PEPTIDE-BASED INFLUENZA VACCINE FORMULATION

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of priority of U.S. Provisional Patent Application No. 60/686,041, filed June 1, 2005, which is incorporated herein by reference.

FIELD OF THE INVENTION

The present invention relates generally to an anti-viral formulation, and in particular relates to a peptide-based influenza vaccine formulation.

BACKGROUND OF THE INVENTION

Influenza is a common infectious disease of the respiratory system associated with the *Orthomyxoviridae* family of viruses. Because of the high degree of variability of the virus, vaccination is typically required on a yearly basis with a reformulated vaccine that takes into account strain variations. Despite the reformulation, it is not possible for a vaccine to include all the different strains actively infecting people in the world during a particular season.

While efficacious vaccines against influenza are currently available, they must be reformulated each year due to antigenic variation in the surface proteins of the virus. One roadblock to reformulation is the relatively long length of time required to formulate and prepare sufficient quantities of vaccine doses for responding to seasonal increases in flu infections. Typically, it can take over six months to prepare a vaccine; occasionally, a new or overlooked influenza strain becomes prominent during that six month period, leading to an epidemic.

Hemagglutinin (HA) is the major surface glycoprotein of influenza virus and a potent immunogen against which viral neutralizing antibodies are directed. Influenza viruses are typed as A or B on the basis of relatively stable intracellular nucleoproteins and envelope associated matrix proteins. Virus subtypes are based on two proteins in the viral envelope, HA and neuraminidase (NA), which undergo constant antigenic change. Fifteen distinct subtypes of HA and 9 subtypes of NA are recognized for influenza A viruses (De Jong, Rimmelzwaan, G.F., Fouchier, R.A.M., and Osterhaus, A.D.M.E. *Journal of Infection*, 2000;40:218-228). The sudden appearance of a new subtype (antigenic shift) has caused three major pandemics in the past century: 1918 (Spanish Flu, H1N1), 1957 (Asian Flu,

H2N2) and 1968 (Hong Kong Flu, H3N2). There has also been recent concern with avian flu, equine flu, and susceptibility in humans.

HA is the major envelope glycoprotein of influenza virus, and mediates the penetration of virus into host cells (Wiley, D.C., et al., *Nature* 1981;289(29):373-377; Wilson, IA, et al., *Nature* 1981;289, 366-373; Caton, et al., *Cell* 1982:417-427). The native HA is formed by the association of three HA monomers which, as a precondition of virus infectivity, are cleaved enzymatically into the amino-terminal HA1 and carboxy-terminal HA2. Based on the three dimensional structure of HA1, antigenic sites have been mapped by determining the amino acid changes of antigenic variants (Wiley, *supra*). The antigenic variations were mostly seen surrounding the receptor binding region of HA, including residues around the antibody inaccessible receptor binding pockets. Thus, there has been a desire to elucidate peptide-based approaches as immunological therapies. However, a major concern of peptide-based subunit vaccines is the ability of linear peptides to induce antibodies that can recognize conformational B cell and T cell epitopes. Monoclonal antibodies to these antigenic sites neutralize influenza virus infectivity when the exact sequences are present. Both T and B cell epitopes are found on these sites (Atassi et al., *Advances in Experimental Medicine and Biology*, 1989;251:49-635-6; Torres et al, *Immunology Letters*, 1988;19(1):49-53).

Multiplication of influenza virus in the presence of monoclonal antibody directed against one of the antigenic sites on HA results in the selection of escape variants, which were a very small minority in the parent virus (Webster et al., *Virology* 1983;126(2):587-99; Webster et al., *Virology* 1980;104(1):139-48; Yewdell et al., Nature 1979;279(5710):246-8). Consistent with these in vitro data, individuals immunized against one strain of virus and infected with another produce a wide array of antigenic variants. These data suggest that population immunity against those epitopes are a driving force for the selection of new strains of influenza viruses.

The vaccine formulation developed each year in the US is determined by the Department of Food and Drug Administration Vaccines and the Related Biologicals Advisory Committee. The World Health Organization (WHO) similarly operates a global surveillance network of laboratories, for detection of new influenza variants (Lavanchy, *Vaccine* 1999; 17:S24-S25). Selection is based on antigenic analysis of recently isolated influenza viruses, the patterns of spread of antigenic variants, and the antibody responses of recently vaccinated individuals. However, because of the delays in vaccine formulation outlined above, it has been difficult to effectively and efficiently produce sufficient quantities of vaccine to meet the

changing requirements in immunization strategies.It is, therefore, desirable to efficiently provide a more efficacious influenza vaccine formulation.

SUMMARY OF THE INVENTION

It is an object of the present invention to obviate or mitigate at least one disadvantage of previous influenza vaccine formulations.

The present invention provides peptide-based influenza vaccine formulations derived from epitopes from influenza HA. Advantageously, the vaccine formulations of the present invention improve the humoral response in animal models when compared with commercial vaccines. Because of the peptide variants in the formulations, the present invention can provide broad protection against different influenza virus strains.

Surprisingly, it was found that peptide-based influenza vaccine formulations in accordance with the present invention, which represent the antigenic diversity of influenza virus in protective HA epitopes, elicit protective immunity that is more broadly reactive than that induced with a commercial vaccine that is based on only a few isolates of influenza.

In a first aspect, the present invention provides a peptide-based anti-influenza formulation comprising at least one peptide selected from the group consisting of SEQ ID NOs: 1 to 248.

In one embodiment of the present invention there is provided a formulation comprising at least four peptide sequences selected from the group consisting of SEQ ID NOs: 1 to 248. In another embodiment, the formulation can comprise at least two peptides selected from the group consisting of SEQ ID NOs: 1 to 64 and at least two peptides selected from the group consisting of SEQ ID NOs: 133 to 180. The formulation can also comprise SEQ ID NOs: 1 to 64 and SEQ ID NOs: 133 to 180.

The present invention also provides a formulation comprising at least one peptide sequence selected from the group consisting of SEQ ID NOs: 185 to 248. In one embodiment, the formulation can comprise at least two peptide sequences from each of at least two of the following groups: SEQ ID NOs: 185 to 200; SEQ ID NOs: 201 to 216; SEQ ID NOs: 217 to 232; or SEQ ID NOs: 233 to 248.

The present invention also provides a formulation comprising at least one peptide sequence selected from the group consisting of SEQ ID NOs: 65 to 128. In one embodiment, the formulation can comprise at least two peptide sequences from each of at least two of the

following groups: SEQ ID NOs: 65 to 80; SEQ ID NOs: 81 to 96; SEQ ID NOs: 97 to 112; or SEQ ID NOs: 113 to 128.

The formulations of the present invention can be used to prepare vaccines. An adjuvant, such as alum, or other substituent may be used in the preparation of the vaccine. The vaccine can be used in the treatment of influenza in animals such as humans, mice, horses or birds.

The formulation of the present invention is broadly reactive against influenza A and B. Advantageously, the formulation of the present invention can be prepared synthetically in as little as 6 weeks.

Other aspects and features of the present invention will become apparent to those ordinarily skilled in the art upon review of the following description of specific embodiments of the invention in conjunction with the accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

Embodiments of the present invention will now be described, by way of example only, with reference to the attached Figures, wherein:

Fig. 1 shows HPLC analysis of equine influenza discosite constructs (INFE-HA-1-V1 to V4; A to D) of the present invention.

Fig. 2 shows HPLC analysis of human influenza A discosite constructs (INF-HA-1-V1 to V4; A to D) of the present invention.

Fig. 3 shows HPLC analysis of human and avian influenza discosite constructs (INF-HA-2-V1 to V4; A to D) of the present invention.

Fig. 4 shows HPLC analysis of influenza B discosite constructs (INF-HB-1-V1 to V4; A to D) of the present invention.

Figs. 5A to 5D illustrate mass spectrometry data for equine influenza discosite constructs (INFE-HA-1-V1 to V4; Figs. 5A to 5D) of the present invention.

Figs. 6A to 6D illustrate mass spectrometry data for human influenza A discosite constructs (INF-HA-1-V1 to V4; Figs. 6A to 6D) of the present invention.

Figs. 7A to 7D illustrate mass spectrometry data for human and avian influenza discosite constructs (INF-HA-2-V1; Figs. 7A to 7D) of the present invention.

Figs. 8A to 8D illustrate mass spectrometry data for human influenza B discosite constructs (INF-HB-1-V1 to V4; Figs. 8A to 8D) of the present invention.

Fig. 9 illustrates induction of humoral immunity by a vaccine of the present invention after immunization.

Fig. 10 shows a survival plot of vaccinated mice against challenge with H3N2.

Fig. 11 shows percent weight loss in challenged mice vaccinated with a vaccine of the present invention INF-01P (INF-HA-1-V1-V4).

Fig. 12 shows induction of humoral immunity by INFE-01P (INFE-HA-1-V1-V4) vaccination in mice as measured by HAI titres.

Fig. 13 illustrates the results of a hemagluttination assay performed in murine vaccine study.

Fig. 14 shows results of the influenza vaccine ELISA test based on data presented in Table 22.

DETAILED DESCRIPTION

Generally, the present invention provides an anti-viral formulation, and more specifically, a peptide-based anti-influenza formulation comprising at least one peptide selected from the group consisting of SEQ ID NOs: 1 to 248.

The formulation of the present invention is a cocktail comprising one or more peptides. The formulation can comprise at least four peptide sequences selected from the group consisting of SEQ ID NOs: 1 to 248. In one example, the formulation can comprise at least two peptides selected from the group consisting of SEQ ID NOs: 1 to 64 and at least two peptides selected from the group consisting of SEQ ID NOs: 133 to 180. A specific example comprises SEQ ID NOs: 1 to 64 and SEQ ID NOs: 133 to 180. In another specific example, the formulation comprises SEQ ID NOs: 1 to 64. In yet another specific example, the formulation comprises SEQ ID NOs: 185 to 248.

In another example, the formulation can comprise 2^n peptide sequences from each of at least two of the following groups: a) SEQ ID NOs: 1 to 16; b) SEQ ID NOs: 17 to 32; c) SEQ ID NOs: 33 to 48: or d) SEQ ID NOs: 49 to 64, wherein n is 1 to 4. Further, the formulation can comprise from at least two of groups a) to d): a) 2^m peptide sequences from SEQ ID NOs: 133 to 140; b) 2^n peptide sequences from SEQ ID NOs: 141 to 156; c) 2^n peptide sequences from SEQ ID NOs: 157 to 172; or d) 2^m peptide sequences from SEQ ID NOs: 173 to 180, wherein m is 1 to 3 and n is 1 to 4. This formulation can be used in the preparation of a human anti-influenza vaccine.

The human anti-influenza formulations described herein can be used alone or in combination.

In another example, the formulation of the present invention can comprise at least one peptide sequence selected from the group consisting of SEQ ID NOs: 185 to 248. As one example, the formulation can comprise 2^n peptide sequences from each of at least two of the following groups: a) SEQ ID NOs: 185 to 200; b) SEQ ID NOs: 201 to 216; c) SEQ ID NOs: 217 to 232; or d) SEQ ID NOs: 233 to 248, wherein n is from 1 to 4. This formulation can be used in the preparation of an equine anti-influenza vaccine.

In another example, the formulation of the present invention can comprise at least one peptide sequence selected from the group consisting of SEQ ID NOs: 65 to 128. As one example, the formulation can comprise 2^n peptide sequences from each of at least two of the following groups: a) SEQ ID NOs: 65 to 80; b) SEQ ID NOs: 81 to 96; c) SEQ ID NOs: 97 to 112; or d) SEQ ID NOs: 113 to 128, wherein n is from 1 to 4. This formulation can be used in the preparation of an avian anti-influenza vaccine.

In an example of a formulation where there is no variability represented by the peptide sequences, the formulation can comprise at least one of SEQ ID NOs: 129 to 132 or SEQ ID NOs: 181 to 184. As one specific example, the formulation can comprise SEQ ID NOs: 129 to 132. In another specific example, the formulation can comprise SEQ ID NOs: 181 to 184.

The present invention also provides a vaccine comprising a formulation including at least one peptide selected from the group consisting of SEQ ID NOs: 1 to 248, together with a pharmaceutically-acceptable diluent or carrier. The vaccine can further comprise an adjuvant which can be, for example, alum.

The formulation can be used for the preparation of a vaccine for preventing or treating influenza in an animal in need thereof. The animal can be human, murine, equine or avian.

The invention relates to the use of a formulation comprising at least two peptides selected from the group consisting of SEQ ID NOs: 1 to 64 and at least two peptides selected from the group consisting of SEQ ID NOs: 133 to 180 for the preparation of a vaccine for treating human influenza.

The invention also relates to the use of a formulation comprising at least one peptide sequence selected from the group consisting of SEQ ID NOs: 185 to 248 for the preparation of a vaccine for treating equine influenza.

The invention further relates to the use of the formulation comprising at least one peptide sequence selected from the group consisting of SEQ ID NOs: 65 to 128 for the preparation of a vaccine for treating avian influenza.

The vaccines prepared in accordance with the present invention can be used for preventing or treating influenza.

The sequences of these peptides are determined based on analysis of the crystal structure of influenza hemagglutinin (HA) protein to determine peptide epitopes. Hemagglutinin is the major surface glycoprotein of influenza virus and a potent immunogen against which viral neutralizing antibodies are directed. The linear peptide epitopes in the cocktail mimic discontinuous epitopes on the HA protein surface. Using bioinformatics software that analyzes the antigenic variation of HA proteins from thousands of human influenza isolates, degenerative peptide cocktails based on these epitopes can be prepared which represent the antigenic variation of HA within these epitopes. Thus, the influenza vaccine formulations of the present invention comprise a cocktail of peptides that represent major epitopes of the HA protein.

The vaccine may be formulated with or without representing variation at specific residues for each peptide. When variation is not represented, the peptide formed may be referred to herein as a Discotope™ construct. A discotope construct is a linear sequence synthetic construct that approximates the position of primary sequence sections that compose discontinuous epitopes. The individual sections are constructed in sequence to elicit immune responses that recognize the discontinuous epitopes found in the original intact protein.

Discontinuous epitopes are composed of two or more segments of the primary sequence of a protein that when properly folded come together and are bound by specific antibodies. They are not recognized by antibodies when the secondary structure is lost and therefore have not been represented by a continuous linear peptide.

When variation is present at particular residues that are known to have different amino acids represented according to different sequences for that particular pathogen, the formulation comprises a number of peptides, which may be collectively referred to herein as a Discosite™ construct.

In order to formulate a mixture of peptides, it is possible to use the method of Torres as outlined in U.S. Patent Application No. 10/072,084, which is herein incorporated by reference.

Design of Discotope/Discosite constructs

In the vaccine formulations of the present invention, influenza hemagglutinin is used to design linear sequences that represent four discontinuous epitopes. Four peptides (discotope constructs) that mimic discontinuous B and T cell epitopes on four antigenic sites of HA were designed. Each discotope construct is synthesized using solid phase peptide synthesis. Sequences of more than 200 human isolates of Influenza A were obtained from Genbank™ and Swiss Protein™ databases and aligned to study the composition of these epitopes.

An influenza vaccine formulation can comprise one or more discotope constructs of SEQ ID NOs: 129 to 132, and/or SEQ ID NOs: 181 to 184.

Design of vaccine formulations

In the context of the present invention, a vaccine formulation is a cocktail of peptides that are used in the preparation of an influenza vaccine. The vaccine can comprise the cocktail of peptides and other substituents known in the art that would be found acceptable for inclusion. These substituents can include, but are not limited to, adjuvants, diluents and/or carriers.

The vaccine formulations of the present invention are particularly suitable for preparing vaccines in the treatment of human, equine and/or avian influenza. However, it will be appreciated that any combination of peptide sequences, or formulations comprising these peptide sequences, may be used in other influenza phenotypes.

Peptide vaccines can be prepared with a pool of one or more peptide sequences representing epitopes contained in the three-dimensional structure of HA (SEQ ID NOs: 1-248). The vaccines comprise one or more discotope constructs (peptides containing non-variable amino acid residues) or one or more discosite constructs (peptides containing variable amino acid residues). A discosite construct of the present invention is derived from one of these epitopes. Thus, a discosite construct formulation comprises one or more peptide sequences derived from the epitope containing the variable residues.

Each discosite construct of the present invention represents 2^x possible peptide sequences based on x varied residues. For example, a discosite construct having 3 or 4 variable residues represents $2^3 = 8$ or $2^4 = 16$ sequences, respectively. Therefore, in the context of the present invention, a discosite construct as referred to herein includes the epitope sequence containing the variable residues and the one or more possible sequences derived therefrom.

The vaccines of the present invention can comprise at least two peptide sequences from a given discosite construct, derived from at least two epitopes contained in the vaccine, for a total of at least 4 peptide sequences (from SEQ ID NOs: 1 to 248) in the vaccine. In some embodiments of the present invention, the human influenza vaccine formulation can comprise at least 4 human influenza-A (INF-HA-1-V1-V4) and/or 4 human influenza-B HA discosite construct sequences (INF-HB-1-V1-V4). This can include at least two peptides from SEQ ID NOs: 1 to 64 and/or at least two peptides from SEQ ID NOs: 133 to 180.

Alternatively, this can include at least two peptide sequences from each of at least two of the following groups: SEQ ID NOs: 1 to 16; SEQ ID NOs: 17 to 32; SEQ ID NOs: 33 to 48: or SEQ ID NOs: 49 to 64; and/or at least two peptide sequences from each of at least two of the following groups: SEQ ID NOs: 133 to 140; SEQ ID NOs: 141 to 156; SEQ ID NOs: 157 to 172: and/or SEQ ID NOs: 173 to 180.

Embodiments of the equine influenza vaccine can comprise at least one peptide sequence derived from the 4 equine discosite constructs (INFE-HA-1-V1-V4; SEQ ID NOs: 185 to 248), In some embodiments, the equine vaccine formulation can comprise at least two peptide sequences from each of at least two of the following groups: SEQ ID NOs: 185 to 200; SEQ ID NOs: 201 to 216; SEQ ID NOs: 217 to 232; and/or SEQ ID NOs: 233 to 248.

Embodiments of the avian influenza vaccine can comprise one or more peptide sequences of the 4 avian discosite constructs (INF-HA-2-V1-V4; SEQ ID NOs; 65 to 128). In some embodiments, the avian vaccine formulation can comprise at least two peptide sequences from each of at least two of the following groups: SEQ ID NOs: 65 to 80; SEQ ID NOs: 81 to 96; SEQ ID NOs: 97 to 112; and/or SEQ ID NOs: 113 to 128.

It will be appreciated by the person of ordinary skill in the art that additional sequences may or may not be added as required.The peptide sequences for use in the vaccine formulations of the present invention are grouped according to the discosite (Tables 1 to 16) or discotope (Tables 17 to 18) construct.

Tables 1 to 4 list discosite constructs of influenza A (human HA-1) epitope sequence.

Tables 5 to 8 list discosite constructs of influenza A (avian HA-2) epitope sequences.

Tables 9 to 12 list discosite constructs of influenza B (human HB-1) epitope sequences.

Tables 13 to 16 list discosite constructs of equine influenza (equine HA-1) epitope sequences.

In each of the discosite constructs listed in the Tables, the variable residue(s) is/are shown below the corresponding residue in the construct.

TABLE 1: Discosite constructs for INF-HA-1-V1

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YACKRGGKSSGSSYPVLNVSY (SEQ ID NO: 1)
----H------------S-TM
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Peptide Name	Sequence	MW
INF-HA-1-V1/1	YACKRGGKSSGSSYPVLNVSY (SEQ ID NO: 1)	2223.47
INF-HA-1-V1/2	YACKRGGKSSGSSYPVLNVSM (SEQ ID NO: 2)	2191.49
INF-HA-1-V1/3	YACKRGGKSSGSSYPVLNVTY (SEQ ID NO: 3)	2237.5
INF-HA-1-V1/4	YACKRGGKSSGSSYPVLNVTM (SEQ ID NO: 4)	2205.52
INF-HA-1-V1/5	YACKRGGKSSGSSYPVLSVSY (SEQ ID NO: 5)	2196.44
INF-HA-1-V1/6	YACKRGGKSSGSSYPVLSVSM (SEQ ID NO: 6)	2164.47
INF-HA-1-V1/7	YACKRGGKSSGSSYPVLSVTY (SEQ ID NO: 7)	2210.47
INF-HA-1-V1/8	YACKRGGKSSGSSYPVLSVTM (SEQ ID NO: 8)	2178.49
INF-HA-1-V1/9	YACKHGGKSSGSSYPVLNVSY (SEQ ID NO: 9)	2204.42
INF-HA-1-V1/10	YACKHGGKSSGSSYPVLNVSM (SEQ ID NO: 10)	2172.45
INF-HA-1-V1/11	YACKHGGKSSGSSYPVLNVTY (SEQ ID NO: 11)	2218.45
INF-HA-1-V1/12	YACKHGGKSSGSSYPVLNVTM (SEQ ID NO: 12)	2186.47
INF-HA-1-V1/13	YACKHGGKSSGSSYPVLSVSY (SEQ ID NO: 13)	2177.4
INF-HA-1-V1/14	YACKHGGKSSGSSYPVLSVSM (SEQ ID NO: 14)	2145.42
INF-HA-1-V1/15	YACKHGGKSSGSSYPVLSVTY (SEQ ID NO: 15)	2191.42
INF-HA-1-V1/16	YACKHGGKSSGSSYPVLSVTM (SEQ ID NO: 16)	2159.45

TABLE 2: Discosite constructs for INF-HA-1-V2

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KKGSVHHPSTITEQTSLYVNA (SEQ ID NO: 17)
-S-------------T--QQ-
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Peptide Name	Sequence	MW
INF-HA-1-V2/1	KKGSVHHPSTITEQTSLYVNA (SEQ ID NO: 17)	2297.53
INF-HA-1-V2/2	KKGSVHHPSTITEQTSLYVQA (SEQ ID NO: 18)	2311.55
INF-HA-1-V2/3	KKGSVHHPSTITEQTSLYQNA (SEQ ID NO: 19)	2326.53
INF-HA-1-V2/4	KKGSVHHPSTITEQTSLYQQA (SEQ ID NO: 20)	2340.55
INF-HA-1-V2/5	KKGSVHHPSTITEQTTLYVNA (SEQ ID NO: 21)	2311.55
INF-HA-1-V2/6	KKGSVHHPSTITEQTTLYVQA (SEQ ID NO: 22)	2325.58
INF-HA-1-V2/7	KKGSVHHPSTITEQTTLYQNA (SEQ ID NO: 23)	2340.55
INF-HA-1-V2/8	KKGSVHHPSTITEQTTLYQQA (SEQ ID NO: 24)	2354.58
INF-HA-1-V2/9	KSGSVHHPSTITEQTSLYVNA (SEQ ID NO: 25)	2256.43
INF-HA-1-V2/10	KSGSVHHPSTITEQTSLYVQA (SEQ ID NO: 26)	2270.46
INF-HA-1-V2/11	KSGSVHHPSTITEQTSLYQNA (SEQ ID NO: 27)	2285.43
INF-HA-1-V2/12	KSGSVHHPSTITEQTSLYQQA (SEQ ID NO: 28)	2299.46

INF-HA-1-V2/13	KSGSVHHPSTITEQTTLYVNA (SEQ ID NO: 29)	2270.46
INF-HA-1-V2/14	KSGSVHHPSTITEQTTLYVQA (SEQ ID NO: 30)	2284.48
INF-HA-1-V2/15	KSGSVHHPSTITEQTTLYQNA (SEQ ID NO: 31)	2299.46
INF-HA-1-V2/16	KSGSVHHPSTITEQTTLYQQA (SEQ ID NO: 32)	2313.48

TABLE 3: Discosite constructs for INF-HA-1-V3

DVLFSVESPNNKNKDPIDTCD (SEQ ID NO: 33)
------K-V-----ES-----

Peptide Name	Sequence	MW
INF-HA-1-V3/1	DVLFSVESPNNKNKDPIDTCD (SEQ ID NO: 33)	2350.52
INF-HA-1-V3/2	DVLFSVESPNNKNKDSIDTCD (SEQ ID NO: 34)	2340.48
INF-HA-1-V3/3	DVLFSVESPNNKNKEPIDTCD (SEQ ID NO: 35)	2364.55
INF-HA-1-V3/4	DVLFSVESPNNKNKESIDTCD (SEQ ID NO: 36)	2354.51
INF-HA-1-V3/5	DVLFSVESVNNKNKDPIDTCD (SEQ ID NO: 37)	2352.54
INF-HA-1-V3/6	DVLFSVESVNNKNKDSIDTCD (SEQ ID NO: 38)	2342.5
INF-HA-1-V3/7	DVLFSVESVNNKNKEPIDTCD (SEQ ID NO: 39)	2366.56
INF-HA-1-V3/8	DVLFSVESVNNKNKESIDTCD (SEQ ID NO: 40)	2356.52
INF-HA-1-V3/9	DVLFSVKSPNNKNKDPIDTCD (SEQ ID NO: 41)	2349.58
INF-HA-1-V3/10	DVLFSVKSPNNKNKDSIDTCD (SEQ ID NO: 42)	2339.54
INF-HA-1-V3/11	DVLFSVKSPNNKNKEPIDTCD (SEQ ID NO: 43)	2363.61
INF-HA-1-V3/12	DVLFSVKSPNNKNKESIDTCD (SEQ ID NO: 44)	2353.57
INF-HA-1-V3/13	DVLFSVKSVNNKNKDPIDTCD (SEQ ID NO: 45)	2351.6
INF-HA-1-V3/14	DVLFSVKSVNNKNKDSIDTCD (SEQ ID NO: 46)	2341.56
INF-HA-1-V3/15	DVLFSVKSVNNKNKEPIDTCD (SEQ ID NO: 47)	2365.62
INF-HA-1-V3/16	DVLFSVKSVNNKNKESIDTCD (SEQ ID NO: 48)	2355.58

TABLE 4: Discosite constructs for INF-HA-1-V4

YVSVSTSRIASRPKVRGQSGR (SEQ ID NO: 49)
--T--S---G---W-------

Peptide Name	Sequence	MW
INF-HA-1-V4/1	YVSVSTSRIASRPKVRGQSGR (SEQ ID NO: 49)	2291.57
INF-HA-1-V4/2	YVSVSTSRIASRPWVRGQSGR (SEQ ID NO: 50)	2349.61
INF-HA-1-V4/3	YVSVSTSRIGSRPKVRGQSGR (SEQ ID NO: 51)	2277.55
INF-HA-1-V4/4	YVSVSTSRIGSRPWVRGQSGR (SEQ ID NO: 52)	2335.58
INF-HA-1-V4/5	YVSVSSSRIASRPKVRGQSGR (SEQ ID NO: 53)	2277.55
INF-HA-1-V4/6	YVSVSSSRIASRPWVRGQSGR (SEQ ID NO: 54)	2335.58
INF-HA-1-V4/7	YVSVSSSRIGSRPKVRGQSGR (SEQ ID NO: 55)	2263.52
INF-HA-1-V4/8	YVSVSSSRIGSRPWVRGQSGR (SEQ ID NO: 56)	2321.56

INF-HA-1-V4/9	YVTVSTSRIASRPKVRGQSGR (SEQ ID NO: 57)	2305.6
INF-HA-1-V4/10	YVTVSTSRIASRPWVRGQSGR (SEQ ID NO: 58)	2363.64
INF-HA-1-V4/11	YVTVSTSRIGSRPKVRGQSGR (SEQ ID NO: 59)	2291.57
INF-HA-1-V4/12	YVTVSTSRIGSRPWVRGQSGR (SEQ ID NO: 60)	2349.61
INF-HA-1-V4/13	YVTVSSSRIASRPKVRGQSGR (SEQ ID NO: 61)	2291.57
INF-HA-1-V4/14	YVTVSSSRIASRPWVRGQSGR (SEQ ID NO: 62)	2349.61
INF-HA-1-V4/15	YVTVSSSRIGSRPKVRGQSGR (SEQ ID NO: 63)	2277.55
INF-HA-1-V4/16	YVTVSSSRIGSRPWVRGQSGR (SEQ ID NO: 64)	2335.58

TABLE 5: Avian influenza A discosite constructs for INF-HA-2-V1

YACKRGGKSSGSSYPVLKVSY (SEQ ID NO: 65)
----Y-----------SRT-

Peptide Name	Sequence	MW
INF-HA-2-V1/1	YACKRGGKSSGSSYPVLKVSY (SEQ ID NO: 65)	2237.54
INF-HA-2-V1/2	YACKRGGKSSGSSYPVLKVTY (SEQ ID NO: 66)	2251.57
INF-HA-2-V1/3	YACKRGGKSSGSSYPVLKRSY (SEQ ID NO: 67)	2294.6
INF-HA-2-V1/4	YACKRGGKSSGSSYPVLKRTY (SEQ ID NO: 68)	2308.62
INF-HA-2-V1/5	YACKRGGKSSGSSYPVLSVSY (SEQ ID NO: 69)	2196.44
INF-HA-2-V1/6	YACKRGGKSSGSSYPVLSVTY (SEQ ID NO: 70)	2210.47
INF-HA-2-V1/7	YACKRGGKSSGSSYPVLSRSY (SEQ ID NO: 71)	2253.5
INF-HA-2-V1/8	YACKRGGKSSGSSYPVLSRTY (SEQ ID NO: 72)	2267.53
INF-HA-2-V1/9	YACKYGGKSSGSSYPVLKVSY (SEQ ID NO: 73)	2244.53
INF-HA-2-V1/10	YACKYGGKSSGSSYPVLKVTY (SEQ ID NO: 74)	2258.56
INF-HA-2-V1/11	YACKYGGKSSGSSYPVLKRSY (SEQ ID NO: 75)	2301.58
INF-HA-2-V1/12	YACKYGGKSSGSSYPVLKRTY (SEQ ID NO: 76)	2315.61
INF-HA-2-V1/13	YACKYGGKSSGSSYPVLSVSY (SEQ ID NO: 77)	2203.43
INF-HA-2-V1/14	YACKYGGKSSGSSYPVLSVTY (SEQ ID NO: 78)	2217.46
INF-HA-2-V1/15	YACKYGGKSSGSSYPVLSRSY (SEQ ID NO: 79)	2260.49
INF-HA-2-V1/16	YACKYGGKSSGSSYPVLSRTY (SEQ ID NO: 80)	2274.51

TABLE 6: Avian influenza A discosite constructs for INF-HA-2-V2 (Discosite B2)

KKGSVHHPSTITEQTSLYVNA (SEQ ID NO: 81)
-S-------------K--QQ-

Peptide Name	Sequence	MW
INF-HA-2-V2/1	KKGSVHHPSTITEQTSLYVNA (SEQ ID NO: 81)	2297.53
INF-HA-2-V2/2	KKGSVHHPSTITEQTSLYVQA (SEQ ID NO: 82)	2311.55
INF-HA-2-V2/3	KKGSVHHPSTITEQTSLYQNA (SEQ ID NO: 83)	2326.53
INF-HA-2-V2/4	KKGSVHHPSTITEQTSLYQQA (SEQ ID NO: 84)	2340.55

INF-HA-2-V2/5	KKGSVHHPSTITEQTKLYVNA (SEQ ID NO: 85)	2338.62
INF-HA-2-V2/6	KKGSVHHPSTITEQTKLYVQA (SEQ ID NO: 86)	2352.65
INF-HA-2-V2/7	KKGSVHHPSTITEQTKLYQNA (SEQ ID NO: 87)	2367.62
INF-HA-2-V2/8	KKGSVHHPSTITEQTKLYQQA (SEQ ID NO: 88)	2381.65
INF-HA-2-V2/9	KSGSVHHPSTITEQTSLYVNA (SEQ ID NO: 89)	2256.43
INF-HA-2-V2/10	KSGSVHHPSTITEQTSLYVQA (SEQ ID NO: 90)	2270.46
INF-HA-2-V2/11	KSGSVHHPSTITEQTSLYQNA (SEQ ID NO: 91)	2285.43
INF-HA-2-V2/12	KSGSVHHPSTITEQTSLYQQA (SEQ ID NO: 92)	2299.46
INF-HA-2-V2/13	KSGSVHHPSTITEQTKLYVNA (SEQ ID NO: 93)	2297.53
INF-HA-2-V2/14	KSGSVHHPSTITEQTKLYVQA (SEQ ID NO: 94)	2311.55
INF-HA-2-V2/15	KSGSVHHPSTITEQTKLYQNA (SEQ ID NO: 95)	2326.53
INF-HA-2-V2/16	KSGSVHHPSTITEQTKLYQQA (SEQ ID NO: 96)	2340.55

TABLE 7: Avian influenza A discosite constructs for INF-HA-2-V3

DVLFSVESPNNKNKDPIDTCD (SEQ ID NO: 97)
------P-N-----EE-----

Peptide Name	Sequence	MW
INF-HA-2-V3/1	DVLFSVESPNNKNKDPIDTCD (SEQ ID NO: 97)	2350.52
INF-HA-2-V3/2	DVLFSVESPNNKNKDEIDTCD (SEQ ID NO: 98)	2382.52
INF-HA-2-V3/3	DVLFSVESPNNKNKEPIDTCD (SEQ ID NO: 99)	2364.55
INF-HA-2-V3/4	DVLFSVESPNNKNKEEIDTCD (SEQ ID NO: 100)	2396.55
INF-HA-2-V3/5	DVLFSVESNNNKNKDPIDTCD (SEQ ID NO: 101)	2367.51
INF-HA-2-V3/6	DVLFSVESNNNKNKDEIDTCD (SEQ ID NO: 102)	2399.51
INF-HA-2-V3/7	DVLFSVESNNNKNKEPIDTCD (SEQ ID NO: 103)	2381.53
INF-HA-2-V3/8	DVLFSVESNNNKNKEEIDTCD (SEQ ID NO: 104)	2413.53
INF-HA-2-V3/9	DVLFSVPSPNNKNKDPIDTCD (SEQ ID NO: 105)	2318.52
INF-HA-2-V3/10	DVLFSVPSPNNKNKDEIDTCD (SEQ ID NO: 106)	2350.52
INF-HA-2-V3/11	DVLFSVPSPNNKNKEPIDTCD (SEQ ID NO: 107)	2332.55
INF-HA-2-V3/12	DVLFSVPSPNNKNKEEIDTCD (SEQ ID NO: 108)	2364.55
INF-HA-2-V3/13	DVLFSVPSNNNKNKDPIDTCD (SEQ ID NO: 109)	2335.51
INF-HA-2-V3/14	DVLFSVPSNNNKNKDEIDTCD (SEQ ID NO: 110)	2367.51
INF-HA-2-V3/15	DVLFSVPSNNNKNKEPIDTCD (SEQ ID NO: 111)	2349.54
INF-HA-2-V3/16	DVLFSVPSNNNKNKEEIDTCD (SEQ ID NO: 112)	2381.53

TABLE 8: Avian influenza A discosite constructs for INF-HA-2-V4

YVSVSTSRIASRPKVRGQSGR (SEQ ID NO: 113)
--T--S---G---W-------

Peptide Name	Sequence	MW
INF-HA-2-V4/1	YVSVSTSRIASRPKVRGQSGR (SEQ ID NO: 113)	2291.57
INF-HA-2-V4/2	YVSVSTSRIASRPWVRGQSGR (SEQ ID NO: 114)	2349.61
INF-HA-2-V4/3	YVSVSTSRIGSRPKVRGQSGR (SEQ ID NO: 115)	2277.55
INF-HA-2-V4/4	YVSVSTSRIGSRPWVRGQSGR (SEQ ID NO: 116)	2335.58
INF-HA-2-V4/5	YVSVSSSRIASRPKVRGQSGR (SEQ ID NO: 117)	2277.55
INF-HA-2-V4/6	YVSVSSSRIASRPWVRGQSGR (SEQ ID NO: 118)	2335.58
INF-HA-2-V4/7	YVSVSSSRIGSRPKVRGQSGR (SEQ ID NO: 119)	2263.52
INF-HA-2-V4/8	YVSVSSSRIGSRPWVRGQSGR (SEQ ID NO: 120)	2321.56
INF-HA-2-V4/9	YVTVSTSRIASRPKVRGQSGR (SEQ ID NO: 121)	2305.6
INF-HA-2-V4/10	YVTVSTSRIASRPWVRGQSGR (SEQ ID NO: 122)	2363.64
INF-HA-2-V4/11	YVTVSTSRIGSRPKVRGQSGR (SEQ ID NO: 123)	2291.57
INF-HA-2-V4/12	YVTVSTSRIGSRPWVRGQSGR (SEQ ID NO: 124)	2349.61
INF-HA-2-V4/13	YVTVSSSRIASRPKVRGQSGR (SEQ ID NO: 125)	2291.57
INF-HA-2-V4/14	YVTVSSSRIASRPWVRGQSGR (SEQ ID NO: 126)	2349.61
INF-HA-2-V4/15	YVTVSSSRIGSRPKVRGQSGR (SEQ ID NO: 127)	2277.55
INF-HA-2-V4/16	YVTVSSSRIGSRPWVRGQSGR (SEQ ID NO: 128)	2335.58

TABLE 9: Human influenza B discosite constructs for INF-HB-1-V1

GSCPNATNRNGDNNKTAINPLTVEVPY (SEQ ID NO: 133)
-------S-S-------T---------

Peptide Name	Sequence	MW
INF-HB-1-V1/1	GSCPNATNRNGDNNKTAINPLTVEVPY (SEQ ID NO: 133)	2860.08
INF-HB-1-V1/2	GSCPNATNRNGDNNKTATNPLTVEVPY (SEQ ID NO: 134)	2848.03
INF-HB-1-V1/3	GSCPNATNRSGDNNKTAINPLTVEVPY (SEQ ID NO: 135)	2833.06
INF-HB-1-V1/4	GSCPNATNRSGDNNKTATNPLTVEVPY (SEQ ID NO: 136)	2821
INF-HB-1-V1/5	GSCPNATSRNGDNNKTAINPLTVEVPY (SEQ ID NO: 137)	2833.06
INF-HB-1-V1/6	GSCPNATSRNGDNNKTATNPLTVEVPY (SEQ ID NO: 138)	2821
INF-HB-1-V1/7	GSCPNATSRSGDNNKTAINPLTVEVPY (SEQ ID NO: 139)	2806.03
INF-HB-1-V1/8	GSCPNATSRSGDNNKTATNPLTVEVPY (SEQ ID NO: 140)	2793.98

TABLE 10: Human influenza B discosite constructs for INF-HB-1-V2

PKDNFHSDNKTQMERLYGDSN (SEQ ID NO: 141)
RN----------KN------

Peptide Name	Sequence	MW
INF-HB-1-V2/1	PKDNFHSDNKTQMERLYGDSN (SEQ ID NO: 141)	2496.63
INF-HB-1-V2/2	PKDNFHSDNKTQMENLYGDSN (SEQ ID NO: 142)	2454.55
INF-HB-1-V2/3	PKDNFHSDNKTQMKRLYGDSN (SEQ ID NO: 143)	2495.69

INF-HB-1-V2/4	PKDNFHSDNKTQMKNLYGDSN (SEQ ID NO: 144)	2453.61
INF-HB-1-V2/5	PNDNFHSDNKTQMERLYGDSN (SEQ ID NO: 145)	2482.56
INF-HB-1-V2/6	PNDNFHSDNKTQMENLYGDSN (SEQ ID NO: 146)	2440.48
INF-HB-1-V2/7	PNDNFHSDNKTQMKRLYGDSN (SEQ ID NO: 147)	2481.62
INF-HB-1-V2/8	PNDNFHSDNKTQMKNLYGDSN (SEQ ID NO: 148)	2439.54
INF-HB-1-V2/9	RKDNFHSDNKTQMERLYGDSN (SEQ ID NO: 149)	2555.7
INF-HB-1-V2/10	RKDNFHSDNKTQMENLYGDSN (SEQ ID NO: 150)	2513.62
INF-HB-1-V2/11	RKDNFHSDNKTQMKRLYGDSN (SEQ ID NO: 151)	2554.76
INF-HB-1-V2/12	RKDNFHSDNKTQMKNLYGDSN (SEQ ID NO: 152)	2512.68
INF-HB-1-V2/13	RNDNFHSDNKTQMERLYGDSN (SEQ ID NO: 153)	2541.63
INF-HB-1-V2/14	RNDNFHSDNKTQMENLYGDSN (SEQ ID NO: 154)	2499.55
INF-HB-1-V2/15	RNDNFHSDNKTQMKRLYGDSN (SEQ ID NO: 155)	2540.69
INF-HB-1-V2/16	RNDNFHSDNKTQMKNLYGDSN (SEQ ID NO: 156)	2498.61

TABLE 11: Human influenza discosite constructs for INF-HB-1-V3

RGKLCPNCFNCTDIICSEGEDLPLIGE (SEQ ID NO: 157)
--------L----L--TK---------

Peptide Name	Sequence	MW
INF-HB-1-V3/1	RGKLCPNCFNCTDIICSEGEDLPLIGE (SEQ ID NO:157)	2940
INF-HB-1-V3/2	RGKLCPNCFNCTDIICSKGEDLPLIGE (SEQ ID NO:158)	2939
INF-HB-1-V3/3	RGKLCPNCFNCTDIICTEGEDLPLIGE (SEQ ID NO:159)	2954
INF-HB-1-V3/4	RGKLCPNCFNCTDIICTKGEDLPLIGE (SEQ ID NO:160)	2953
INF-HB-1-V3/5	RGKLCPNCFNCTDLICSEGEDLPLIGE (SEQ ID NO:161)	2940
INF-HB-1-V3/6	RGKLCPNCFNCTDLICSKGEDLPLIGE (SEQ ID NO:162)	2939
INF-HB-1-V3/7	RGKLCPNCFNCTDLICTEGEDLPLIGE (SEQ ID NO:163)	2954
INF-HB-1-V3/8	RGKLCPNCFNCTDLICTKGEDLPLIGE (SEQ ID NO:164)	2953
INF-HB-1-V3/9	RGKLCPNCLNCTDIICSEGEDLPLIGE (SEQ ID NO:165)	2906
INF-HB-1-V3/10	RGKLCPNCLNCTDIICSKGEDLPLIGE (SEQ ID NO:166)	2905
INF-HB-1-V3/11	RGKLCPNCLNCTDIICTEGEDLPLIGE (SEQ ID NO:167)	2920
INF-HB-1-V3/12	RGKLCPNCLNCTDIICTKGEDLPLIGE (SEQ ID NO:168)	2919
INF-HB-1-V3/13	RGKLCPNCLNCTDLICSEGEDLPLIGE (SEQ ID NO:169)	2906
INF-HB-1-V3/14	RGKLCPNCLNCTDLICSKGEDLPLIGE (SEQ ID NO:170)	2905
INF-HB-1-V3/15	RGKLCPNCLNCTDLICTEGEDLPLIGE (SEQ ID NO:171)	2920
INF-HB-1-V3/16	RGKLCPNCLNCTDLICTKGEDLPLIGE (SEQ ID NO:172)	2919

TABLE 12: Human Influenza B discosite constructs for INF-HB-1-V4

KFTSSANGIGGFPNQTEDEGLKQSGR (SEQ ID NO: 173)
------------D----G--P----

Peptide Name	Sequence	MW
INF-HB-1-V4/1	KFTSSANGIGGFPNQTEDEGLKQSGR (SEQ ID NO:173)	2725.88
INF-HB-1-V4/2	KFTSSANGIGGFPNQTEDEGLPQSGR (SEQ ID NO:174)	2694.82
INF-HB-1-V4/3	KFTSSANGIGGFPNQTEDGGLKQSGR (SEQ ID NO:175)	2653.82
INF-HB-1-V4/4	KFTSSANGIGGFPNQTEDGGLPQSGR (SEQ ID NO:176)	2622.76
INF-HB-1-V4/5	KFTSSANGIGGFPDQTEDEGLKQSGR (SEQ ID NO:177)	2726.87
INF-HB-1-V4/6	KFTSSANGIGGFPDQTEDEGLPQSGR (SEQ ID NO:178)	2695.81
INF-HB-1-V4/7	KFTSSANGIGGFPDQTEDGGLKQSGR (SEQ ID NO:179)	2654.8
INF-HB-1-V4/8	KFTSSANGIGGFPDQTEDGGLPQSGR (SEQ ID NO:180)	2623.75

TABLE 13: Equine influenza discosite constructs for INFE-HA-1-V1

SACKRRSASSNAAFPQMNKTM (SEQ ID NO: 185)
----------Y---T-SY

Peptide Name	Sequence	MW
INFE-HA-1-V1/1	SACKRRSASSNAAFPQMNKTM (SEQ ID NO: 185)	2286.62
INFE-HA-1-V1/2	SACKRRSASSNAAFPQMNKTY (SEQ ID NO: 186)	2318.6
INFE-HA-1-V1/3	SACKRRSASSNAAFPQMNKSM (SEQ ID NO: 187)	2272.6
INFE-HA-1-V1/4	SACKRRSASSNAAFPQMNKSY (SEQ ID NO: 188)	2304.57
INFE-HA-1-V1/5	SACKRRSASSNAAFPQMTKTM (SEQ ID NO: 189)	2273.62
INFE-HA-1-V1/6	SACKRRSASSNAAFPQMTKTY (SEQ ID NO: 190)	2305.6
INFE-HA-1-V1/7	SACKRRSASSNAAFPQMTKSM (SEQ ID NO: 191)	2259.6
INFE-HA-1-V1/8	SACKRRSASSNAAFPQMTKSY (SEQ ID NO: 192)	2291.57
INFE-HA-1-V1/9	SACKRRSASSNAAYPQMNKTM (SEQ ID NO: 193)	2302.62
INFE-HA-1-V1/10	SACKRRSASSNAAYPQMNKTY (SEQ ID NO: 194)	2334.6
INFE-HA-1-V1/11	SACKRRSASSNAAYPQMNKSM (SEQ ID NO: 195)	2288.6
INFE-HA-1-V1/12	SACKRRSASSNAAYPQMNKSY (SEQ ID NO: 196)	2320.57
INFE-HA-1-V1/13	SACKRRSASSNAAYPQMTKTM (SEQ ID NO: 197)	2289.62
INFE-HA-1-V1/14	SACKRRSASSNAAYPQMTKTY (SEQ ID NO: 198)	2321.6
INFE-HA-1-V1/15	SACKRRSASSNAAYPQMTKSM (SEQ ID NO: 199)	2275.6
INFE-HA-1-V1/16	SACKRRSASSNAAYPQMTKSY (SEQ ID NO: 200)	2307.57

TABLE 14: Equine influenza discosite constructs for INFE-HA-1-V2

SSTDNAIHHSSSNQEQTKLYVQE (SEQ ID NO: 201)
-N-------P---T-------S-

Peptide Name	Sequence	MW
INFE-HA-1-V2/1	SSTDNAIHHSSSNQEQTKLYVQE (SEQ ID NO: 201)	2603.67
INFE-HA-1-V2/2	SSTDNAIHHSSSNQEQTKLYVSE (SEQ ID NO: 202)	2562.62
INFE-HA-1-V2/3	SSTDNAIHHSSSNTEQTKLYVQE (SEQ ID NO: 203)	2576.64

INFE-HA-1-V2/4	SSTDNAIHHSSSNTEQTKLYVSE (SEQ ID NO: 204)	2535.59
INFE-HA-1-V2/5	SSTDNAIHHPSSNQEQTKLYVQE (SEQ ID NO: 205)	2613.71
INFE-HA-1-V2/6	SSTDNAIHHPSSNQEQTKLYVSE (SEQ ID NO: 206)	2572.66
INFE-HA-1-V2/7	SSTDNAIHHPSSNTEQTKLYVQE (SEQ ID NO: 207)	2586.68
INFE-HA-1-V2/8	SSTDNAIHHPSSNTEQTKLYVSE (SEQ ID NO: 208)	2545.63
INFE-HA-1-V2/9	SNTDNAIHHSSSNQEQTKLYVQE (SEQ ID NO: 209)	2630.69
INFE-HA-1-V2/10	SNTDNAIHHSSSNQEQTKLYVSE (SEQ ID NO: 210)	2589.64
INFE-HA-1-V2/11	SNTDNAIHHSSSNTEQTKLYVQE (SEQ ID NO: 211)	2603.67
INFE-HA-1-V2/12	SNTDNAIHHSSSNTEQTKLYVSE (SEQ ID NO: 212)	2562.62
INFE-HA-1-V2/13	SNTDNAIHHPSSNQEQTKLYVQE (SEQ ID NO: 213)	2640.73
INFE-HA-1-V2/14	SNTDNAIHHPSSNQEQTKLYVSE (SEQ ID NO: 214)	2599.68
INFE-HA-1-V2/15	SNTDNAIHHPSSNTEQTKLYVQE (SEQ ID NO: 215)	2613.71
INFE-HA-1-V2/16	SNTDNAIHHPSSNTEQTKLYVSE (SEQ ID NO: 216)	2572.66

TABLE 15: Equine influenza discosite constructs for INFE-HA-1-V3

DQFQEESPNNRNFDPDDNCE (SEQ ID NO: 217)
---L-F---T---P------

Peptide Name	Sequence	MW
INFE-HA-1-V3/1	DQFQEESPNNRNFDPDDNCE (SEQ ID NO: 217)	2413.36
INFE-HA-1-V3/2	DQFQEESPNNRNFPPDDNCE (SEQ ID NO: 218)	2395.39
INFE-HA-1-V3/3	DQFQEESPNTRNFDPDDNCE (SEQ ID NO: 219)	2400.36
INFE-HA-1-V3/4	DQFQEESPNTRNFPPDDNCE (SEQ ID NO: 220)	2382.39
INFE-HA-1-V3/5	DQFQEFSPNNRNFDPDDNCE (SEQ ID NO: 221)	2431.42
INFE-HA-1-V3/6	DQFQEFSPNNRNFPPDDNCE (SEQ ID NO: 222)	2413.45
INFE-HA-1-V3/7	DQFQEFSPNTRNFDPDDNCE (SEQ ID NO: 223)	2418.42
INFE-HA-1-V3/8	DQFQEFSPNTRNFPPDDNCE (SEQ ID NO: 224)	2400.45
INFE-HA-1-V3/9	DQFLEESPNNRNFDPDDNCE (SEQ ID NO: 225)	2398.39
INFE-HA-1-V3/10	DQFLEESPNNRNFPPDDNCE (SEQ ID NO: 226)	2380.42
INFE-HA-1-V3/11	DQFLEESPNTRNFDPDDNCE (SEQ ID NO: 227)	2385.39
INFE-HA-1-V3/12	DQFLEESPNTRNFPPDDNCE (SEQ ID NO: 228)	2367.42
INFE-HA-1-V3/13	DQFLEFSPNNRNFDPDDNCE (SEQ ID NO: 229)	2416.45
INFE-HA-1-V3/14	DQFLEFSPNNRNFPPDDNCE (SEQ ID NO: 230)	2398.48
INFE-HA-1-V3/15	DQFLEFSPNTRNFDPDDNCE (SEQ ID NO: 231)	2403.45
INFE-HA-1-V3/16	DQFLEFSPNTRNFPPDDNCE (SEQ ID NO: 232)	2385.48

TABLE 16: Equine influenza discosite constructs for INFE-HA-1-V4

RITVSTSRPGARPWVRGQSGR (SEQ ID NO: 233)
-----S----S--Q-N-----

Peptide Name	Sequence	MW
INFE-HA-1-V4/1	RITVSTSRPGARPWVRGQSGR (SEQ ID NO: 233)	2324.61
INFE-HA-1-V4/2	RITVSTSRPGARPWVNGQSGR (SEQ ID NO: 234)	2282.52
INFE-HA-1-V4/3	RITVSTSRPGARPQVRGQSGR (SEQ ID NO: 235)	2266.53
INFE-HA-1-V4/4	RITVSTSRPGARPQVNGQSGR (SEQ ID NO: 236)	2224.44
INFE-HA-1-V4/5	RITVSTSRPGSRPWVRGQSGR (SEQ ID NO: 237)	2340.61
INFE-HA-1-V4/6	RITVSTSRPGSRPWVNGQSGR (SEQ ID NO: 238)	2298.52
INFE-HA-1-V4/7	RITVSTSRPGSRPQVRGQSGR (SEQ ID NO: 239)	2282.52
INFE-HA-1-V4/8	RITVSTSRPGSRPQVNGQSGR (SEQ ID NO: 240)	2240.44
INFE-HA-1-V4/9	RITVSSSRPGARPWVRGQSGR (SEQ ID NO: 241)	2310.58
INFE-HA-1-V4/10	RITVSSSRPGARPWVNGQSGR (SEQ ID NO: 242)	2268.5
INFE-HA-1-V4/11	RITVSSSRPGARPQVRGQSGR (SEQ ID NO: 243)	2252.5
INFE-HA-1-V4/12	RITVSSSRPGARPQVNGQSGR (SEQ ID NO: 244)	2210.42
INFE-HA-1-V4/13	RITVSSSRPGSRPWVRGQSGR (SEQ ID NO: 245)	2326.58
INFE-HA-1-V4/14	RITVSSSRPGSRPWVNGQSGR (SEQ ID NO: 246)	2284.5
INFE-HA-1-V4/15	RITVSSSRPGSRPQVRGQSGR (SEQ ID NO: 247)	2268.5
INFE-HA-1-V4/16	RITVSSSRPGSRPQVNGQSGR (SEQ ID NO: 248)	2226.42

Tables 17 and 18 list discotope construct sequences (for influenza A and B, respectively) which can be used in the preparation of a vaccine in accordance with the present invention.

TABLE 17: Influenza A Discotope constructs

Peptide Name	Sequence	MW
INF-HA-1-M1	YACKRGGKSSGSSYPVLNVSY (SEQ ID NO: 129)	2223.47
INF-HA-1-M2	KKGSVHHPSTITEQTSLYVNA (SEQ ID NO: 130)	2297.53
INF-HA-1-M3	DVLFSVESPNNKNKDPIDTCD (SEQ ID NO: 131)	2350.52
INF-HA-1-M4	YVSVSTSRIASRPKVRGQSGR (SEQ ID NO: 132)	2291.57

TABLE 18: Influenza B Discotope constructs

Peptide Name	Sequence	MW
INF-HB-1-M1	GSCPNVANGNGDNNKTAINPVTVEVPY (SEQ ID NO: 181)	2744.95
INF-HB-1-M2	PKDNFHSDDKTQMERLYGDSN (SEQ ID NO: 182)	2497.61
INF-HB-1-M3	RGKLCPNCFNCTDIICSEGEDLPLIGE (SEQ ID NO: 183)	2940.36
INF-HB-1-M4	KFTSSANGIGGFPNQTEDEGLKQSGR (SEQ ID NO: 184)	2725.88

Peptide Synthesis

Peptides were synthesized using standard solid-phase peptide chemistry. The peptides were synthesized by solid phase peptide synthesis (SPPS) using 9-fluoroenylmethoxycarbonyl (Fmoc) chemistry on Pioneer™ automated peptide synthesizer, utilizing pre-loaded Fmoc protected NovaSyn™ TGT resin (NovaBiochem) as described. Where variability at a given position is desired, mixture of two amino acids is placed at that position. This is repeated each time during the synthesis wherever the variability is desired. While 1M solution of 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) and N-Hydroxybenzotriazole (HOBt) in dimethylformamide (DMF), and 1 M solution of diisopropylethyl amine (DIPEA) in DMF was used for coupling amino acids, 20% piperidine in DMF was used for deblocking amino acids during the synthesis.

Coupling was allowed to occur for one hour at room temperature. After the last amino acid was coupled, the resin was taken out from synthesizer and washed on a sintered glass funnel several times with DMF, with 2-propanol and with dichloromethylene (DCM), and dried under high vacuum. The peptide mixtures are cleaved and deprotected by the addition of a solution containing TFA / water / phenol / thioanisole /EDT /TIS [82:5:5:5:2:1]. The resin was incubated at room temperature for 4 hours. Cleavage mixture was then filtered under reduced pressure into a flask containing a 10-fold volume of cold ether. Resin was also rinsed twice with TFA into the same ether solution.

Following incubation for 30 minutes in a freezer to further assist precipitation, the sample was centrifuged at 1,000Xg for 5 minutes, and the ether removed. This extraction process was repeated three times. Following a final ether extraction, the residual organic solvent was evaporated under nitrogen gas, and the peptide mixture was re-dissolved in water and purified by using high performance liquid chromatography (HPLC). Excess of the solvent was removed by using a rotor evaporator, and finally lyophilized to dry powder. Mass spectrometry (MS) and amino acid analysis were performed on all the discotope constructs to ensure that they have the appropriate peptide content.

Figures 1 to 4 illustrate exemplary HPLC data from the discosite constructs of the present invention. Each HPLC plot corresponds to a particular discosite construct formulation, containing a cocktail of peptides in the respective discosite construct. Figs. 1A to 1D correspond to discosite constructs INFE-HA-1-V1 to V4 (SEQ ID NOs: 185 to 248), respectively. Figs. 2A to 2D correspond to discosite constructs INF-HA-1-V1 to V4 (SEQ ID NOs: 1 to 64), respectively. Figs. 3A to 3D correspond to discosite constructs INF-HA-2-V1 to

V4 (SEQ ID NOs: 65 to 128), respectively. Figs. 4A to 4D correspond to discosite constructs INF-HB-1-V1 to V4 (SEQ ID NOs: 133 to 180), respectively.

Figures 5 to 8 illustrate MS data from the discosite constructs of the present invention. As with the HPLC plots, each MS plot corresponds to a particular discosite construct formulation containing a cocktail of peptides in the respective discosite construct. Figs. 5A to 5D correspond to discosite constructs INFE-HA-1-V1 to V4 (SEQ ID NOs: 185 to 248), respectively. Figs. 6A to 6D correspond to discosite constructs INF-HA-1-V1 to V4 (SEQ ID NOs: 1 to 64), respectively. Figs. 7A to 7D correspond to discosite constructs INF-HA-2-V1 to V4 (SEQ ID NOs: 65 to 128), respectively. Figs. 8A to 8D correspond to discosite constructs INF-HB-1-V1 to V4 (SEQ ID NOs: 133 to 180), respectively.

Examples

Two exemplary vaccines, INF-01P (INF-HA-1-V1-V4) and INFE-01P (INFE-HA-V1-V4), were prepared in accordance with the present invention. The vaccines were prepared based on formulations comprising peptide sequences derived from epitopes from influenza A and as listed in Tables 1 to 4 (SEQ ID NOs: 1 to 64) and Tables 13 to 16 (SEQ ID NOs: 185 to 248), and summarized in Tables 19 and 20, below. Using a murine model, the vaccines were tested: a) to determine whether addition of adjuvant enhances immunity against challenge; b) to determine the humoral response induced by candidate vaccines in comparison to commercial vaccine in a murine model; and c) to assess the range of protection elicited by the vaccine against influenza challenge using different influenza virus strains.

Vaccination schedule: Groups of mice were vaccinated subcutaneously at the base of the tail; mice receiving the commercial vaccine were immunized intramuscularly (as recommended). Mice were similarly boosted two additional times, at three week intervals. Two weeks after the last immunization, the mice were challenged with a lethal dose of H3N2. Mice were monitored daily after challenge for weight and signs of infection.

Adjuvants: The following adjuvants were used to boost immune responses in combination with the vaccine: Ribi (Cedarlane,1:1 ratio Ribi:vaccine), Alum (Sigma, equal volumes of 500ng/ml and vaccine), and Montanide (Seppic, 1:1 ratio montanide:vaccine). In the present example, Alum was used as an adjuvant, although any suitable adjuvant can be used.

EXAMPLE 1

In the present example, B6 mice were immunized with INF-01P vaccine plus either Alum, Ribi, or Montanide, or the commercial vaccine (2004-2005 season). Sera was obtained from vaccinated mice one day prior to challenge with virus. Mice were challenged with pathogenic A/HK/1/68-MA20c virus and followed for three weeks post-challenge.

The INF-01P vaccine is based on 4 human influenza sequence discosite construct formulations as shown in Table 19:

TABLE 19: INF-01P influenza vaccine formulation

INF-HA-1-V1 (SEQ ID NO:1) YACKRGGKSSGSSYPVLNVSY (SEQ ID NOs: 1 to 16) ----H----------S-TM
INF-HA-1-V2 (SEQ ID NO: 17) KKGSVHHPSTITEQTSLYVNA (SEQ ID NOs: 17 to 32) -S----------T--QQ-
INF-HA-1-V3 (SEQ ID NO: 33) DVLFSVESPNNKNKDPIDTCD (SEQ ID NOs: 33 to 48) ------K-V-----ES-----
INF-HA-1-V4 (SEQ ID NO: 49) YVSVSTSRIASRPKVRGQSGR (SEQ ID NOs: 49 to 64) --T--S---G---W-------

Figure 9 shows the induction of humoral immunity by INF-01P vaccination as measured by HAI titres. As shown in this example, mice immunized with INF-01P plus Alum vaccine had increased humoral immunity as compared to mice immunized with INF-01P plus Ribi or INF-01P plus Montanide, and compared to the current influenza vaccine.

Figure 10 shows a survival plot of INF-01P-vaccinated mice against challenge with H3N2. In this example, mice immunized with INF-01P plus Alum vaccine are better protected and have a better survival rate against challenge compared to INF-01P plus Ribi or INF-01P plus Montanide.

Figure 11 illustrates percent weight loss in challenged mice vaccinated with INF-01P. As shown in the present example, mice immunized with INF-01P plus Alum were more protected against weight loss than mice immunized with INF-01P plus Ribi or INF-01P plus Montanide.

EXAMPLE 2

B6 mice were immunized with INFE-01P (equine flu) vaccine plus either Alum or the commercial vaccine (2004-2005 season). Sera from the mice were tested for HAI activity against several influenza strains (H3N2 A/Hong Kong/1/68g, H1N1 A/FM/1/47, H5N1 A/Hong Kong/213/2003, B/Mass/3/66, and H1N1 A/New Caledonia/20/1999). Sera were obtained after the first vaccination.

The INFE-01P vaccine is based on 4 equine influenza sequence discosite construct formulations as shown in Table 20:

TABLE 20: INFE-01P Equine influenza vaccine formulation

INFE-HA-1-V1 (SEQ ID NO: 185) SACKRRSASSNAAFPQMNKTM (SEQ ID NOs: 185 to 200) ----------Y---T-SY
INFE-HA-1-V2 (SEQ ID NO: 201) SSTDNAIHHSSSNQEQTKLYVQE (SEQ ID NOs: 201 to 216) -N-------P---T-------S-
INFE-HA-1-V3 (SEQ ID NO: 217) DQFQEESPNNRNFDPDDNCE (SEQ ID NOs: 217 to 232) ---L-F---T---P------
INFE-HA-1-V4 (SEQ ID NO: 233) RITVSTSRPGARPWVRGQSGR (SEQ ID NOs: 233 to 248) -----S----S--Q-N-----

Figure 12 illustrates humoral immunity in mice immunized with INFE-01P, as measured by HAI titres. As illustrated, humoral immunity was induced in mice immunized with this exemplary equine vaccine formulation against several strains of influenza virus, and as compared to the commercial vaccine or adjuvant only (control) mice.

EXAMPLE 3

Hemagglutination (HAI) assays:

The immunogenicity of the individual and combined discotope constructs was evaluated in mice. Mice immunized with the four discotope constructs collectively developed antibodies that could inhibit viral hemagglutinination activity. Influenza-based discotope

constructs were shown to successfully mimic discontinuous epitopes in that antibodies were elicited that inhibited hemagglutination of red blood cells by influenza virus.

A standard HAI assay was used to measure induction of functionally relevant antibodies against HA. Numerous distinct strains of influenza were used to test HAI titres induced by vaccine candidates in order to determine the breadth of immunity induced by the vaccine preparations.

Figure 13 illustrates the results of a hemagluttination assay performed in murine vaccine study. Each vaccine group received a different vaccine formulation or phosphate buffered saline (negative control). When incubated with virus (H3 Subtype influenza), blood will hemagluttinate (cloudy); when agglutination is protected or inhibited, the RBC remain in a pellet (dark circle).

These results exhibit the strength of hemagluttination inhibition exhibited by the VBI candidate vaccines, as compared to a licenced commercial vaccine for influenza. Note that each of the VBI candidate vaccines and adjuvant combinations exhibit equal or greater hemagluttination inhibition (HI) as does the commercial vaccine (up to 1/40 – 1/80). The discosite construct immunogens with Alum adjuvant (row 12) demonstrate detectable HI even at dilutions up to 1/320.

Table 21: Reciprocal Titre for Different Treatments

Immunogen	Reciprocal Titre
Unvaccinated Control	10
Commercial Vaccine	80
Discosite Immunogens + Montanide	80
Discotope Immunogens + Ribi	160
Discosite Immunogens + Alum	320

Figure 14 shows results of the Influenza Vaccine ELISA test. This is based on data presented in Table 22.

Table 22: Influenza Vaccine ELISA Test Results

	1/50	**1/100**	**1/300**	**1/900**	**1/2700**	**1/8100**
G1	1.538	1.295	0.921	0.665	0.358	0.134
G2	1.965	1.319	0.818	0.595	0.455	0.145
G3	1.559	1.321	0.771	0.583	0.367	0.121
G4	1.427	1.24	0.854	0.564	0.446	0.149
G5	1.258	1.13	0.667	0.456	0.335	0.141
G6	1.298	0.926	0.623	0.45	0.329	0.13

G7	1.416	0.912	0.571	0.421	0.251	0.135
G8	1.4	1.177	0.753	0.453	0.271	0.16
G9	1.686	1.355	0.795	0.477	0.291	0.193
G10	1.21	0.95	0.613	0.475	0.296	0.176
G11	2.82	2.29	1.467	0.77	0.455	0.236
G12	2.959	2.944	2.699	2.174	1.146	0.559
G13	0.694	0.547	0.343	0.289	0.253	0.16

G1 = Discosite construct IFN-1 (SEQ ID NOs: 1 to 16)+Ribi; G2 = Discosite construct IFN-2 (SEQ ID NOs: 17 to 32)+Ribi; G3 = Discosite construct IFN-3 (SEQ ID NOs: 33 to 48)+Ribi; G4 = Discosite construct IFN-4 (SEQ ID NOs: 49 to 64)+Ribi; G5 = Discotope construct 1 (SEQ ID NO: 129)+ Ribi; G6 = Discotope construct 2 (SEQ ID NO: 130)+ Ribi; G7 = Discotope construct 3 (SEQ ID NO: 131)+ Ribi; G8 = Discotope construct 4 (SEQ ID NO: 132)+ Ribi; G9 = Control (Ribi alone); G10 = Discosite construct IFN-1-4+Ribi; G11 = Discotope construct 1-4+ Ribi; G12 = Discosite construct IFN-1-4+Montanide; and G13 = Commercial Vaccine.

Collectively, these data suggest a fundamentally new approach to influenza vaccine development that results in faster vaccine production and broader protection than current influenza vaccines. This approach may be of value to the development of a pandemic influenza vaccine.

The above-described embodiments of the present invention are intended to be examples only. Alterations, modifications and variations may be effected to the particular embodiments by those of skill in the art without departing from the scope of the invention, which is defined solely by the claims appended hereto.

All references are herein incorporated by reference.

What is claimed is:

1. A peptide-based anti-influenza formulation comprising at least one peptide selected from the group consisting of SEQ ID NOs: 1 to 248.

2. The formulation of claim 1 comprising at least four peptide sequences selected from the group consisting of SEQ ID NOs: 1 to 248.

3. The formulation of claim 1 comprising at least two peptides selected from the group consisting of SEQ ID NOs: 1 to 64 and at least two peptides selected from the group consisting of SEQ ID NOs: 133 to 180.

4. The formulation of claim 3 comprising SEQ ID NOs: 1 to 64 and SEQ ID NOs: 133 to 180.

5. The formulation of claim 1 comprising at least one peptide sequence selected from the group consisting of SEQ ID NOs: 185 to 248.

6. The formulation of claim 1 comprising at least one peptide sequence selected from the group consisting of SEQ ID NOs: 65 to 128.

7. The formulation of claim 1 comprising 2^n peptide sequences from each of at least two of the following groups:

a) SEQ ID NOs: 1 to 16;

b) SEQ ID NOs: 17 to 32;

c) SEQ ID NOs: 33 to 48: or

d) SEQ ID NOs: 49 to 64,

wherein n is 1 to 4.

8. The formulation of claim 1 comprising from at least two of groups a) to d):

a) 2^m peptide sequences from SEQ ID NOs: 133 to 140;

b) 2^n peptide sequences from SEQ ID NOs: 141 to 156;

c) 2^n peptide sequences from SEQ ID NOs: 157 to 172; or

d) 2^m peptide sequences from SEQ ID NOs: 173 to 180,

wherein m is 1 to 3 and n is 1 to 4.

9. The formulation of claim 1 comprising 2^n peptide sequences from each of at least two of the following groups:

a) SEQ ID NOs: 185 to 200;

b) SEQ ID NOs: 201 to 216;

c) SEQ ID NOs: 217 to 232; or

d) SEQ ID NOs: 233 to 248,

wherein n is from 1 to 4.

10. The formulation of claim 1 comprising 2^n peptide sequences from each of at least two of the following groups:

a) SEQ ID NOs: 65 to 80;

b) SEQ ID NOs: 81 to 96;

c) SEQ ID NOs: 97 to 112; or

d) SEQ ID NOs: 113 to 128,

wherein n is from 1 to 4.

11. The formulation of claim 1 comprising at least one of SEQ ID NOs: 129 to 132 or SEQ ID NOs: 181 to 184.

12. The formulation of claim 11 comprising SEQ ID NOs: 129 to 132.

13. The formulation of claim 11 comprising SEQ ID NOs: 181 to 184.

14. The formulation of claim 1 comprising SEQ ID NOs: 1 to 64.

15. The formulation of claim 1 comprising SEQ ID NOs: 185 to 248.

16. A vaccine comprising the formulation of claim 1 together with a pharmaceutically-acceptable diluent or carrier.

17. The vaccine of claim 16 further comprising an adjuvant.

18. The vaccine of claim 17 wherein the adjuvant is alum.

19. Use of the formulation of claim 1 for the preparation of a vaccine for preventing or treating influenza in an animal in need thereof.

20. The use according to claim 19 wherein the animal is human, murine, equine or avian.

21. Use of the formulation of claim 3 for the preparation of a vaccine for treating human influenza.

22. Use of the formulation of claim 5 for the preparation of a vaccine for treating equine influenza.

23. Use of the formulation of claim 6 for the preparation of a vaccine for treating avian influenza.

24. Use of the vaccine of claim 16 for preventing or treating influenza.

25. A vaccine comprising the formulation of claim 3.

26. The vaccine of claim 25 further comprising an adjuvant.

27. The vaccine of claim 26 wherein the adjuvant is alum.

28. A method of preventing or treating influenza in an animal in need thereof comprising administering to the animal an effective amount of the vaccine of claim 16.