length = 10
FEATURE               Location/Qualifiers
REGION                1..10
                      note = Description of Artificial Sequence: Synthetic peptide
source                1..10
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 2
GTFEFTSFFY                                                         10

SEQ ID NO: 3          moltype = AA  length = 9
FEATURE               Location/Qualifiers
REGION                1..9
                      note = Description of Artificial Sequence: Synthetic peptide
source                1..9
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 3
YSHGTGTGY                                                          9

SEQ ID NO: 4          moltype = AA  length = 18
FEATURE               Location/Qualifiers
REGION                1..18
                      note = Description of Artificial Sequence: Synthetic peptide
source                1..18
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 4
GLPVGGNEKK AKLANVVR                                                18

SEQ ID NO: 5          moltype = AA  length = 17
FEATURE               Location/Qualifiers
REGION                1..17
                      note = Description of Artificial Sequence: Synthetic peptide
source                1..17
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 5
GMMMGMFNML STVLGVS                                                 17

SEQ ID NO: 6          moltype = AA  length = 17
FEATURE               Location/Qualifiers
REGION                1..17
                      note = Description of Artificial Sequence: Synthetic peptide
source                1..17
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 6
LQLFIKDYRY TYRCHRG                                                 17

SEQ ID NO: 7          moltype = AA  length = 9
FEATURE               Location/Qualifiers
REGION                1..9
                      note = Description of Artificial Sequence: Synthetic peptide
source                1..9
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 7
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RRAIATPGM                                                    9

SEQ ID NO: 8          moltype = AA  length = 9
FEATURE               Location/Qualifiers
REGION                1..9
                      note = Description of Artificial Sequence: Synthetic peptide
source                1..9
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 8
FMYSDFHFI                                                    9

SEQ ID NO: 9          moltype = AA  length = 18
FEATURE               Location/Qualifiers
REGION                1..18
                      note = Description of Artificial Sequence: Synthetic peptide
source                1..18
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 9
MRRNYFTAEV SHCRATEY                                          18

SEQ ID NO: 10         moltype = AA  length = 11
FEATURE               Location/Qualifiers
REGION                1..11
                      note = Description of Artificial Sequence: Synthetic peptide
source                1..11
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 10
QLMWALGENM A                                                 11

SEQ ID NO: 11         moltype = AA  length = 17
FEATURE               Location/Qualifiers
REGION                1..17
                      note = Description of Artificial Sequence: Synthetic peptide
source                1..17
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 11
DVVNFVSMEF SLTDPRL                                           17

SEQ ID NO: 12         moltype = AA  length = 17
FEATURE               Location/Qualifiers
REGION                1..17
                      note = Description of Artificial Sequence: Synthetic peptide
source                1..17
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 12
KWGMEMRRCL LQSLQQI                                           17

SEQ ID NO: 13         moltype = AA  length = 10
FEATURE               Location/Qualifiers
REGION                1..10
                      note = Description of Artificial Sequence: Synthetic peptide
source                1..10
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 13
AEIEDLIFLA                                                   10

SEQ ID NO: 14         moltype = AA  length = 9
FEATURE               Location/Qualifiers
REGION                1..9
                      note = Description of Artificial Sequence: Synthetic peptide
source                1..9
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 14
CTELKLSDY                                                    9

SEQ ID NO: 15         moltype = AA  length = 9
FEATURE               Location/Qualifiers
REGION                1..9
                      note = Description of Artificial Sequence: Synthetic peptide
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source                1..9
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 15
CTELKLTDQ                                                    9

SEQ ID NO: 16         moltype = AA  length = 9
FEATURE               Location/Qualifiers
REGION                1..9
                      note = Description of Artificial Sequence: Synthetic peptide
source                1..9
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 16
CTELKLTDY                                                    9

SEQ ID NO: 17         moltype = AA  length = 14
FEATURE               Location/Qualifiers
REGION                1..14
                      note = Description of Artificial Sequence: Synthetic peptide
source                1..14
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 17
ELRSRYWAIR TRSG                                              14

SEQ ID NO: 18         moltype = AA  length = 14
FEATURE               Location/Qualifiers
REGION                1..14
                      note = Description of Artificial Sequence: Synthetic peptide
source                1..14
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 18
ELKSRYWAIR TRSG                                              14

SEQ ID NO: 19         moltype = AA  length = 9
FEATURE               Location/Qualifiers
REGION                1..9
                      note = Description of Artificial Sequence: Synthetic peptide
source                1..9
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 19
GMDPRMCSL                                                    9

SEQ ID NO: 20         moltype = AA  length = 9
FEATURE               Location/Qualifiers
REGION                1..9
                      note = Description of Artificial Sequence: Synthetic peptide
source                1..9
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 20
ILKGKFQTA                                                    9

SEQ ID NO: 21         moltype = AA  length = 9
FEATURE               Location/Qualifiers
REGION                1..9
                      note = Description of Artificial Sequence: Synthetic peptide
source                1..9
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 21
ILRGSIAHK                                                    9

SEQ ID NO: 22         moltype = AA  length = 9
FEATURE               Location/Qualifiers
REGION                1..9
                      note = Description of Artificial Sequence: Synthetic peptide
source                1..9
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 22
ILRGSVAHK                                                    9
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SEQ ID NO: 23          moltype = AA  length = 10
FEATURE                Location/Qualifiers
REGION                 1..10
                       note = Description of Artificial Sequence: Synthetic peptide
source                 1..10
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 23
LELRSRYWAI                                                        10

SEQ ID NO: 24          moltype = AA  length = 9
FEATURE                Location/Qualifiers
REGION                 1..9
                       note = Description of Artificial Sequence: Synthetic peptide
source                 1..9
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 24
LIFLARSAL                                                         9

SEQ ID NO: 25          moltype = AA  length = 9
FEATURE                Location/Qualifiers
REGION                 1..9
                       note = Description of Artificial Sequence: Synthetic peptide
source                 1..9
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 25
RGINDRNFW                                                         9

SEQ ID NO: 26          moltype = AA  length = 16
FEATURE                Location/Qualifiers
REGION                 1..16
                       note = Description of Artificial Sequence: Synthetic peptide
source                 1..16
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 26
FLARSALILR GSVAHK                                                 16

SEQ ID NO: 27          moltype = AA  length = 10
FEATURE                Location/Qualifiers
REGION                 1..10
                       note = Description of Artificial Sequence: Synthetic peptide
source                 1..10
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 27
RMVLSAFDER                                                        10

SEQ ID NO: 28          moltype = AA  length = 18
FEATURE                Location/Qualifiers
REGION                 1..18
                       note = Description of Artificial Sequence: Synthetic peptide
source                 1..18
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 28
TLELRSGYWA IRTRSGGN                                               18

SEQ ID NO: 29          moltype = AA  length = 18
FEATURE                Location/Qualifiers
REGION                 1..18
                       note = Description of Artificial Sequence: Synthetic peptide
source                 1..18
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 29
IAYERMCNIL KGKFQTAA                                               18

SEQ ID NO: 30          moltype = AA  length = 17
FEATURE                Location/Qualifiers
REGION                 1..17
                       note = Description of Artificial Sequence: Synthetic peptide
source                 1..17
                       mol_type = protein
```

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                    organism = synthetic construct
SEQUENCE: 30
FLARSALILR GSVAHKS                                              17

SEQ ID NO: 31       moltype = AA  length = 9
FEATURE             Location/Qualifiers
REGION              1..9
                    note = Description of Artificial Sequence: Synthetic peptide
source              1..9
                    mol_type = protein
                    organism = synthetic construct
SEQUENCE: 31
FQGRGVFEL                                                       9

SEQ ID NO: 32       moltype = AA  length = 10
FEATURE             Location/Qualifiers
REGION              1..10
                    note = Description of Artificial Sequence: Synthetic peptide
source              1..10
                    mol_type = protein
                    organism = synthetic construct
SEQUENCE: 32
GQISIQPTFS                                                      10

SEQ ID NO: 33       moltype = AA  length = 25
FEATURE             Location/Qualifiers
REGION              1..25
                    note = Description of Artificial Sequence: Synthetic peptide
source              1..25
                    mol_type = protein
                    organism = synthetic construct
SEQUENCE: 33
WHSNLNDATY QRTRALVRTG MDPRM                                      25

SEQ ID NO: 34       moltype = AA  length = 25
FEATURE             Location/Qualifiers
REGION              1..25
                    note = Description of Artificial Sequence: Synthetic peptide
source              1..25
                    mol_type = protein
                    organism = synthetic construct
SEQUENCE: 34
WHSNLNDTTY QRTRALVRTG MDPRM                                      25

SEQ ID NO: 35       moltype = AA  length = 9
FEATURE             Location/Qualifiers
REGION              1..9
                    note = Description of Artificial Sequence: Synthetic peptide
source              1..9
                    mol_type = protein
                    organism = synthetic construct
SEQUENCE: 35
CVNGSCFTV                                                       9

SEQ ID NO: 36       moltype = AA  length = 10
FEATURE             Location/Qualifiers
REGION              1..10
                    note = Description of Artificial Sequence: Synthetic peptide
source              1..10
                    mol_type = protein
                    organism = synthetic construct
SEQUENCE: 36
RMVLASTTAK                                                      10

SEQ ID NO: 37       moltype = AA  length = 9
FEATURE             Location/Qualifiers
REGION              1..9
                    note = Description of Artificial Sequence: Synthetic peptide
source              1..9
                    mol_type = protein
                    organism = synthetic construct
SEQUENCE: 37
TYQRTRALV                                                       9

SEQ ID NO: 38       moltype = AA  length = 9
FEATURE             Location/Qualifiers
```

-continued

```
REGION                1..9
                      note = Description of Artificial Sequence: Synthetic peptide
source                1..9
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 38
CVNGSCYTV                                                      9

SEQ ID NO: 39         moltype = AA  length = 14
FEATURE               Location/Qualifiers
REGION                1..14
                      note = Description of Artificial Sequence: Synthetic peptide
source                1..14
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 39
ELKSRYWAIR TRSG                                                14

SEQ ID NO: 40         moltype = AA  length = 9
FEATURE               Location/Qualifiers
REGION                1..9
                      note = Description of Artificial Sequence: Synthetic peptide
source                1..9
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 40
FMYSDLHFI                                                      9

SEQ ID NO: 41         moltype = AA  length = 9
FEATURE               Location/Qualifiers
REGION                1..9
                      note = Description of Artificial Sequence: Synthetic peptide
source                1..9
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 41
FMYTDFHFI                                                      9

SEQ ID NO: 42         moltype = AA  length = 9
FEATURE               Location/Qualifiers
REGION                1..9
                      note = Description of Artificial Sequence: Synthetic peptide
source                1..9
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 42
GRDPRMCSL                                                      9

SEQ ID NO: 43         moltype = AA  length = 10
FEATURE               Location/Qualifiers
REGION                1..10
                      note = Description of Artificial Sequence: Synthetic peptide
source                1..10
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 43
GTFEFTSYFY                                                     10

SEQ ID NO: 44         moltype = AA  length = 9
FEATURE               Location/Qualifiers
REGION                1..9
                      note = Description of Artificial Sequence: Synthetic peptide
source                1..9
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 44
IIKGKFQTA                                                      9

SEQ ID NO: 45         moltype = AA  length = 9
FEATURE               Location/Qualifiers
REGION                1..9
                      note = Description of Artificial Sequence: Synthetic peptide
source                1..9
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 45
```

-continued

```
ILKGKFQIA                                                    9

SEQ ID NO: 46          moltype = AA  length = 9
FEATURE                Location/Qualifiers
REGION                 1..9
                       note = Description of Artificial Sequence: Synthetic peptide
source                 1..9
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 46
ILRGSVAHK                                                    9

SEQ ID NO: 47          moltype = AA  length = 10
FEATURE                Location/Qualifiers
REGION                 1..10
                       note = Description of Artificial Sequence: Synthetic peptide
source                 1..10
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 47
LQLRSRYWAI                                                   10

SEQ ID NO: 48          moltype = AA  length = 10
FEATURE                Location/Qualifiers
REGION                 1..10
                       note = Description of Artificial Sequence: Synthetic peptide
source                 1..10
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 48
LELRSRHWAI                                                   10

SEQ ID NO: 49          moltype = AA  length = 9
FEATURE                Location/Qualifiers
REGION                 1..9
                       note = Description of Artificial Sequence: Synthetic peptide
source                 1..9
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 49
LVFLARSAL                                                    9

SEQ ID NO: 50          moltype = AA  length = 9
FEATURE                Location/Qualifiers
REGION                 1..9
                       note = Description of Artificial Sequence: Synthetic peptide
source                 1..9
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 50
RWINDRNFW                                                    9

SEQ ID NO: 51          moltype = AA  length = 9
FEATURE                Location/Qualifiers
REGION                 1..9
                       note = Description of Artificial Sequence: Synthetic peptide
source                 1..9
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 51
YSHWTGTGY                                                    9

SEQ ID NO: 52          moltype = AA  length = 9
FEATURE                Location/Qualifiers
REGION                 1..9
                       note = Description of Artificial Sequence: Synthetic peptide
source                 1..9
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 52
YSHGSGTGY                                                    9

SEQ ID NO: 53          moltype = AA  length = 16
FEATURE                Location/Qualifiers
REGION                 1..16
                       note = Description of Artificial Sequence: Synthetic peptide
```

-continued

```
source                1..16
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 53
FLARSALVLR GSVAHK                                              16

SEQ ID NO: 54         moltype = AA  length = 18
FEATURE               Location/Qualifiers
REGION                1..18
                      note = Description of Artificial Sequence: Synthetic peptide
source                1..18
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 54
IAYERMCNII KGKFQTAA                                            18

SEQ ID NO: 55         moltype = AA  length = 18
FEATURE               Location/Qualifiers
REGION                1..18
                      note = Description of Artificial Sequence: Synthetic peptide
source                1..18
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 55
IAYERMCNIL KVKFQTAA                                            18

SEQ ID NO: 56         moltype = AA  length = 18
FEATURE               Location/Qualifiers
REGION                1..18
                      note = Description of Artificial Sequence: Synthetic peptide
source                1..18
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 56
IAYERMCNIL KGKFKTAA                                            18

SEQ ID NO: 57         moltype = AA  length = 18
FEATURE               Location/Qualifiers
REGION                1..18
                      note = Description of Artificial Sequence: Synthetic peptide
source                1..18
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 57
IAYERMCNIL KGKFQIAA                                            18

SEQ ID NO: 58         moltype = AA  length = 17
FEATURE               Location/Qualifiers
REGION                1..17
                      note = Description of Artificial Sequence: Synthetic peptide
source                1..17
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 58
DVVNFVSMEF SLTYPRL                                             17

SEQ ID NO: 59         moltype = AA  length = 17
FEATURE               Location/Qualifiers
REGION                1..17
                      note = Description of Artificial Sequence: Synthetic peptide
source                1..17
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 59
DVVNFVSMEF SLTDQRL                                             17

SEQ ID NO: 60         moltype = AA  length = 17
FEATURE               Location/Qualifiers
REGION                1..17
                      note = Description of Artificial Sequence: Synthetic peptide
source                1..17
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 60
FLARSALVLR GSVAHKS                                             17
```

-continued

```
SEQ ID NO: 61          moltype = AA  length = 17
FEATURE                Location/Qualifiers
REGION                 1..17
                       note = Description of Artificial Sequence: Synthetic peptide
source                 1..17
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 61
KLGMEMRRCL LQSLQQI                                          17

SEQ ID NO: 62          moltype = AA  length = 17
FEATURE                Location/Qualifiers
REGION                 1..17
                       note = Description of Artificial Sequence: Synthetic peptide
source                 1..17
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 62
KWGMEMRRCL LQSLQQV                                          17

SEQ ID NO: 63          moltype = AA  length = 17
FEATURE                Location/Qualifiers
REGION                 1..17
                       note = Description of Artificial Sequence: Synthetic peptide
source                 1..17
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 63
LQLFIKDFRY TYRCHRG                                          17

SEQ ID NO: 64          moltype = AA  length = 17
FEATURE                Location/Qualifiers
REGION                 1..17
                       note = Description of Artificial Sequence: Synthetic peptide
source                 1..17
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 64
LQLFIKDYRY TYRCLRG                                          17

SEQ ID NO: 65          moltype = AA  length = 17
FEATURE                Location/Qualifiers
REGION                 1..17
                       note = Description of Artificial Sequence: Synthetic peptide
source                 1..17
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 65
LQLFIKDYRY TYRCPRG                                          17

SEQ ID NO: 66          moltype = AA  length = 17
FEATURE                Location/Qualifiers
REGION                 1..17
                       note = Description of Artificial Sequence: Synthetic peptide
source                 1..17
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 66
LQLFIKDYRY TYRCHRV                                          17

SEQ ID NO: 67          moltype = AA  length = 9
FEATURE                Location/Qualifiers
REGION                 1..9
                       note = Description of Artificial Sequence: Synthetic peptide
source                 1..9
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 67
FQGPGVFEL                                                   9

SEQ ID NO: 68          moltype = AA  length = 10
FEATURE                Location/Qualifiers
REGION                 1..10
                       note = Description of Artificial Sequence: Synthetic peptide
source                 1..10
                       mol_type = protein
```

-continued

```
                    organism = synthetic construct
SEQUENCE: 68
SQISIQPTFS                                                        10

SEQ ID NO: 69       moltype = AA  length = 10
FEATURE             Location/Qualifiers
REGION              1..10
                    note = Description of Artificial Sequence: Synthetic peptide
source              1..10
                    mol_type = protein
                    organism = synthetic construct
SEQUENCE: 69
GQVSIQPTFS                                                        10

SEQ ID NO: 70       moltype = AA  length = 10
FEATURE             Location/Qualifiers
REGION              1..10
                    note = Description of Artificial Sequence: Synthetic peptide
source              1..10
                    mol_type = protein
                    organism = synthetic construct
SEQUENCE: 70
GQISVQPTFS                                                        10

SEQ ID NO: 71       moltype = AA  length = 10
FEATURE             Location/Qualifiers
REGION              1..10
                    note = Description of Artificial Sequence: Synthetic peptide
source              1..10
                    mol_type = protein
                    organism = synthetic construct
SEQUENCE: 71
GQNSIQPTFS                                                        10

SEQ ID NO: 72       moltype = AA  length = 25
FEATURE             Location/Qualifiers
REGION              1..25
                    note = Description of Artificial Sequence: Synthetic peptide
source              1..25
                    mol_type = protein
                    organism = synthetic construct
SEQUENCE: 72
WHSNLNDTTY QRTRALVRTG MDPRM                                       25

SEQ ID NO: 73       moltype = AA  length = 25
FEATURE             Location/Qualifiers
REGION              1..25
                    note = Description of Artificial Sequence: Synthetic peptide
source              1..25
                    mol_type = protein
                    organism = synthetic construct
SEQUENCE: 73
WHSNLNDSTY QRTRALVRTG MDPRM                                       25

SEQ ID NO: 74       moltype = AA  length = 25
FEATURE             Location/Qualifiers
REGION              1..25
                    note = Description of Artificial Sequence: Synthetic peptide
source              1..25
                    mol_type = protein
                    organism = synthetic construct
SEQUENCE: 74
WHSNLNDATY QRKRALVRTG MDPRM                                       25

SEQ ID NO: 75       moltype = AA  length = 25
FEATURE             Location/Qualifiers
REGION              1..25
                    note = Description of Artificial Sequence: Synthetic peptide
source              1..25
                    mol_type = protein
                    organism = synthetic construct
SEQUENCE: 75
WHSNLNDATY QRTRSLVRTG MDPRM                                       25

SEQ ID NO: 76       moltype = AA  length = 25
FEATURE             Location/Qualifiers
```

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```
REGION                  1..25
                        note = Description of Artificial Sequence: Synthetic peptide
source                  1..25
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 76
WHSNLNDATY QRTRAIVRTG MDPRM                                          25

SEQ ID NO: 77           moltype = AA  length = 25
FEATURE                 Location/Qualifiers
REGION                  1..25
                        note = Description of Artificial Sequence: Synthetic peptide
source                  1..25
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 77
WHSNLNDATY QRTRALVRSG MDPRM                                          25

SEQ ID NO: 78           moltype = AA  length = 25
FEATURE                 Location/Qualifiers
REGION                  1..25
                        note = Description of Artificial Sequence: Synthetic peptide
source                  1..25
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 78
WHSNLNDATY QRTRALVRTG RDPRM                                          25

SEQ ID NO: 79           moltype = AA  length = 9
FEATURE                 Location/Qualifiers
REGION                  1..9
                        note = Description of Artificial Sequence: Synthetic peptide
source                  1..9
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 79
FYIQMCTEL                                                            9

SEQ ID NO: 80           moltype = AA  length = 10
FEATURE                 Location/Qualifiers
REGION                  1..10
                        note = Description of Artificial Sequence: Synthetic peptide
source                  1..10
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 80
TYNAELLVLL                                                           10

SEQ ID NO: 81           moltype = AA  length = 71
FEATURE                 Location/Qualifiers
REGION                  1..71
                        note = Description of Artificial Sequence: Synthetic
                         polypeptide
source                  1..71
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 81
GALNNRFQIK GVELKSKTYQ RTRALVRVKR ELRSRYWAIR TRSGRVKRFY IQMCTELRVK  60
RTYNAELLVL L                                                         71

SEQ ID NO: 82           moltype = AA  length = 14
FEATURE                 Location/Qualifiers
REGION                  1..14
                        note = Description of Artificial Sequence: Synthetic peptide
source                  1..14
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 82
ELRSRHWAIR TRSG                                                      14

SEQ ID NO: 83           moltype = AA  length = 14
FEATURE                 Location/Qualifiers
REGION                  1..14
                        note = Description of Artificial Sequence: Synthetic peptide
source                  1..14
                        mol_type = protein
```

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```
                    organism = synthetic construct
SEQUENCE: 83
ELRSRYWASR TRSG                                                    14

SEQ ID NO: 84       moltype = AA  length = 9
FEATURE             Location/Qualifiers
REGION              1..9
                    note = Description of Artificial Sequence: Synthetic peptide
source              1..9
                    mol_type = protein
                    organism = synthetic construct
SEQUENCE: 84
FMFSDFHFI                                                          9

SEQ ID NO: 85       moltype = AA  length = 9
FEATURE             Location/Qualifiers
REGION              1..9
                    note = Description of Artificial Sequence: Synthetic peptide
source              1..9
                    mol_type = protein
                    organism = synthetic construct
SEQUENCE: 85
VLRGSIAHK                                                          9

SEQ ID NO: 86       moltype = AA  length = 9
FEATURE             Location/Qualifiers
REGION              1..9
                    note = Description of Artificial Sequence: Synthetic peptide
source              1..9
                    mol_type = protein
                    organism = synthetic construct
SEQUENCE: 86
LTFLARSAL                                                          9

SEQ ID NO: 87       moltype = AA  length = 18
FEATURE             Location/Qualifiers
REGION              1..18
                    note = Description of Artificial Sequence: Synthetic peptide
source              1..18
                    mol_type = protein
                    organism = synthetic construct
SEQUENCE: 87
VAYERMCNIL KGKFQTAA                                                18

SEQ ID NO: 88       moltype = AA  length = 18
FEATURE             Location/Qualifiers
REGION              1..18
                    note = Description of Artificial Sequence: Synthetic peptide
source              1..18
                    mol_type = protein
                    organism = synthetic construct
SEQUENCE: 88
VAYERMCNII KGKFQTAA                                                18

SEQ ID NO: 89       moltype = AA  length = 18
FEATURE             Location/Qualifiers
REGION              1..18
                    note = Description of Artificial Sequence: Synthetic peptide
source              1..18
                    mol_type = protein
                    organism = synthetic construct
SEQUENCE: 89
VAYERMCNIL KGKFKTAA                                                18

SEQ ID NO: 90       moltype = AA  length = 18
FEATURE             Location/Qualifiers
REGION              1..18
                    note = Description of Artificial Sequence: Synthetic peptide
source              1..18
                    mol_type = protein
                    organism = synthetic construct
SEQUENCE: 90
VAYERMCNIL KGKFQIAA                                                18

SEQ ID NO: 91       moltype = AA  length = 18
FEATURE             Location/Qualifiers
```

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```
REGION                  1..18
                        note = Description of Artificial Sequence: Synthetic peptide
source                  1..18
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 91
VAYERMCNIL KGKFQTAV                                              18

SEQ ID NO: 92           moltype = AA  length = 17
FEATURE                 Location/Qualifiers
REGION                  1..17
                        note = Description of Artificial Sequence: Synthetic peptide
source                  1..17
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 92
KWGMELRRCL LQSLQQI                                               17

SEQ ID NO: 93           moltype = AA  length = 10
FEATURE                 Location/Qualifiers
REGION                  1..10
                        note = Description of Artificial Sequence: Synthetic peptide
source                  1..10
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 93
SQISVQPTFS                                                       10

SEQ ID NO: 94           moltype = AA  length = 10
FEATURE                 Location/Qualifiers
REGION                  1..10
                        note = Description of Artificial Sequence: Synthetic peptide
source                  1..10
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 94
GQVSVQPTFS                                                       10

SEQ ID NO: 95           moltype = AA  length = 20
FEATURE                 Location/Qualifiers
REGION                  1..20
                        note = Description of Artificial Sequence: Synthetic peptide
source                  1..20
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 95
LEAGCKNFFP RSFTSCGSLE                                            20

SEQ ID NO: 96           moltype = AA  length = 12
FEATURE                 Location/Qualifiers
REGION                  1..12
                        note = Description of Artificial Sequence: Synthetic peptide
source                  1..12
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 96
CRRRRRREAE AC                                                    12

SEQ ID NO: 97           moltype = AA  length = 8
FEATURE                 Location/Qualifiers
REGION                  1..8
                        note = Description of Artificial Sequence: Synthetic peptide
source                  1..8
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 97
GGGGGGGG                                                         8

SEQ ID NO: 98           moltype = AA  length = 12
FEATURE                 Location/Qualifiers
REGION                  1..12
                        note = Description of Artificial Sequence: Synthetic peptide
source                  1..12
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 98
```

-continued

```
GSAGSAAGSG EF                                                    12

SEQ ID NO: 99          moltype = AA  length = 20
FEATURE                Location/Qualifiers
REGION                 1..20
                       note = Description of Artificial Sequence: Synthetic peptide
source                 1..20
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 99
GGGGSGGGGS GGGGSGGGGS                                            20

SEQ ID NO: 100         moltype = AA  length = 17
FEATURE                Location/Qualifiers
REGION                 1..17
                       note = Description of Artificial Sequence: Synthetic peptide
source                 1..17
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 100
ISQAVHAAHA EINEAGR                                               17

SEQ ID NO: 101         moltype = AA  length = 13
FEATURE                Location/Qualifiers
REGION                 1..13
                       note = Description of Artificial Sequence: Synthetic peptide
source                 1..13
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 101
AKFVAAWTLK AAA                                                   13

SEQ ID NO: 102         moltype = AA  length = 15
FEATURE                Location/Qualifiers
REGION                 1..15
                       note = Description of Artificial Sequence: Synthetic peptide
MOD_RES                3
                       note = L-cyclohexylalanine
MOD_RES                14
                       note = Aminocaproic acid
source                 1..15
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 102
AKXVAAWTLK AAAXC                                                 15

SEQ ID NO: 103         moltype = AA  length = 17
FEATURE                Location/Qualifiers
REGION                 1..17
                       note = Description of Artificial Sequence: Synthetic peptide
source                 1..17
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 103
GALNNRFQIK GVELKSK                                               17

SEQ ID NO: 104         moltype = DNA  length = 36519
FEATURE                Location/Qualifiers
misc_feature           1..36519
                       note = Description of Artificial Sequence: Synthetic
                        polynucleotide
source                 1..36519
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 104
ccatcttcaa taatatacct caaacttttt gtgcgcgtta atatgcaaat gaggcgtttg  60
aatttgggga ggaagggcgg tgattggtcg agggatgagc gaccgttagg ggcggggcga  120
gtgacgtttt gatgacgtgg ttgcgaggag gagccagttt gcaagttctc gtgggaaaag  180
tgacgtcaaa cgaggtgtgg tttgaacacg gaaatactca attttcccgc gctctctgac  240
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gatgagaaaa tcatcatcgc ttccgggaac gagattctgg aactggtggt aaatgccatg  660
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atgggcgacg accctccgga gccccccacc ccatttgaga caccttcgct gcacgatttg  720
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ctacctgcgc caccaccccc agtaccgcga ccagcgagtg gcgcagctgc tcaggctcct  31020
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atcattggga tcgtgaacat tctggcctgc accctcatct cctttgtgat ttacccctgc  31260
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aaagaatcac ttacttgaaa tctgatacca ggtctctgtc catgttttct gccaacacca  32040
cctcactccc ctcttcccag ctctggtact gcaggccccg gcgggctgca aacttcctcc  32100
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gtgagcattg ccaaatgtaa gattgaaata agcatgctgg ctagacccgg tgatatcttc  35640
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cgatgccatg cggatgaagc acaaaatttt caggtgcgta aaaaatgtaa ttactcccct  36120
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gagctctaac ctgtccgccc gctctctgct caatatatag ccccagatct acactgacgt  36300
aaaggccaaa gtctaaaaat acccgccaaa taatcacaca cgcccagcac acgcccagaa  36360
accggtgaca cactcagaaa aatacgcgca cttcctcaaa cggccaaact gccgtcattt  36420
ccgggttccc acgctacgtc atcaaaacac gactttcaaa ttccgtcgac cgttaaaaac  36480
atcacccgcc ccgcccctaa cggtcgccgc tcccgcagcc aatcaccttc ctccctcccc  36540
aaattcaaac agctcatttg catattaacg cgcaccaaaa gtttgaggta tattattgat  36600
gatg                                                               36604
```

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SEQ ID NO: 106          moltype = AA  length = 948
FEATURE                 Location/Qualifiers
REGION                  1..948
                        note = Description of Artificial Sequence: Synthetic
                         polypeptide
source                  1..948
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 106
ELRSRYWAIR TRSGRVKREL RSRHWAIRTR SGRVKRELRS RYWASRTRSG RVKRFMYSDF  60
HFIRVKRFMY SDLHFIRVKR FMYTDFHFIR VKRFMFSDFH FIRVKRGTFE FTSFFYRVKR  120
GTFEFTSYFY RVKRILKGKF QTARVKRIIK GKFQTARVKR ILKGKFQIAR VKRILRGSIA  180
HKRVKRILRG SVAHKRVKRV LRGSIAHKRV KRLIFLARSA LRVKRLVFLA RSALRVKRLT  240
FLARSALRVK RYSHGTGTGY RVKRYSHWTG TGYRVKRYSH GSGTGYRVKR FLARSALILR  300
GSVAHKRVKR FLARSALVLR GSVAHKRVKR IAYERMCNIL KGKFQTAARV KRVAYERMCN  360
ILKGKFQTAA RVKRVAYERM CNIIKGKFQT AARVKRVAYE RMCNILKGKF KTAARVKRVA  420
YERMCNILKG KFQIAARVKR VAYERMCNIL KGKFQTAVRV KRDVVNFVSM EFSLTDPRLR  480
VKRDVVNFVS MEFSLTYPRL RVKRDVVNFV SMEFSLTDQR LRVKRFLARS ALILRGSVAH  540
KSRVKRFLAR SALVLRGSVA HKSRVKRKWG MEMRRCLLQS LQQIRVKRKL GMEMRRCLLQ  600
SLQQIRVKRK WGMEMRRCLL QSLQQVRVKR KWGMELRRCL LQSLQQIRVK RFQGRGVFEL  660
RVKRGQISIQ PTFSRVKRSQ ISVQPTFSRV KRGQVSVQPT FSRVKRGQIS VQPTFSRVKR  720
WHSNLNDATY QRTRALVRTG MDPRMRVKRW HSNLNDTTYQ RTRALVRTGM DPRMRVKRWH  780
SNLNDSTYQR TRALVRTGMD PRMRVKRWHS NLNDATYQRK RALVRTGMDP RMRVKRWHSN  840
LNDATYQRTR SLVRTGMDPR MRVKRWHSNL NDATYQRTRA IVRTGMDPRM RVKRWHSNLN  900
DATYQRTRAL VRSGMDPRMR VKRWHSNLND ATYQRTRALV RTGRDPRM               948

SEQ ID NO: 107          moltype = AA  length = 50
FEATURE                 Location/Qualifiers
REGION                  1..50
                        note = Description of Artificial Sequence: Synthetic
                         polypeptide
REGION                  1..50
                        note = MISC_FEATURE - This sequence may encompass 1-10 'Gly
                         Gly Gly Gly Ser' repeating units
source                  1..50
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 107
GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS             50

SEQ ID NO: 108          moltype = AA  length = 100
FEATURE                 Location/Qualifiers
REGION                  1..100
                        note = Description of Artificial Sequence: Synthetic
                         polypeptide
REGION                  1..100
                        note = MISC_FEATURE - This sequence may encompass 1-20 'Glu
                         Ala Ala Ala Lys' repeating units
source                  1..100
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 108
EAAAKEAAAK EAAAKEAAAK EAAAKEAAAK EAAAKEAAAK EAAAKEAAAK EAAAKEAAAK  60
EAAAKEAAAK EAAAKEAAAK EAAAKEAAAK EAAAKEAAAK                       100

SEQ ID NO: 109          moltype = AA  length = 40
FEATURE                 Location/Qualifiers
REGION                  1..40
                        note = Description of Artificial Sequence: Synthetic
                         polypeptide
REGION                  1..40
                        note = MISC_FEATURE - This sequence may encompass 1-20 'Xaa
                         Pro' repeating units
source                  1..40
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 109
XPXPXPXPXP XPXPXPXPXP XPXPXPXPXP XPXPXPXPXP                       40

SEQ ID NO: 110          moltype = AA  length = 4
FEATURE                 Location/Qualifiers
REGION                  1..4
                        note = Description of Artificial Sequence: Synthetic peptide
source                  1..4
                        mol_type = protein
```

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                     organism = synthetic construct
SEQUENCE: 110
RVKR                                                              4

SEQ ID NO: 111       moltype = AA  length = 6
FEATURE              Location/Qualifiers
REGION               1..6
                     note = Description of Artificial Sequence: Synthetic peptide
MOD_RES              1
                     note = S-[2,3-bis(palmitoyloxy)-(2-R,S)-propyl]-N-palmitoyl
                      -(R)-cysteine
source               1..6
                     mol_type = protein
                     organism = synthetic construct
SEQUENCE: 111
CSKKKK                                                            6

SEQ ID NO: 112       moltype = AA  length = 5
FEATURE              Location/Qualifiers
REGION               1..5
                     note = Description of Artificial Sequence: Synthetic peptide
MOD_RES              1
                     note = Tripalmitoyl-S-glyceryl-cysteine
source               1..5
                     mol_type = protein
                     organism = synthetic construct
SEQUENCE: 112
CSSNA                                                             5

SEQ ID NO: 113       moltype = AA  length = 6
FEATURE              Location/Qualifiers
REGION               1..6
                     note = Description of Artificial Sequence: Synthetic peptide
MOD_RES              1
                     note = Tripalmitoyl-S-glyceryl-cysteine
source               1..6
                     mol_type = protein
                     organism = synthetic construct
SEQUENCE: 113
CSKKKK                                                            6

SEQ ID NO: 114       moltype = AA  length = 6
FEATURE              Location/Qualifiers
REGION               1..6
                     note = Description of Artificial Sequence: Synthetic peptide
MOD_RES              1
                     note = Dipalmitoyl-S-glyceryl-cysteine
source               1..6
                     mol_type = protein
                     organism = synthetic construct
SEQUENCE: 114
CSKKKK                                                            6

SEQ ID NO: 115       moltype = AA  length = 16
FEATURE              Location/Qualifiers
REGION               1..16
                     note = Description of Artificial Sequence: Synthetic peptide
source               1..16
                     mol_type = protein
                     organism = synthetic construct
SEQUENCE: 115
GALNNRFQIK GVELKS                                                 16

SEQ ID NO: 116       moltype = AA  length = 560
FEATURE              Location/Qualifiers
source               1..560
                     mol_type = protein
                     organism = Influenza B virus
SEQUENCE: 116
MSNMDIDGIN TGTIDKTPEE ITSGTSGTTR PIIRPATLAP PSNKRTRNPS PERATTSSED  60
DVGRKTQKKQ TPTEIKKSVY NMVVKLGEFY NQMMVKAGLN DDMERNLIQN AHAVERILLA  120
ATDDKKTEFQ KKKNARDVKE GKEEIDHNKT GGTFYKMVRD DKTIYFSPIR ITFLKEEVKT  180
MYKTTMGSDG FSGLNHIMIG HSQMNDVCFQ RSKALKRVGL DPSLISTFAG STVPRRSGAT  240
GVAIKGGGTL VAEAIRFIGR AMADRGLLRD IKAKTAYEKI LLNLKNKCSA PQQKALVDQV  300
IGSRNPGIAD IEDLTLLARS MVVVRPSVAS KVVLPISIYA KIPQLGFNVE EYSMVGYEAM  360
ALYNMATPVS ILRMGDDAKD KSQLFFMSCF GAAYEDLRVL SALTGTEFKP RSALKCKGFH  420
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VPAKEQVEGM GAALMSIKLQ FWAPMTRSGG NEVGGDGGSG QISCSPVFAV ERPIALSKQA  480
VRRMLSMNIE GRDADVKGNL LKMMNDSMAK KTSGNAFIGK KMFQISDKNK TNPIEIPIKQ  540
TIPNFFFGRD TAEDYDDLDY                                              560

SEQ ID NO: 117          moltype = AA  length = 560
FEATURE                 Location/Qualifiers
source                  1..560
                        mol_type = protein
                        organism = Influenza B virus
SEQUENCE: 117
MSNMDIDGIN TGTIDKTPEE ITSGTSGTTR PIIRPATLAP PSNKRTRNPS PERATTSSEA  60
DVGRKTQKKQ TPTEIKKSVY NMVVKLGEFY NQMMVKAGLN DDMERNLIQN AHAVERILLA  120
ATDDKKTEFQ KKKNARDVKE GKEEIDHNKT GGTFYKMVRD DKTIYFSPIR ITFLKEEVKT  180
MYKTTMGSDG FSGLNHIMIG HSQMNDVCFQ RSKALKRVGL DPSLISTFAG STLPRRSGAT  240
GVAIKGGGTL VAEAIRFIGR AMADRGLLRD IKAKTAYEKI LLNLKNKCSA PQQKALVDQV  300
IGSRNPGIAD IEDLTLLARS MVVVRPSVAS KVVLPISIYA KIPQLGFNVE EYSMVGYEAM  360
ALYNMATPVS ILRMGDDAKD KSQLFFMSCF GAAYEDLRVL SALTGTEFKP RSALKCKGFH  420
VPAKEQVEGM GAALMSIKLQ FWAPMTRSGG NEVGGDGGSG QISCSPVFAV ERPIALSKQA  480
VRRMLSMNIE GRDADVKGNL LKMMNDSMAK KTNGNAFIGK KMFQISDKNK TNPVEIPIKQ  540
TIPNFFFGRD TAEDYDDLDY                                              560

SEQ ID NO: 118          moltype = AA  length = 560
FEATURE                 Location/Qualifiers
source                  1..560
                        mol_type = protein
                        organism = Influenza B virus
SEQUENCE: 118
MSNMDIDGIN TGTIDKTPEE ITSGTSGTTR PIIRPATLAP PSNKRTRNPS PERATTSSED  60
DVGRKTQKKQ TPTEIKKSVY NMVVKLGEFY NQMMVKAGLN DDMERNLIQN AYAVERILLA  120
ATDDKKTEFQ KKKNARDVKE GKEEIDHNKT GGTFYKMVRD DKTIYFSPIR ITFLKEEVKT  180
MYKTTMGSDG FSGLNHIMIG HSQMNDVCFQ RSKALKRVGL DPSLISTFAG STVPRRSGAT  240
GVAIKGGGTL VAEAIRFIGR AMADRGLLRD IKAKTAYEKI LLNLKNKCSA PQQKALVDQV  300
IGSRNPGIAD IEDLTLLARS MVVVRPSVAS KVVLPISIYA KIPQLGFNVE EYSMVGYEAM  360
ALYNMATPVS ILRMGDDAKD KSQLFFMSCF GAAYEDLRVL SALTGTEFKP RSALKCKGFH  420
VPAKEQVEGM GAALMSIKLQ FWAPMTRSGG NEAGGDGGSG QISCSPVFAV ERPIALSKQA  480
VRRMLSMNIE GRDADVKGNL LKMMNDSMAK KTSGNAFIGK KMFQISDKNK TNPIEIPIKQ  540
TIPNFFFGRD TAEDYDDLDY                                              560
```

1. A polypeptide comprising a sequence that comprises a first sequence, second sequence, and third sequence, wherein each of the first sequence, second sequence and third sequence must be a different SEQ ID NO and comprises at least 55% identity to a sequence selected from the sequences set forth in the group consisting of SEQ ID NOs: 40, 58, 61, 43, 51, 93, 22, 49, 82, 88, and 34, and wherein the sequence is from the influenza virus.

2. The polypeptide of claim **1**, wherein:

the first sequence comprises at least 55% sequence identity to the sequence if SEQ ID NO: 51;

the second sequence comprises at least 55% sequence identity to the sequence of SEQ ID NO: 58; and

the third sequence comprises at least 55% sequence identity to the sequence of SEQ ID NO: 93.

3. The polypeptide of claim **2**, wherein the polypeptide further comprises a fourth sequence comprising at least 55% sequence identity to the sequence of SEQ ID NO: 61, 22, 49, 34, 40, or 82.

4. The polypeptide of claim **2**, wherein the polypeptide further comprises:

a fourth sequence comprises at least 55% sequence identity to the sequence of SEQ ID NO: 61;

a fifth sequence comprising at least 55% sequence identity to the sequence of SEQ ID NO: 22;

a sixth sequence comprising at least 55% sequence identity to the sequence of SEQ ID NO: 49;

a seventh sequence comprising at least 55% sequence identity to the sequence of SEQ ID NO: 34;

an eighth sequence comprising at least 55% sequence identity to the sequence of SEQ ID NO: 40;

a ninth sequence comprising at least 55% sequence identity to the sequence of SEQ ID NO: 43; and

a tenth sequence comprising at least 55% sequence identity to the sequence of SEQ ID NO: 82.

5. The polypeptide of claim **1**, wherein the polypeptide comprises:

at least 3 sequences comprising at least 55% sequence identity to the sequence of SEQ ID NO: 51;

at least 3 sequences comprising at least 55% sequence identity to the sequence of SEQ ID NO: 58; and

at least 4 sequences comprising at least 55% sequence identity to the sequence of SEQ ID NO: 93.

6. The polypeptide of claim **1**, wherein the first sequence and second sequence are directly linked.

7. The polypeptide of claim **1**, wherein at least two of the first, second, third, and fourth sequence are linked by a linker.

8. The polypeptide of claim **7**, wherein the linker comprises the sequence RVKR (SEQ ID NO: 110).

9. The polypeptide of claim **1**, wherein the polypeptide is an isolated polypeptide.

10. The polypeptide of claim **1**, wherein the polypeptide comprises the sequences of SEQ ID NOs: 51, 58, 61, 93, 22, 49, and 34.

11. A polynucleotide encoding the polypeptide of claim **1**.

12. A composition comprising the polynucleotide of claim **11**.

13. A vector comprising the polynucleotide of claim **11**.

14. A composition comprising the vector of claim **13**.

15. A virus comprising the polynucleotide of claim **11**.

16. The virus of claim **15**, wherein the virus is an adenovirus.

17. A composition comprising the virus of claim **15**.

18. A pharmaceutical composition comprising the virus of claim **15**, and a pharmaceutically acceptable carrier or excipient.

19. A method comprising administering to a subject the composition of claim **12**.

20. A method comprising administering to a subject the pharmaceutical composition of claim **18**.

* * * * *