NEUTRALIZING ANTI-INFLUENZA A VIRUS ANTIBODIES AND USES THEREOF

This application claims the benefit of priority of United States provisional Application Nos. 61/083,838 and 61/181,582, filed July 25, 2008, and May 27, 2009, respectively, the disclosures of which are hereby incorporated by reference as if written herein in their entirety.

BACKGROUND

The neutralizing antibody response to Influenza A virus is thought to be specific for a given viral subtype. There are 16 influenza A subtypes defined by their hemagglutinins (HAs). The 16 HAs, H1 – H16, can be classified into two groups. Group 1 consists of H1, H2, H5, H6, H8, H9, H11, H12, H13, and H16 subtypes, and group 2 includes H3, H4, H7, H10, H14 and H15 subtypes. While all subtypes are present in birds, mostly H1, H2 and H3 subtypes cause disease in humans. H5, H7 and H9 subtypes are causing sporadic severe infections in humans and may generate a new pandemic. H1 and H3 viruses continuously evolve generating new variants, a phenomenon called antigenic drift. As a consequence, antibodies produced in response to past viruses are poorly or non protective against new drifted viruses. A consequence is that a new vaccine has to be produced every year against H1 and H3 viruses that are predicted to emerge, a process that is very costly as well as not always efficient. The same applies to the production of a H5 influenza vaccine. Indeed it is not clear whether the current H5 vaccines based on the Vietnam or Indonesia influenza A virus isolates will protect against a future pandemic H5 virus.

For these reasons it would be highly desirable to have a vaccine that induces broadly neutralizing antibodies capable of neutralizing all influenza A virus subtypes as well as their yearly variants (reviewed by Gerhard et al., 2006). In addition broadly neutralizing heterosubtypic antibodies could be used in preventive or therapeutic settings.

Antibodies that recognize influenza A virus have been characterized. Antibodies to M2 (an invariant small protein expressed on infected cells but not on infectious viruses) have shown some protective effect *in vivo*, possibly by targeting infected cells for destruction by NK cells or cytotoxic T cells. However, the HA is the primary target of neutralizing antibodies. It comprises a large ectodomain of ≈500 amino acids that is cleaved by host-derived enzymes to generate 2 polypeptides that remain linked by a disulfide bond. The

larger N-terminal fragment (HA1, 320-330 amino acids) forms a membrane-distal globular domain that contains the receptor-binding site and most determinants recognized by virus-neutralizing antibodies. The smaller C-terminal portion (HA2, ≈180 amino acids) forms a stem-like structure that anchors the globular domain to the cellular or viral membrane. The degree of sequence homology between subtypes is smaller in the HA1 polypeptides (34%–59% homology between subtypes) than in the HA2 polypeptide (51%–80% homology). The most conserved region is the sequence around the cleavage site, particularly the HA2 N-terminal 11 amino acids, termed fusion peptide, which is conserved among all influenza A virus subtypes. Part of this region is exposed as a surface loop in the HA precursor molecule (HA0), but becomes inaccessible when HA0 is cleaved into HA1/HA2. In summary there are conserved regions among different HA subtypes especially in the HA1-HA2 joining region and in the HA2 region. However these regions may be poorly accessible to neutralizing antibodies.

There has only been limited success in identifying antibodies that neutralize more than one subtype of influenza A virus and their breath of neutralization is narrow and their potency is low. Okuno et al, immunized mice with influenza virus A/Okuda/57 (H2N2) and isolated a monoclonal antibody (C179) that binds to a conserved conformational epitope in HA2 and neutralizes the group 1 H2, H1 and H5 subtype influenza A viruses *in vitro* and *in vivo* in animal models (Okuno et al.,1993; Smirnov et al., 1999; Smirnov et al., 2000).

Recently Gioia et al., described the presence of H5N1 virus neutralizing antibodies in the serum of some individuals that received a conventional seasonal influenza vaccine. (Gioia et al., 2008). The authors suggest that the neutralizing activity might be due to antibodies to neuraminidase (N1). However, monoclonal antibodies were not isolated and target epitopes were not characterized. It is not clear whether the serum antibodies neutralize other subtypes of influenza A virus.

Despite decades of research, there are no marketed antibodies that broadly neutralize or inhibit influenza A virus infection or attenuate disease caused by influenza A virus. Therefore, there is a need to identify new antibodies that protect again multiple subtypes of influenza A virus.

SUMMARY OF THE INVENTION

The invention is based, in part, on the isolation from individuals vaccinated with the seasonal influenza vaccine of naturally occurring human monoclonal antibodies that bind to

HA and neutralize infection of more than one subtype of influenza A virus, as well as novel epitopes to which the antibodies of the invention bind. Accordingly, in one aspect of the invention, the invention comprises an antibody and antigen binding fragments thereof that neutralize infection of more than one subtype of influenza A virus, selected from group 1 and group 2 subtypes.

In one embodiment of the invention, the invention comprises an antibody, or an antigen binding fragment thereof, that neutralizes infection of a group 1 subtype and a group 2 subtype of influenza A virus. In another embodiment of the invention, the invention comprises an antibody, or an antigen binding fragment thereof, comprising at least one complementarity determining region (CDR) sequence having at least 95% sequence identity to any one of SEQ ID NOs: 1-6 or 17-22, wherein the antibody neutralizes influenza A virus.

In yet another embodiment of the invention, the invention comprises a heavy chain CDR1 with the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 17; a heavy chain CDR2 with the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 18; and a heavy chain CDR3 with the amino acid sequence of SEQ ID NO: 3 or SEQ ID NO: 19, wherein the antibody neutralizes influenza A virus. In yet another embodiment of the invention, the invention comprises an antibody, or an antigen binding fragment thereof, comprising a light chain CDR1 with the amino acid sequence of SEQ ID NO: 4 or SEQ ID NO: 20; a light chain CDR2 with the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 21; and a light chain CDR3 with the amino acid sequence of SEQ ID NO: 6 or SEQ ID NO: 22, wherein the antibody neutralizes influenza A virus.

In still another embodiment of the invention, the invention comprises an antibody, or an antigen binding fragment thereof, wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 13 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 14; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 33 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 14; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 29 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 30; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 35 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 30, and wherein the antibody neutralizes influenza A virus. The invention further comprises an antibody, or an antigen binding fragment thereof, wherein the antibody is FI6 variant 1 or FI6 variant 2.

In yet another embodiment of the invention, the invention comprises an antibody, or antigen binding fragment thereof, that neutralizes infection of a group 1 subtype and a group 2 subtype of influenza A virus, wherein the antibody or fragment thereof is expressed by an immortalized B cell clone.

In another aspect, the invention comprises a nucleic acid molecule comprising a polynucleotide encoding an antibody or antibody fragment of the invention. In yet another aspect, the invention comprises a vector comprising a nucleic acid molecule of the invention or a cell expressing an antibody of the invention or an antigen binding fragment thereof. In still another aspect, the invention comprises an isolated or purified immunogenic polypeptide comprising an epitope that binds to an antibody or antigen binding fragment of the invention.

The invention further comprises a pharmaceutical composition comprising an antibody of the invention or an antigen binding fragment thereof, a nucleic acid molecule of the invention, a vector comprising a nucleic acid molecule of the invention, a cell expressing an antibody or an antibody fragment of the invention, or an immunogenic polypeptide of the invention, and a pharmaceutically acceptable diluent or carrier. The invention also comprises a pharmaceutical composition comprising a first antibody or an antigen binding fragment thereof, and a second antibody, or an antigen binding fragment thereof, wherein the first antibody is an antibody of the invention, and the second antibody is an antibody, or an antigen binding fragment thereof, that neutralizes influenza A virus infection.

Use of an antibody of the invention, or an antigen binding fragment thereof, a nucleic acid of the invention, a vector comprising a nucleic acid of the invention, a cell expressing a vector of the invention, an isolated or purified immunogenic polypeptide comprising an epitope that binds to an antibody or antibody fragment of the invention, or a pharmaceutical composition of the invention (i) in the manufacture of a medicament for the treatment of influenza A virus infection, (ii) in a vaccine, or (iii) in diagnosis of influenza A virus infection is also contemplated to be within the scope of the invention. Further, use of an antibody of the invention, or an antigen binding fragment thereof, for monitoring the quality of anti-influenza A virus vaccines by checking that the antigen of said vaccine contains the specific epitope in the correct conformation is also contemplated to be within the scope of the invention.

In another aspect, the invention comprises a method of reducing influenza A virus infection or lowering the risk of influenza A virus infection comprising administering to a

subject in need thereof, a therapeutically effective amount of an antibody or an antigen binding antibody fragment of the invention.

In a further aspect, the invention comprises an epitope which specifically binds to an antibody of the invention, or an antigen binding fragment thereof, for use (i) in therapy, (ii) in the manufacture of a medicament for treating influenza A virus infection, (iii) as a vaccine, or (iv) in screening for ligands able to neutralise influenza A virus infection.

DETAILED DESCRIPTION OF THE INVENTION

The invention is based, in part, on the discovery and isolation, from individuals that were vaccinated with the seasonal influenza A vaccine, of naturally occurring human antibodies that broadly neutralize influenza A virus of different subtypes as well as novel epitopes to which the antibodies of the invention bind. Such antibodies are desirable, as only one or few antibodies are required in order to neutralize different subtypes of influenza A virus. In addition, the epitopes recognized by such antibodies may be part of a vaccine capable of inducing broad protection against both seasonal and candidate pandemic isolates of different subtypes.

Accordingly, in one aspect, the invention provides an antibody and antigen binding fragments thereof that neutralize at least two influenza A viruses in group 1 and group 2 subtypes. In one embodiment, the invention provides an antibody, or an antigen binding fragment thereof, that neutralizes infection of a group 1 subtype and a group 2 subtype of influenza A virus.

In another aspect of the invention, it provides a neutralizing antibody and antigen binding fragments thereof having broad specificity against HA of different influenza A virus subtypes. In one embodiment, the antibody, or antigen binding fragments of the invention specifically binds to an epitope in the stem region of HA that is conserved among two or more influenza A virus subtypes selected from group 1 and group 2. In another embodiment, the antibody, or antigen binding fragments of the invention, specifically binds to a polypeptide comprising the amino acid sequence of any one of SEQ ID NOs: 37, 38, 39 or 40.

Human monoclonal antibodies, the immortalized B cell clones or the transfected host cells that secrete antibodies of the invention, and nucleic acid encoding the antibodies of the invention are also included within the scope of the invention.

As used herein, the terms "antigen binding fragment," "fragment," and "antibody fragment" are used interchangeably to refer to any fragment of an antibody of the invention that retains the antigen-binding activity of the antibody. Exemplary antibody fragments include, but are not limited to, a single chain antibody, Fab, Fab', F(ab')2, Fv or scFv. The term "antibody" as used herein includes both antibodies and antigen binding fragments thereof.

As used herein, the term "broad specificity" is used to refer to an antibody or an antigen binding fragment of the invention that can bind and/or neutralize one or more group 1 subtype and one or more group 2 subtype of influenza A virus.

As used herein, a "neutralizing antibody" is one that can neutralize, *i.e.*, prevent, inhibit, reduce, impede or interfere with, the ability of a pathogen to initiate and/or perpetuate an infection in a host. The terms "neutralizing antibody" and "an antibody that neutralizes" or "antibodies that neutralize" are used interchangeably herein. These antibodies can be used, alone or in combination, as prophylactic or therapeutic agents upon appropriate formulation, in association with active vaccination, as a diagnostic tool, or as a production tool as described herein.

The antibody, or antigen binding fragments, of the invention neutralizes one or more influenza A virus from group 1 (H1, H2, H5, H6, H8, H9, H11, H12, H13, and H16 and their variants) and one or more influenza A virus from group 2 (H3, H4, H7, H10, H14 and H15 and their variants) subtypes. In one embodiment, exemplary group 1 subtypes include H1, H2, H5, H6, and H9 and exemplary group 2 subtypes include H3 and H7.

The antibody and antibody fragment of the invention is capable of neutralizing various combinations of influenza A virus subtypes. In one embodiment, the antibody can neutralize influenza A virus H1 and H3 subtypes, or H2 and H3 subtypes, or H3 and H5 subtypes, or H3 and H9 subtypes, or H1 and H7 subtypes, or H2 and H7 subtypes, or H5 and H7 subtypes, or H7 and H9 subtypes.

In another embodiment, the antibody and antibody fragment of the invention can neutralize influenza A virus H1, H2 and H3 subtypes, or H1, H3 and H5 subtypes, or H1, H3 and H9 subtypes, or H2, H3 and H5 subtypes, or H2, H3 and H9 subtypes, or H3, H5 and H9 subtypes, or H1, H2 and H7 subtypes, or H1, H5 and H7 subtypes, or H1, H7 and H9 subtypes, or H2, H5 and H7 subtypes, or H2, H7 and H9 subtypes, or H5, H7 and H9 subtypes, or H1, H3 and H7 subtypes, or H2, H3 and H7 subtypes, or H3, H5 and H7 subtypes, or H3, H7 and H9 subtypes.

In yet another embodiment, the antibody can neutralize influenza A virus H1, H2, H3 and H7 subtypes, or H1, H3, H5 and H7 subtypes, or H1, H3, H7 and H9 subtypes, or H2, H3, H5 and H7 subtypes, or H2, H3, H7 and H9 subtypes, or H3, H5, H7 and H9 subtypes or H1, H2, H3 and H5 subtypes, or H1, H2, H3 and H9 subtypes, or H1, H3, H5 and H9 subtypes, or H2, H3, H5 and H9 subtypes, or H1, H2, H5 and H7 subtypes, or H1, H2, H7 and H9 subtypes, or H1, H5, H7 and H9 subtypes, or H2, H5, H7 and H9 subtypes.

In still another embodiment, the antibody of the invention can neutralize influenza A virus H1, H2, H3, H5 and H7 subtypes, or H1, H2, H3, H7 and H9 subtypes, or H1, H3, H5, H7 and H9 subtypes, or H2, H3, H5, H7 and H9 subtypes, or H1, H2, H3, H5 and H9 subtypes, or H1, H2, H5, H7 and H9 subtypes, or H1, H2, H3, H5, H7 and H9 subtypes. In yet another embodiment, the antibody and antigen binding fragments of the invention neutralize one or more of the above combinations in addition to neutralizing influenza A virus H6 subtype.

The antibody and antigen binding fragment of the invention have high neutralizing potency. The concentration of the antibody of the invention required for 50% neutralization of influenza A virus, can, for example, be about 50 μg/ml or less. In one embodiment, the concentration of the antibody of the invention required for 50% neutralization of influenza A virus is about 50, 45, 40, 35, 30, 25, 20, 17.5, 15, 12.5, 11, 10, 9, 8, 7, 6, 5, 4.5, 4, 3.5, 3, 2.5, 2, 1.5 or about 1 μg/ml or less. In another embodiment, the concentration of the antibody of the invention required for 50% neutralization of influenza A virus is about 0.9, 0.8, 0.75, 0.7, 0.65, 0.6, 0.55, 0.5, 0.45, 0.4, 0.35, 0.3, 0.25, 0.2, 0.15, 0.1, 0.075, 0.05, 0.04, 0.03, 0.02, 0.01, 0.008, 0.006, 0.004, 0.003, 0.002 or about 0.001 μg/ml or less. This means that only low concentrations of antibody are required for 50% neutralization of influenza A virus. Specificity and potency can be measured using a standard neutralization assay as known to one of skill in the art.

Antibodies of the invention

The invention provides an antibody having particularly broad specificity to HA and that neutralizes one or more influenza A virus subtypes from group 1 and one or more influenza A virus subtypes from group 2. The antibody of the invention binds to an epitope in a region of HA that is conserved among two or more influenza A virus subtypes selected from group 1 and group 2.

In one embodiment, the invention provides an antibody, *e.g.*, an isolated antibody or a purified antibody, that specifically binds to a conserved epitope in the stem region of HA of group 1 and group 2 influenza A virus subtypes and interferes with viral replication or spreading. The invention also provides an antibody, *e.g.*, an isolated antibody or a purified antibody, that specifically binds to a conserved epitope in the stem region of HA of group 1 and group 2 subtypes and inhibits virus entry into a cell. Without being bound to any theory, in an exemplary embodiment the antibody or antigen binding fragments of the invention bind to a conserved epitope in the stem region of influenza A virus and inhibit virus entry into a cell by interfering with the fusion step. An epitope or antigenic determinant of a protein corresponds to those parts of the molecule that are specifically recognized by the binding site (or paratope) of an antibody. Epitopes are thus relational entities that require complementary paratopes for their operational recognition. An epitope that is conserved among different variants of a protein means that the same paratope can specifically recognize these different variants by contacting the same parts of the molecules.

The antibodies of the invention may be monoclonal, for example, human monoclonal antibodies, or recombinant antibodies. The invention also provides fragments of the antibodies of the invention, particularly fragments that retain the antigen-binding activity of the antibodies. Although the specification, including the claims, may, in some places, refer explicitly to antigen binding fragment(s), antibody fragment(s), variant(s) and/or derivative(s) of antibodies, it is understood that the term "antibody" or "antibody of the invention" includes all categories of antibodies, namely, antigen binding fragment(s), antibody fragment(s), variant(s) and derivative(s) of antibodies.

In one embodiment, the antibodies and antibody fragments of the invention neutralize a combination of two or more influenza A virus subtypes of group 1 and group 2. Exemplary influenza A virus subtypes include, but are not limited to, H5N1 (A/Vietnam/1203/04), H1N1 (A/New Caledonia/20/99), H1N1 (A/Salomon Island/3/2006), H3N2 (A/Wyoming/3/03) and H9N2 (A/chicken/Hong Kong/G9/97). In another embodiment, the antibodies neutralize and/or are specific for a combination of 3, 4, 5, 6, 7 or more group 1 and group 2 influenza A virus subtypes.

In an exemplary embodiment, the invention comprises an antibody, or an antibody fragment thereof, that is specific for influenza A virus subtypes H1 and H3 (e.g. H1N1 and H3N2); or H1, H3, H5, and H9 (e.g. H1N1, H3N2, H5N1 and H9N2). In yet another embodiment, the antibody or antibody fragments thereof is specific for H1, H3, H5, H7 and

H9 (e.g.H1N1, H3N2, H5N1, H7N1, H7N7, H9N2). Other exemplary combinations of subtypes of influenza A virus are provided earlier in the application.

The SEQ ID numbers for the amino acid sequence for the heavy chain variable region (V_H) and the light chain variable region (V_L) of exemplary antibodies of the invention as well as the SEQ ID numbers for the nucleic acid sequences encoding them are listed in Table 1.

Table 1. SEQ ID Numbers for V_H and V_L Polypeptides and Polynucleotides for Exemplary Influenza A Virus Neutralizing Antibodies

	SEQ ID NOs. for V_H and V_L Polypeptides and Polynucleotides			
	V_H Polypeptide	**V_L Polypeptide**	**V_H Polynucleotide**	**V_L Polynucleotide**
FI6 variant 1	13	14	15	16
FI6 variant 2	33	14	34	16
FI28 variant 1	29	30	31	32
FI28 variant 2	35	30	36	32

In one embodiment, an antibody or antibody fragment of the invention comprises a heavy chain variable region having an amino acid sequence that is about 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to the sequence recited in any one of SEQ ID NOs: 13, 33, 29 or 35. In another embodiment, an antibody or antibody fragment of the invention comprises a light chain variable region having an amino acid sequence that is about 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to the sequence recited in SEQ ID NOs: 14 or 30.

In yet another embodiment, the heavy chain variable region of an antibody of the invention may be encoded by a nucleic acid that has a sequence that is about 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to the sequence recited in any one of SEQ ID NOs: 15, 34, 31 or 36. In yet another embodiment, the light chain variable region of an antibody of the invention may be encoded by a nucleic acid that has a sequence that is about 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to the sequence recited in SEQ ID NOs: 16 or 32.

The CDRs of the antibody heavy chains are referred to as CDRH1, CDRH2 and CDRH3, respectively. Similarly, the CDRs of the antibody light chains are referred to as CDRL1, CDRL2 and CDRL3, respectively. The positions of the CDR amino acids are

defined according to the IMGT numbering system as: CDR1 – IMGT positions 27 to 38, CDR2 – IMGT positions 56 to 65 and CDR3 – IMGT positions 105 to 117.

Table 2 provides the SEQ ID numbers for the amino acid sequences of the six CDRs of the heavy and light chains, respectively, of the exemplary antibodies of the invention.

Table 2. SEQ ID Numbers for CDR Polypeptides of Exemplary Influenza A Virus Neutralizing Antibodies

	SEQ ID NOs. for CDR Polypeptides					
	CDRH1	**CDRH2**	**CDRH3**	**CDRL1**	**CDRL2**	**CDRL3**
FI6 variant 1	1	2	3	4	5	6
FI6 variant 2	1	2	3	4	5	6
FI28 variant 1	17	18	19	20	21	22
FI28 variant 2	17	18	19	20	21	22

In one embodiment, an antibody or antibody fragment of the invention comprises at least one CDR with a sequence that has at least 95% sequence identity to any one of SEQ ID NOs: 1-6 or 17-22,

In another embodiment, the invention provides an antibody comprising a heavy chain comprising one or more (*i.e.* one, two or all three) heavy chain CDRs from FI6 variant 1, FI6 variant 2, FI28 variant 1 or FI28 variant 2. In an exemplary embodiment, the antibody or antigen binding fragment of the invention comprises a heavy chain CDR1 with the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 17; a heavy chain CDR2 with the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 18; and a heavy chain CDR3 with the amino acid sequence of SEQ ID NO: 3 or SEQ ID NO: 19. In certain embodiments, an antibody or antibody fragment as provided herein comprises a heavy chain comprising (i) SEQ ID NO: 1 for CDRH1, SEQ ID NO: 2 for CDRH2 and SEQ ID NO: 3 for CDRH3, or (ii) SEQ ID NO: 17 for CDRH1, SEQ ID NO: 18 for CDRH2 and SEQ ID NO: 19 for CDRH3.

Also provided is an antibody comprising a light chain comprising one or more (*i.e.* one, two or all three) light chain CDRs from FI6 variant 1, FI6 variant 2, FI28 variant 1 or FI28 variant 2. In an exemplary embodiment, the antibody or antigen binding fragment of the invention comprises a light chain CDR1 with the amino acid sequence of SEQ ID NO: 4 or SEQ ID NO: 20; a light chain CDR2 with the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 21; and a light chain CDR3 with the amino acid sequence of SEQ ID NO: 6 or

SEQ ID NO: 22. In certain embodiments, an antibody as provided herein comprises a light chain comprising (i) SEQ ID NO: 4 for CDRL1, SEQ ID NO: 5 for CDRL2 and SEQ ID NO: 6 for CDRL3, or (ii) SEQ ID NO: 20 for CDRL1, SEQ ID NO: 21 for CDRL2 and SEQ ID NO: 22 for CDRL3.

In one embodiment, an antibody of the invention, or antigen binding fragment thereof, comprises all of the CDRs of antibody FI6 variant 1 as listed in Table 2, and neutralizes influenza A virus infection. In another embodiment, an antibody of the invention, or antigen binding fragment thereof, comprises all of the CDRs of antibody FI6 variant 2 as listed in Table 2, and neutralizes influenza A virus infection. In yet another embodiment, an antibody of the invention, or antigen binding fragment thereof, comprises all of the CDRs of antibody FI28 variant 1 as listed in Table 2, and neutralizes influenza A virus infection. In still another embodiment, an antibody of the invention, or antigen binding fragment thereof, comprises all of the CDRs of antibody FI28 variant 2 as listed in Table 2, and neutralizes influenza A virus infection.

Exemplary antibodies of the invention include, but are not limited to, FI6 variant 1, FI6 variant 2, FI28 variant 1 or FI28 variant 2.

The invention further comprises an antibody, or fragment thereof, that binds to the same epitope as an antibody of the invention, or an antibody that competes with an antibody or antigen binding fragment of the invention.

Antibodies of the invention also include hybrid antibody molecules that comprise one or more CDRs from an antibody of the invention and one or more CDRs from another antibody to the same epitope. In one embodiment, such hybrid antibodies comprise three CDRs from an antibody of the invention and three CDRs from another antibody to the same epitope. Exemplary hybrid antibodies comprise i) the three light chain CDRs from an antibody of the invention and the three heavy chain CDRs from another antibody to the same epitope, or ii) the three heavy chain CDRs from an antibody of the invention and the three light chain CDRs from another antibody to the same epitope.

Variant antibodies are also included within the scope of the invention. Thus, variants of the sequences recited in the application are also included within the scope of the invention. Such variants include natural variants generated by somatic mutation *in vivo* during the immune response or *in vitro* upon culture of immortalized B cell clones. Alternatively,

variants may arise due to the degeneracy of the genetic code, as mentioned above or may be produced due to errors in transcription or translation.

Further variants of the antibody sequences having improved affinity and/or potency may be obtained using methods known in the art and are included within the scope of the invention. For example, amino acid substitutions may be used to obtain antibodies with further improved affinity. Alternatively, codon optimization of the nucleotide sequence may be used to improve the efficiency of translation in expression systems for the production of the antibody. Further, polynucleotides comprising a sequence optimized for antibody specificity or neutralizing activity by the application of a directed evolution method to any of the nucleic acid sequences of the invention are also within the scope of the invention.

In one embodiment variant antibody sequences may share 70% or more (*i.e.* 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or more) amino acid sequence identity with the sequences recited in the application. In some embodiments such sequence identity is calculated with regard to the full length of the reference sequence (*i.e.* the sequence recited in the application). In some further embodiments, percentage identity, as referred to herein, is as determined using BLAST version 2.1.3 using the default parameters specified by the NCBI (the National Center for Biotechnology Information; http://www.ncbi.nlm.nih.gov/) [Blosum 62 matrix; gap open penalty=11 and gap extension penalty=1].

In another aspect, the invention also includes nucleic acid sequences encoding part or all of the light and heavy chains and CDRs of the antibodies of the present invention. Provided herein are nucleic acid sequences encoding part or all of the light and heavy chains and CDRs of exemplary antibodies of the invention. Table 1 provides the SEQ ID numbers for the nucleic acid sequences encoding the heavy chain and light chain variable regions of the exemplary antibodies of the invention. For example, nucleic acid sequences provided herein include SEQ ID NO: 15 (encoding the FI6 variant 1 heavy chain variable region), SEQ ID NO: 34 (encoding the FI6 variant 2 heavy chain variable region), SEQ ID NO: 16 (encoding the FI6 variant 1 and FI6 variant 2 light chain variable region), SEQ ID NO: 31 (encoding the FI28 variant 1 heavy chain variable region); SEQ ID NO: 36 (encoding the FI28 variant 2 heavy chain variable region) and SEQ ID NO: 32 (encoding the FI28 variant 1 and variant 2 light chain variable region).

Table 3 provides the SEQ ID numbers for the nucleic acid sequences encoding the CDRs of the exemplary antibodies of the invention. Due to the redundancy of the genetic code, variants of these sequences will exist that encode the same amino acid sequences.

Table 3. SEQ ID Numbers for CDR Polynucleotides of Exemplary Influenza A Virus Neutralizing Antibodies

	SEQ ID NOs. for CDR Polynucleotides					
	CDRH1	**CDRH2**	**CDRH3**	**CDRL1**	**CDRL2**	**CDRL3**
FI6 variant 1	7	8	9	10	11	12
FI6 variant 2	7	8	9	10	11	12
FI28 variant 1	23	24	25	26	27	28
FI28 variant 2	23	24	25	26	27	28

In one embodiment, nucleic acid sequences according to the invention include nucleic acid sequences having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% identity to the nucleic acid encoding a heavy or light chain of an antibody of the invention. In another embodiment, a nucleic acid sequence of the invention has the sequence of a nucleic acid encoding a heavy or light chain CDR of an antibody of the invention. For example, a nucleic acid sequence according to the invention comprises a sequence that is at least 75% identical to the nucleic acid sequences of SEQ ID NOs: 7-12, 15, 16, 34, 23-28, 31, 32 or 36.

Further included within the scope of the invention are vectors, for example expression vectors, comprising a nucleic acid sequence according to the invention. Cells transformed with such vectors are also included within the scope of the invention. Examples of such cells include but are not limited to, eukaryotic cells, *e.g.* yeast cells, animal cells or plant cells. In one embodiment the cells are mammalian, *e.g.* human, CHO, HEK293T, PER.C6, NS0, myeloma or hybridoma cells.

The invention also relates to monoclonal antibodies that bind to an epitope capable of binding the antibodies of the invention, including, but not limited to, a monoclonal antibody selected from the group consisting FI6 variant 1, FI6 variant 2, FI28 variant 1 and FI28 variant 2.

Monoclonal and recombinant antibodies are particularly useful in identification and purification of the individual polypeptides or other antigens against which they are directed.

The antibodies of the invention have additional utility in that they may be employed as reagents in immunoassays, radioimmunoassays (RIA) or enzyme-linked immunosorbent assays (ELISA). In these applications, the antibodies can be labelled with an analytically-detectable reagent such as a radioisotope, a fluorescent molecule or an enzyme. The antibodies may also be used for the molecular identification and characterization (epitope mapping) of antigens.

Antibodies of the invention can be coupled to a drug for delivery to a treatment site or coupled to a detectable label to facilitate imaging of a site comprising cells of interest, such as cells infected with influenza A virus. Methods for coupling antibodies to drugs and detectable labels are well known in the art, as are methods for imaging using detectable labels. Labelled antibodies may be employed in a wide variety of assays, employing a wide variety of labels. Detection of the formation of an antibody-antigen complex between an antibody of the invention and an epitope of interest (an influenza A virus epitope) can be facilitated by attaching a detectable substance to the antibody. Suitable detection means include the use of labels such as radionuclides, enzymes, coenzymes, fluorescers, chemiluminescers, chromogens, enzyme substrates or co-factors, enzyme inhibitors, prosthetic group complexes, free radicals, particles, dyes, and the like. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material is luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S, or ^{3}H. Such labelled reagents may be used in a variety of well-known assays, such as radioimmunoassays, enzyme immunoassays, *e.g.*, ELISA, fluorescent immunoassays, and the like. (See US 3,766,162; US 3,791,932; US 3,817,837; and US 4,233,402 for example).

An antibody according to the invention may be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent, or a radioactive metal ion or radioisotope. Examples of radioisotopes include, but are not limited to, I-131, I-123, I-125, Y-90, Re-188, Re-186, At-211, Cu-67, Bi-212, Bi-213, Pd-109, Tc-99, In-111, and the like. Such antibody conjugates can be used for modifying a given biological response; the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug

moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin.

Techniques for conjugating such therapeutic moiety to antibodies are well known. See, for example, Arnon *et al.* (1985) "Monoclonal Antibodies for Immunotargeting of Drugs in Cancer Therapy," in *Monoclonal Antibodies and Cancer Therapy*, ed. Reisfeld *et al.* (Alan R. Liss, Inc.), pp. 243-256; ed. Hellstrom *et al.* (1987) "Antibodies for Drug Delivery," in *Controlled Drug Delivery*, ed. Robinson *et al.* (2d ed; Marcel Dekker, Inc.), pp. 623-653; Thorpe (1985) "Antibody Carriers of Cytotoxic Agents in Cancer Therapy: A Review," in *Monoclonal Antibodies '84: Biological and Clinical Applications*, ed. Pinchera *et al.* pp. 475-506 (Editrice Kurtis, Milano, Italy, 1985); "Analysis, Results, and Future Prospective of the Therapeutic Use of Radiolabeled Antibody in Cancer Therapy," in *Monoclonal Antibodies for Cancer Detection and Therapy*, ed. Baldwin *et al.* (Academic Press, New York, 1985), pp. 303-316; and Thorpe *et al.* (1982) *Immunol. Rev.* 62:119-158.

Alternatively, an antibody, or antibody fragment thereof, can be conjugated to a second antibody, or antibody fragment thereof, to form an antibody heteroconjugate as described in US 4,676,980. In addition, linkers may be used between the labels and the antibodies of the invention (e.g. US 4,831,175). Antibodies or, antigen-binding fragments thereof may be directly labelled with radioactive iodine, indium, yttrium, or other radioactive particle known in the art (e.g. US 5,595,721). Treatment may consist of a combination of treatment with conjugated and non-conjugated antibodies administered simultaneously or subsequently (e.g. WO00/52031; WO00/52473).

Antibodies of the invention may also be attached to a solid support. Additionally, antibodies of the invention, or functional antibody fragments thereof, can be chemically modified by covalent conjugation to a polymer to, for example, increase their circulating half-life. Examples of polymers, and methods to attach them to peptides, are shown in US 4,766,106; US 4,179,337; US 4,495,285 and US 4,609,546. In some embodiments the polymers may be selected from polyoxyethylated polyols and polyethylene glycol (PEG). PEG is soluble in water at room temperature and has the general formula: $R(O--CH_2--CH_2)_n O--R$ where R can be hydrogen, or a protective group such as an alkyl or alkanol group. In one embodiment the protective group may have between 1 and 8 carbons. In a further embodiment the protective group is methyl. The symbol n is a positive integer. In one embodiment n is between 1 and 1,000. In another embodiment n is between 2 and 500. In

one embodiment the PEG has an average molecular weight between 1,000 and 40,000. In a further embodiment the PEG has a molecular weight between 2,000 and 20,000. In yet a further embodiment the PEG has a molecular weight between 3,000 and 12,000. In one embodiment PEG has at least one hydroxy group. In another embodiment the PEG has a terminal hydroxy group. In yet another embodiment it is the terminal hydroxy group which is activated to react with a free amino group on the inhibitor. However, it will be understood that the type and amount of the reactive groups may be varied to achieve a covalently conjugated PEG/antibody of the present invention.

Water-soluble polyoxyethylated polyols are also useful in the present invention. They include polyoxyethylated sorbitol, polyoxyethylated glucose, polyoxyethylated glycerol (POG), and the like. In one embodiment, POG is used. Without being bound by any theory, because the glycerol backbone of polyoxyethylated glycerol is the same backbone occurring naturally in, for example, animals and humans in mono-, di-, triglycerides, this branching would not necessarily be seen as a foreign agent in the body. In some embodiments POG has a molecular weight in the same range as PEG. Another drug delivery system that can be used for increasing circulatory half-life is the liposome. Methods of preparing liposome delivery systems are discussed in Gabizon et al. (1982), Cafiso (1981) and Szoka (1980). Other drug delivery systems are known in the art and are described in, for example, referenced in Poznansky et al. (1980) and Poznansky (1984).

Antibodies of the invention may be provided in purified form. Typically, the antibody will be present in a composition that is substantially free of other polypeptides *e.g.* where less than 90% (by weight), usually less than 60% and more usually less than 50% of the composition is made up of other polypeptides.

Antibodies of the invention may be immunogenic in non-human (or heterologous) hosts *e.g.* in mice. In particular, the antibodies may have an idiotope that is immunogenic in non-human hosts, but not in a human host. Antibodies of the invention for human use include those that cannot be easily isolated from hosts such as mice, goats, rabbits, rats, non-primate mammals, *etc.* and cannot generally be obtained by humanisation or from xeno-mice.

Antibodies of the invention can be of any isotype (*e.g.* IgA, IgG, IgM *i.e.* an α, γ or μ heavy chain), but will generally be IgG. Within the IgG isotype, antibodies may be IgG1, IgG2, IgG3 or IgG4 subclass. Antibodies of the invention may have a κ or a λ light chain.

Production of antibodies

Antibodies according to the invention can be made by any method known in the art. For example, the general methodology for making monoclonal antibodies using hybridoma technology is well known (Kohler, G. and Milstein, C,. 1975; Kozbar et al. 1983). In one embodiment, the alternative EBV immortalisation method described in WO2004/076677 is used.

Using the method described in WO2004/076677, B cells producing the antibody of the invention can be transformed with EBV in the presence of a polyclonal B cell activator. Transformation with EBV is a standard technique and can easily be adapted to include polyclonal B cell activators.

Additional stimulants of cellular growth and differentiation may optionally be added during the transformation step to further enhance the efficiency. These stimulants may be cytokines such as IL-2 and IL-15. In one aspect, IL-2 is added during the immortalisation step to further improve the efficiency of immortalisation, but its use is not essential. The immortalized B cells produced using these methods can then be cultured using methods known in the art and antibodies isolated therefrom.

Using the method described in UK Patent Application 0819376.5, single plasma cells can be cultured in microwell culture plates. Antibodies can be isolated from the single plasma cell cultures. Further, from single plasma cell cultures, RNA can be extracted and single cell PCR can be performed using methods known in the art. The VH and VL regions of the antibodies can be amplified by RT-PCR, sequenced and cloned into an expression vector that is then transfected into HEK293T cells or other host cells. The cloning of nucleic acid in expression vectors, the transfection of host cells, the culture of the transfected host cells and the isolation of the produced antibody can be done using any methods known to one of skill in the art.

The antibodies may be further purified, if desired, using filtration, centrifugation and various chromatographic methods such as HPLC or affinity chromatography. Techniques for purification of antibodies, *e.g.*, monoclonal antibodies, including techniques for producing pharmaceutical-grade antibodies, are well known in the art.

Fragments of the antibodies of the invention can be obtained from the antibodies by methods that include digestion with enzymes, such as pepsin or papain, and/or by cleavage of disulfide bonds by chemical reduction. Alternatively, fragments of the antibodies can be

obtained by cloning and expression of part of the sequences of the heavy or light chains. Antibody "fragments" may include Fab, Fab', $F(ab')_2$ and Fv fragments. The invention also encompasses single-chain Fv fragments (scFv) derived from the heavy and light chains of an antibody of the invention *e.g.* the invention includes a scFv comprising the CDRs from an antibody of the invention. Also included are heavy or light chain monomers and dimers, single domain heavy chain antibodies, single domain light chain antibodies, as well as single chain antibodies, *e.g.* single chain Fv in which the heavy and light chain variable domains are joined by a peptide linker.

Antibody fragments of the invention may impart monovalent or multivalent interactions and be contained in a variety of structures as described above. For instance, scFv molecules may be synthesized to create a trivalent "triabody" or a tetravalent "tetrabody." The scFv molecules may include a domain of the Fc region resulting in bivalent minibodies. In addition, the sequences of the invention may be a component of multispecific molecules in which the sequences of the invention target the epitopes of the invention and other regions of the molecule bind to other targets. Exemplary molecules include, but are not limited to, bispecific Fab2, trispecific Fab3, bispecific scFv, and diabodies (Holliger and Hudson, 2005, Nature Biotechnology 9: 1126-1136).

Standard techniques of molecular biology may be used to prepare DNA sequences encoding the antibodies or antibody fragments of the present invention. Desired DNA sequences may be synthesised completely or in part using oligonucleotide synthesis techniques. Site-directed mutagenesis and polymerase chain reaction (PCR) techniques may be used as appropriate.

Any suitable host cell/vector system may be used for expression of the DNA sequences encoding the antibody molecules of the present invention or fragments thereof. Bacterial, for example *E. coli*, and other microbial systems may be used, in part, for expression of antibody fragments such as Fab and $F(ab')_2$ fragments, and especially Fv fragments and single chain antibody fragments, for example, single chain Fvs. Eukaryotic, *e.g.* mammalian, host cell expression systems may be used for production of larger antibody molecules, including complete antibody molecules. Suitable mammalian host cells include, but are not limited to, CHO, HEK293T, PER.C6, NS0, myeloma or hybridoma cells.

The present invention also provides a process for the production of an antibody molecule according to the present invention comprising culturing a host cell comprising a

vector encoding a nucleic acid of the present invention under conditions suitable for leading to expression of protein from DNA encoding the antibody molecule of the present invention, and isolating the antibody molecule.

The antibody molecule may comprise only a heavy or light chain polypeptide, in which case only a heavy chain or light chain polypeptide coding sequence needs to be used to transfect the host cells. For production of products comprising both heavy and light chains, the cell line may be transfected with two vectors, a first vector encoding a light chain polypeptide and a second vector encoding a heavy chain polypeptide. Alternatively, a single vector may be used, the vector including sequences encoding light chain and heavy chain polypeptides.

Alternatively, antibodies according to the invention may be produced by i) expressing a nucleic acid sequence according to the invention in a host cell, and ii) isolating the expressed antibody product. Additionally, the method may include iii) purifying the antibody.

Screening of Transformed B Cells, Cultured Single Plasma Cells and Transfected HEK293T Cells

Transformed B cells and cultured single plasma cells may be screened for those producing antibodies of the desired specificity or function.

The screening step may be carried out by any immunoassay, for example, ELISA, by staining of tissues or cells (including transfected cells), by neutralization assay or by one of a number of other methods known in the art for identifying desired specificity or function. The assay may select on the basis of simple recognition of one or more antigens, or may select on the additional basis of a desired function *e.g.* to select neutralizing antibodies rather than just antigen-binding antibodies, to select antibodies that can change characteristics of targeted cells, such as their signalling cascades, their shape, their growth rate, their capability of influencing other cells, their response to the influence by other cells or by other reagents or by a change in conditions, their differentiation status, *etc.*

Individual transformed B cell clones may then be produced from the positive transformed B cell culture. The cloning step for separating individual clones from the mixture of positive cells may be carried out using limiting dilution, micromanipulation, single cell deposition by cell sorting or another method known in the art.

Nucleic acid from the cultured single plasma cells can be isolated, cloned and expressed in HEK293T cells or other host cells using methods known in the art.

The immortalized B cell clones or the transfected HEK293T cells of the invention can be used in various ways *e.g.* as a source of monoclonal antibodies, as a source of nucleic acid (DNA or mRNA) encoding a monoclonal antibody of interest, for research, *etc.*

The invention provides a composition comprising immortalized B memory cells or transfected host cells that produce antibodies that neutralize at least two different subtypes of influenza A virus selected from group 1 and group 2 subtypes.

Epitopes

As mentioned above, the antibodies of the invention can be used to map the epitopes to which they bind. The inventors have discovered that the antibodies neutralizing influenza A virus infection are directed towards epitopes found on HA. In one embodiment, the antibodies are directed to one or more epitopes in the stem region of HA that are conserved among one or more group 1 and group 2 subtypes of influenza A virus. The epitopes to which the antibodies of the invention bind may be linear (continuous) or conformational (discontinuous). In one embodiment, the antibodies and antibody fragments of the invention bind a region of the polypeptide comprising SEQ ID NOs: 37, 38, 39 or 40, as discussed herein.

The epitopes recognized by the antibodies of the present invention may have a number of uses. The epitope and mimotopes thereof in purified or synthetic form can be used to raise immune responses (*i.e.* as a vaccine, or for the production of antibodies for other uses) or for screening sera for antibodies that immunoreact with the epitope or mimotopes thereof. In one embodiment such an epitope or mimotope, or antigen comprising such an epitope or mimotope may be used as a vaccine for raising an immune response. The antibodies and antibody fragments of the invention can also be used in a method of monitoring the quality of vaccines. In particular the antibodies can be used to check that the antigen in a vaccine contains the correct immunogenic epitope in the correct conformation.

The epitope may also be useful in screening for ligands that bind to said epitope. Such ligands, include but are not limited to antibodies; including those from camels, sharks and other species, fragments of antibodies, peptides, phage display technology products, aptamers, adnectins or fragments of other viral or cellular proteins, may block the epitope and so prevent infection. Such ligands are encompassed within the scope of the invention.

Recombinant Expression

The immortalized B cell clone or the cultured plasma cell of the invention may also be used as a source of nucleic acid for the cloning of antibody genes for subsequent recombinant expression. Expression from recombinant sources is more common for pharmaceutical purposes than expression from B cells or hybridomas *e.g.* for reasons of stability, reproducibility, culture ease, *etc.*

Thus the invention provides a method for preparing a recombinant cell, comprising the steps of: (i) obtaining one or more nucleic acids (*e.g.* heavy and/or light chain mRNAs) from the B cell clone or the cultured single plasma cell that encodes the antibody of interest; (ii) inserting the nucleic acid into an expression vector and (iii) transfecting the vector into a host cell in order to permit expression of the antibody of interest in that host cell.

Similarly, the invention provides a method for preparing a recombinant cell, comprising the steps of: (i) sequencing nucleic acid(s) from the B cell clone or the cultured single plasma cell that encodes the antibody of interest; and (ii) using the sequence information from step (i) to prepare nucleic acid(s) for insertion into a host cell in order to permit expression of the antibody of interest in that host cell. The nucleic acid may, but need not, be manipulated between steps (i) and (ii) to introduce restriction sites, to change codon usage, and/or to optimise transcription and/or translation regulatory sequences.

The invention also provides a method of preparing a transfected host cell, comprising the step of transfecting a host cell with one or more nucleic acids that encode an antibody of interest, wherein the nucleic acids are nucleic acids that were derived from an immortalized B cell clone or a cultured single plasma cell of the invention. Thus the procedures for first preparing the nucleic acid(s) and then using it to transfect a host cell can be performed at different times by different people in different places (*e.g.* in different countries).

These recombinant cells of the invention can then be used for expression and culture purposes. They are particularly useful for expression of antibodies for large-scale pharmaceutical production. They can also be used as the active ingredient of a pharmaceutical composition. Any suitable culture technique can be used, including but not limited to static culture, roller bottle culture, ascites fluid, hollow-fiber type bioreactor cartridge, modular minifermenter, stirred tank, microcarrier culture, ceramic core perfusion, *etc.*

Methods for obtaining and sequencing immunoglobulin genes from B cells or plasma cells are well known in the art (*e.g.* see Chapter 4 of *Kuby Immunology*, 4th edition, 2000).

The transfected host cell may be a eukaryotic cell, including yeast and animal cells, particularly mammalian cells (*e.g.* CHO cells, NS0 cells, human cells such as PER.C6 (Jones et al 2003) or HKB-11 (Cho et al. 2001; Cho et al. 2003) cells, myeloma cells (US 5,807,715; US 6,300,104 *etc.*)), as well as plant cells. Preferred expression hosts can glycosylate the antibody of the invention, particularly with carbohydrate structures that are not themselves immunogenic in humans. In one embodiment the transfected host cell may be able to grow in serum-free media. In a further embodiment the transfected host cell may be able to grow in culture without the presence of animal-derived products. The transfected host cell may also be cultured to give a cell line.

The invention provides a method for preparing one or more nucleic acid molecules (*e.g.* heavy and light chain genes) that encode an antibody of interest, comprising the steps of: (i) preparing an immortalized B cell clone or culturing a plasma cell according to the invention; (ii) obtaining from the B cell clone or the cultured single plasma cell nucleic acid that encodes the antibody of interest. The invention also provides a method for obtaining a nucleic acid sequence that encodes an antibody of interest, comprising the steps of: (i) preparing an immortalized B cell clone or culturing a single plasma cell according to the invention; (ii) sequencing nucleic acid from the B cell clone or the cultured plasma cell that encodes the antibody of interest.

The invention also provides a method of preparing nucleic acid molecule(s) that encodes an antibody of interest, comprising the step of obtaining the nucleic acid that was obtained from a transformed B cell clone or a cultured plasma cell of the invention. Thus the procedures for first obtaining the B cell clone or the cultured plasma cell, and then obtaining nucleic acid(s) from the B cell clone or the cultured plasma cell can be performed at different times by different people in different places (*e.g.* in different countries).

The invention provides a method for preparing an antibody (*e.g.* for pharmaceutical use), comprising the steps of: (i) obtaining and/or sequencing one or more nucleic acids (*e.g.* heavy and light chain genes) from the selected B cell clone or the cultured plasma cell expressing the antibody of interest; (ii) inserting the nucleic acid(s) into or using the nucleic acid(s) sequence(s) to prepare an expression vector; (iii) transfect a host cell that can express the antibody of interest; (iv) culturing or sub-culturing the transfected host cells under

conditions where the antibody of interest is expressed; and, optionally, (v) purifying the antibody of interest.

The invention also provides a method of preparing an antibody comprising the steps of: culturing or sub-culturing a transfected host cell population under conditions where the antibody of interest is expressed and, optionally, purifying the antibody of interest, wherein said transfected host cell population has been prepared by (i) providing nucleic acid(s) encoding a selected antibody of interest that is produced by a B cell clone or a cultured plasma cell prepared as described above, (ii) inserting the nucleic acid(s) into an expression vector, (iii) transfecting the vector in a host cell that can express the antibody of interest, and (iv) culturing or sub-culturing the transfected host cell comprising the inserted nucleic acids to produce the antibody of interest. Thus the procedures for first preparing the recombinant host cell and then culturing it to express antibody can be performed at very different times by different people in different places (*e.g.* in different countries).

Pharmaceutical Compositions

The invention provides a pharmaceutical composition containing the antibodies and/or antibody fragments of the invention and/or nucleic acid encoding such antibodies and/or the epitopes recognised by the antibodies of the invention. A pharmaceutical composition may also contain a pharmaceutically acceptable carrier to allow administration. The carrier should not itself induce the production of antibodies harmful to the individual receiving the composition and should not be toxic. Suitable carriers may be large, slowly metabolised macromolecules such as proteins, polypeptides, liposomes, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers and inactive virus particles.

Pharmaceutically acceptable salts can be used, for example mineral acid salts, such as hydrochlorides, hydrobromides, phosphates and sulphates, or salts of organic acids, such as acetates, propionates, malonates and benzoates.

Pharmaceutically acceptable carriers in therapeutic compositions may additionally contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents or pH buffering substances, may be present in such compositions. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries and suspensions, for ingestion by the subject.

Within the scope of the invention, forms of administration may include those forms suitable for parenteral administration, *e.g.* by injection or infusion, for example by bolus injection or continuous infusion. Where the product is for injection or infusion, it may take the form of a suspension, solution or emulsion in an oily or aqueous vehicle and it may contain formulatory agents, such as suspending, preservative, stabilising and/or dispersing agents. Alternatively, the antibody molecule may be in dry form, for reconstitution before use with an appropriate sterile liquid.

Once formulated, the compositions of the invention can be administered directly to the subject. In one embodiment the compositions are adapted for administration to human subjects.

The pharmaceutical compositions of this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intraperitoneal, intrathecal, intraventricular, transdermal, transcutaneous, topical, subcutaneous, intranasal, enteral, sublingual, intravaginal or rectal routes. Hyposprays may also be used to administer the pharmaceutical compositions of the invention. Typically, the therapeutic compositions may be prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared.

Direct delivery of the compositions will generally be accomplished by injection, subcutaneously, intraperitoneally, intravenously or intramuscularly, or delivered to the interstitial space of a tissue. The compositions can also be administered into a lesion. Dosage treatment may be a single dose schedule or a multiple dose schedule. Known antibody-based pharmaceuticals provide guidance relating to frequency of administration *e.g.* whether a pharmaceutical should be delivered daily, weekly, monthly, *etc.* Frequency and dosage may also depend on the severity of symptoms.

Compositions of the invention may be prepared in various forms. For example, the compositions may be prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared (*e.g.* a lyophilised composition, like Synagis™ and Herceptin™, for reconstitution with sterile water containing a preservative). The composition may be prepared for topical administration *e.g.* as an ointment, cream or powder. The composition may be prepared for oral administration *e.g.* as a tablet or capsule, as a spray, or as a syrup (optionally flavoured).

The composition may be prepared for pulmonary administration *e.g.* as an inhaler, using a fine powder or a spray. The composition may be prepared as a suppository or pessary. The composition may be prepared for nasal, aural or ocular administration *e.g.* as drops. The composition may be in kit form, designed such that a combined composition is reconstituted just prior to administration to a subject. For example, a lyophilised antibody can be provided in kit form with sterile water or a sterile buffer.

It will be appreciated that the active ingredient in the composition will be an antibody molecule, an antibody fragment or variants and derivatives thereof. As such, it will be susceptible to degradation in the gastrointestinal tract. Thus, if the composition is to be administered by a route using the gastrointestinal tract, the composition will need to contain agents which protect the antibody from degradation but which release the antibody once it has been absorbed from the gastrointestinal tract.

A thorough discussion of pharmaceutically acceptable carriers is available in Gennaro (2000) *Remington: The Science and Practice of Pharmacy,* 20th edition, ISBN: 0683306472.

Pharmaceutical compositions of the invention generally have a pH between 5.5 and 8.5, in some embodiments this may be between 6 and 8, and in further embodiments about 7. The pH may be maintained by the use of a buffer. The composition may be sterile and/or pyrogen free. The composition may be isotonic with respect to humans. In one embodiment pharmaceutical compositions of the invention are supplied in hermetically-sealed containers.

Pharmaceutical compositions will include an effective amount of one or more antibodies of the invention and/or a polypeptide comprising an epitope that binds an antibody of the invention *i.e.* an amount that is sufficient to treat, ameliorate, or prevent a desired disease or condition, or to exhibit a detectable therapeutic effect. Therapeutic effects also include reduction in physical symptoms. The precise effective amount for any particular subject will depend upon their size and health, the nature and extent of the condition, and the therapeutics or combination of therapeutics selected for administration. The effective amount for a given situation is determined by routine experimentation and is within the judgment of a clinician. For purposes of the present invention, an effective dose will generally be from about 0.01mg/kg to about 50mg/kg, or about 0.05 mg/kg to about 10 mg/kg of the compositions of the present invention in the individual to which it is administered. Known antibody-based pharmaceuticals provide guidance in this respect *e.g.* Herceptin™ is administered by intravenous infusion of a 21 mg/ml solution, with an initial loading dose of

4mg/kg body weight and a weekly maintenance dose of 2mg/kg body weight; Rituxan™ is administered weekly at 375 mg/m^2; *etc.*

In one embodiment compositions can include more than one (*e.g.* 2, 3, *etc.*) antibodies of the invention to provide an additive or synergistic therapeutic effect. In another embodiment, the composition may comprise one or more (*e.g.* 2, 3, *etc.*) antibodies of the invention and one or more (*e.g.* 2, 3, *etc.*) additional antibodies against influenza A virus. For example, one antibody may bind to a HA epitope, while another may bind to a different epitope on HA, or to an epitope on the neuraminidase and/or matrix proteins. Further, the administration of antibodies of the invention together with an influenza A vaccine or with antibodies of specificities other than influenza A virus are within the scope of the invention. The antibodies of the invention can be administered either combined/simultaneously or at separate times from an influenza vaccine or from antibodies of specificities other than influenza A virus.

In another embodiment, the invention provides a pharmaceutical composition comprising two or more antibodies, wherein the first antibody is an antibody of the invention and is specific for an HA epitope, and the second antibody is specific for a neuraminidase epitope, a second HA epitope and/or a matrix epitope. For example, the invention provides a pharmaceutical composition comprising two or more antibodies, wherein the first antibody is specific for an epitope in the stem of an influenza A virus HA, and the second antibody is specific for a neuraminidase epitope, a second HA epitope (for example, an epitope in the globular head of HA, a second epitope in the stem of HA), and/or a matrix epitope. The second epitope in the stem or the epitope in the globular head of the influenza A virus HA may, but need not, be conserved among more than one influenza A virus subtype.

In yet another embodiment, the invention provides a pharmaceutical composition comprising two or more antibodies, wherein the first antibody is specific for a neuraminidase epitope, and the second antibody is specific for a second neuraminidase epitope, a HA epitope and/or a matrix epitope.

In still another embodiment, the invention provides a pharmaceutical composition comprising two or more antibodies, wherein the first antibody is specific for a matrix epitope, and the second antibody is specific for a second matrix epitope, an epitope on HA and/or neuraminidase.

Exemplary antibodies of the invention specific for an Influenza A virus target protein include, but are not limited to, FI6 variant 1, FI6 variant 2, FI28 variant 1 or FI28 variant 2.

In one embodiment, the invention provides a pharmaceutical composition comprising the antibody FI6 variant 1 or an antigen binding fragment thereof, and a pharmaceutically acceptable carrier. In another embodiment, the invention provides a pharmaceutical composition comprising the antibody FI6 variant 2 or an antigen binding fragment thereof, and a pharmaceutically acceptable carrier. In yet another embodiment, the invention provides a pharmaceutical composition comprising the antibody FI28 variant 1 or an antigen binding fragment thereof, and a pharmaceutically acceptable carrier. In still another embodiment, the invention provides a pharmaceutical composition comprising the antibody FI28 variant 2 or an antigen binding fragment thereof, and a pharmaceutically acceptable carrier.

Antibodies of the invention may be administered (either combined or separately) with other therapeutics *e.g.* with chemotherapeutic compounds, with radiotherapy, *etc.* In one embodiment, the therapeutic compounds include anti-viral compounds such as Tamiflu™. Such combination therapy provides an additive or synergistic improvement in therapeutic efficacy relative to the individual therapeutic agents when administered alone. The term "synergy" is used to describe a combined effect of two or more active agents that is greater than the sum of the individual effects of each respective active agent. Thus, where the combined effect of two or more agents results in "synergistic inhibition" of an activity or process, it is intended that the inhibition of the activity or process is greater than the sum of the inhibitory effects of each respective active agent. The term "synergistic therapeutic effect" refers to a therapeutic effect observed with a combination of two or more therapies wherein the therapeutic effect (as measured by any of a number of parameters) is greater than the sum of the individual therapeutic effects observed with the respective individual therapies.

Antibodies may be administered to those subjects who have previously shown no response to treatment for influenza A virus infection, *i.e.* have been shown to be refractive to anti-influenza treatment. Such treatment may include previous treatment with an anti-viral agent. This may be due to, for example, infection with an anti-viral resistant strain of influenza A virus.

In one embodiment, a composition of the invention may include antibodies of the invention, wherein the antibodies may make up at least 50% by weight (*e.g.* 60%, 70%, 75%,

80%, 85%, 90%, 95%, 97%, 98%, 99% or more) of the total protein in the composition. In such a composition, the antibodies are in purified form.

The invention provides a method of preparing a pharmaceutical, comprising the steps of: (i) preparing an antibody of the invention; and (ii) admixing the purified antibody with one or more pharmaceutically-acceptable carriers.

The invention also provides a method of preparing a pharmaceutical, comprising the step of admixing an antibody with one or more pharmaceutically-acceptable carriers, wherein the antibody is a monoclonal antibody that was obtained from a transformed B cell or a cultured plasma cell of the invention. Thus the procedures for first obtaining the monoclonal antibody and then preparing the pharmaceutical can be performed at very different times by different people in different places (*e.g.* in different countries).

As an alternative to delivering antibodies or B cells for therapeutic purposes, it is possible to deliver nucleic acid (typically DNA) that encodes the monoclonal antibody (or active fragment thereof) of interest derived from the B cell or the cultured plasma cell to a subject, such that the nucleic acid can be expressed in the subject *in situ* to provide a desired therapeutic effect. Suitable gene therapy and nucleic acid delivery vectors are known in the art.

Compositions of the invention may be immunogenic compositions, and in some embodiments may be vaccine compositions comprising an antigen comprising an epitope recognized by an antibody of the invention. Vaccines according to the invention may either be prophylactic (*i.e.* to prevent infection) or therapeutic (*i.e.* to treat infection). In one embodiment, the invention provides a vaccine comprising a polypeptide comprising the amino acid sequence of SEQ ID NOs: 37, 38, 39 or 40.

Compositions may include an antimicrobial, particularly if packaged in a multiple dose format. They may comprise detergent *e.g.* a Tween (polysorbate), such as Tween 80. Detergents are generally present at low levels *e.g.* <0.01%. Compositions may also include sodium salts (*e.g.* sodium chloride) to give tonicity. A concentration of 10±2mg/ml NaCl is typical.

Further, compositions may comprise a sugar alcohol (*e.g.* mannitol) or a disaccharide (*e.g.* sucrose or trehalose) *e.g.* at around 15-30mg/ml (*e.g.* 25 mg/ml), particularly if they are to be lyophilised or if they include material which has been reconstituted from lyophilised

material. The pH of a composition for lyophilisation may be adjusted to around 6.1 prior to lyophilisation.

The compositions of the invention may also comprise one or more immunoregulatory agents. In one embodiment, one or more of the immunoregulatory agents include(s) an adjuvant.

The epitope compositions of the invention may elicit both a cell mediated immune response as well as a humoral immune response in order to effectively address influenza A virus infection. This immune response may induce long lasting (*e.g.* neutralizing) antibodies and a cell mediated immunity that can quickly respond upon exposure to influenza A virus.

Medical Treatments and Uses

The antibodies and antibody fragments of the invention or derivatives and variants thereof may be used for the treatment of influenza A virus infection, for the prevention of influenza A virus infection or for the diagnosis of influenza A virus infection.

Methods of diagnosis may include contacting an antibody or an antibody fragment with a sample. Such samples may be tissue samples taken from, for example, nasal passages, sinus cavities, salivary glands, lung, liver, pancreas, kidney, ear, eye, placenta, alimentary tract, heart, ovaries, pituitary, adrenals, thyroid, brain or skin. The methods of diagnosis may also include the detection of an antigen/antibody complex.

The invention therefore provides (i) an antibody, an antibody fragment, or variants and derivatives thereof according to the invention, (ii) an immortalized B cell clone according to the invention, (iii) an epitope capable of binding an antibody of the invention or (iv) a ligand, preferably an antibody, capable of binding an epitope that binds an antibody of the invention for use in therapy.

The invention also provides a method of treating a subject comprising administering to the subject an antibody, an antibody fragment, or variants and derivatives thereof according to the invention, or, a ligand, preferably an antibody, capable of binding an epitope that binds an antibody of the invention. In one embodiment, the method results in reduced influenza A virus infection in the subject. In another embodiment, the method prevents, reduces the risk or delays influenza A virus infection in the subject.

The invention also provides the use of (i) an antibody, an antibody fragment, or variants and derivatives thereof according to the invention, (ii) an immortalized B cell clone

according to the invention, (iii) an epitope capable of binding an antibody of the invention, or (iv) a ligand, preferably an antibody, that binds to an epitope capable of binding an antibody of the invention, in the manufacture of a medicament for the prevention or treatment of influenza A virus infection.

The invention provides a composition of the invention for use as a medicament for the prevention or treatment of a influenza A virus infection. It also provides the use of an antibody of the invention and/or a protein comprising an epitope to which such an antibody binds in the manufacture of a medicament for treatment of a subject and/or diagnosis in a subject. It also provides a method for treating a subject, comprising the step of administering to the subject a composition of the invention. In some embodiments the subject may be a human. One way of checking efficacy of therapeutic treatment involves monitoring disease symptoms after administration of the composition of the invention. Treatment can be a single dose schedule or a multiple dose schedule.

In one embodiment, an antibody, antibody fragment, immortalized B cell clone, epitope or composition according to the invention is administered to a subject in need of such treatment. Such a subject includes, but is not limited to, one who is particularly at risk of or susceptible to influenza A virus infection, including, for example, an immunocompromised subject. The antibody or antibody fragment of the invention can also be used in passive immunisation or active vaccination.

Antibodies and fragments thereof as described in the present invention may also be used in a kit for the diagnosis of influenza A virus infection. Further, epitopes capable of binding an antibody of the invention may be used in a kit for monitoring the efficacy of vaccination procedures by detecting the presence of protective anti-influenza A virus antibodies. Antibodies, antibody fragment, or variants and derivatives thereof, as described in the present invention may also be used in a kit for monitoring vaccine manufacture with the desired immunogenicity.

The invention also provides a method of preparing a pharmaceutical, comprising the step of admixing a monoclonal antibody with one or more pharmaceutically-acceptable carriers, wherein the monoclonal antibody is a monoclonal antibody that was obtained from a transfected host cell of the invention. Thus the procedures for first obtaining the monoclonal antibody (*e.g.* expressing it and/or purifying it) and then admixing it with the pharmaceutical

carrier(s) can be performed at very different times by different people in different places (*e.g.* in different countries).

Starting with a transformed B cell or a cultured plasma cell of the invention, various steps of culturing, sub-culturing, cloning, sub-cloning, sequencing, nucleic acid preparation *etc.* can be performed in order to perpetuate the antibody expressed by the transformed B cell or the cultured plasma cell, with optional optimization at each step. In a preferred embodiment, the above methods further comprise techniques of optimization (*e.g.* affinity maturation or optimization) applied to the nucleic acids encoding the antibody. The invention encompasses all cells, nucleic acids, vectors, sequences, antibodies *etc.* used and prepared during such steps.

In all these methods, the nucleic acid used in the expression host may be manipulated to insert, delete or amend certain nucleic acid sequences. Changes from such manipulation include, but are not limited to, changes to introduce restriction sites, to amend codon usage, to add or optimise transcription and/or translation regulatory sequences, *etc.* It is also possible to change the nucleic acid to alter the encoded amino acids. For example, it may be useful to introduce one or more (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, *etc.*) amino acid substitutions, deletions and/or insertions into the antibody's amino acid sequence. Such point mutations can modify effector functions, antigen-binding affinity, post-translational modifications, immunogenicity, *etc.*, can introduce amino acids for the attachment of covalent groups (*e.g.* labels) or can introduce tags (*e.g.* for purification purposes). Mutations can be introduced in specific sites or can be introduced at random, followed by selection (*e.g.* molecular evolution). For instance, one or more nucleic acids encoding any of the CDR regions, heavy chain variable regions or light chain variable regions of antibodies of the invention can be randomly or directionally mutated to introduce different properties in the encoded amino acids. Such changes can be the result of an iterative process wherein initial changes are retained and new changes at other nucleotide positions are introduced. Moreover, changes achieved in independent steps may be combined. Different properties introduced into the encoded amino acids may include, but are not limited to, enhanced affinity.

General

The term "comprising" encompasses "including" as well as "consisting" *e.g.* a composition "comprising" X may consist exclusively of X or may include something additional *e.g.* X + Y.

The word "substantially" does not exclude "completely" *e.g.* a composition which is "substantially free" from Y may be completely free from Y. Where necessary, the word "substantially" may be omitted from the definition of the invention.

The term "about" in relation to a numerical value x means, for example, $x\pm10\%$.

The term "disease" as used herein is intended to be generally synonymous, and is used interchangeably with, the terms "disorder" and "condition" (as in medical condition), in that all reflect an abnormal condition of the human or animal body or of one of its parts that impairs normal functioning, is typically manifested by distinguishing signs and symptoms, and causes the human or animal to have a reduced duration or quality of life.

As used herein, reference to "treatment" of a subject or patient is intended to include prevention, prophylaxis and therapy. The terms "subject" or "patient" are used interchangeably herein to mean all mammals including humans. Examples of subjects include humans, cows, dogs, cats, horses, goats, sheep, pigs, and rabbits. In one embodiment, the patient is a human.

This description may contain subject matter that falls outside the scope of the claims. This subject matter is included to aid understanding of the invention.

EXAMPLES

Exemplary embodiments of the present invention are provided in the following examples. The following examples are presented only by way of illustration and to assist one of ordinary skill in using the invention. The examples are not intended in any way to otherwise limit the scope of the invention.

Example 1. Generation and Characterization of Influenza A Virus Broadly Neutralizing Antibodies from Plasma Cells

To identify individuals that may produce heterosubtypic antibodies in responses to the seasonal influenza vaccine (containing H1 and H3 HAs), we screened by ELISPOT circulating plasma cells collected on day 7 after boost for their capacity to secrete antibodies that bound to vaccine or to an unrelated H5 HA (A/VN/1203/04). Strikingly, while in four of the five donors tested H5-specific plasma cells were undetectable, in one donor 14% of IgG-secreting plasma cells produced antibodies to H5, while 57% produced antibodies to the vaccine. $CD138^+$ plasma cells were isolated from peripheral blood mononuclear cells (PBMCs) collected 7 days after vaccination using magnetic micro-beads followed by cell-sorting using a FACSAria machine. Limiting numbers of plasma cells were seeded in microwell culture plates. The culture supernatants were tested in three parallel ELISAs using

as antigens recombinant H5 or H9 HAs and the irrelevant antigen tetanus toxoid. Out of the 4,928 culture supernatants screened, 12 bound to H5 but not H9 HA, 25 to H9 but not H5 HA and 54 to both H5 and H9. Some the 54 cultures with highest OD signal were subjected to RT-PCR and two paired VH and VL genes were retrieved.

The VH and VL genes were cloned into expression vectors and recombinant antibodies were produced by transfecting HEK293T cells. The two monoclonal antibodies, FI6 and FI28, shared most V, D and J gene fragments (IGHV3-30*01, IGHD3-9*01, IGHJ4*02 and IGKV4-1*01), but differed in the N regions, in the IGKJ usage and in the pattern of somatic mutations and were therefore not clonally related.

The specificity of recombinant antibodies was investigated by ELISA using a panel of HAs belonging to different subtypes. Remarkably, FI6 bound all influenza A HA subtypes tested including group 1 (H1, H5 and H9) and group 2 (H3 and H7), while it did not bind HA from influenza B virus. In contrast FI28 bound only to the three group 1 HA (H1, H5 and H9).

Table 4.

	Binding to HA by ELISA (% of subtype specific control antibodies)				
	H1	**H3**	**H5**	**H7**	**H9**
	A/NC/ 20/99	A/BR/ 10/07	A/VN/ 1203/04	A/NL/ 219/03	A/HK/ 1073/99
FI6	85.9	68.5	73.7	87.9	98.7
FI28	59.4	1.3	46.3	-0.5	87.7

Given the homology of VH and VL sequences of the two antibodies, shuffling experiments were performed using H and L chains of FI6, FI28 and 7I13, a hCMV-specific antibody that uses the same V, D and J elements of the H chain. While binding to H7 required the pairing of FI6 H and L chains, binding to H5 was maintained when the FI6 and FI28 L chains were shuffled. In addition H5 binding was also observed when the H chain of FI6 was paired to the unrelated 7I13 L chain. In contrast H5 binding was not observed when the homologous 7I13 H chain was paired with FI6 L chain. Without being bound by any particular theory, these results suggest that the main contribution to H5 binding is from the H chain, while H7 binding requires a precise pairing between H and L chains of FI6.

FI6 and FI28 were then tested for their capacity to neutralize group 1 and group 2 influenza A subtypes using pseudotyped viruses (Table 5) as well as infectious viruses (Table 6). Remarkably FI6 neutralized all pseudoviruses tested, including six H5 isolates belonging to the antigenically divergent clades 0, 1, 2.1, 2.2 and 2.3, and two H7 avian isolates. In addition FI6 neutralized all infectious viruses tested, including two H3N2 viruses and four H1N1 viruses spanning several decades, up to the recent H1N1 pandemic isolate A/CA/04/09 (Table 6). FI28 neutralized all H5 pseudoviruses but did not neutralize H7 pseudoviruses as well as all the infectious viruses tested. The neutralizing titers on pseudoviruses were higher than titers on infectious viruses.

Table 5.

	Neutralization of HA-pseudotypes (IC90, μg/ml)							
	H5N1						**H7N1**	
	A/HK/ 491/97	A/HK/ 213/03	A/VN/ 1203/04	A/INDO/ 5/05	A/WS/ MONG/05	A/AH/ 1/05	A/ck/IT/ 13474/99	A/ck/FPV/ Ro/34
FI6	0.07	0.02	0.02	0.31	0.03	0.05	1.87	0.09
FI28	0.05	0.33	0.02	0.35	0.04	0.05	>100	>100

Table 6.

	Neutralization of infectious viruses (IC50, μg/ml)					
	H1N1				**H3N2**	
	A/PR/ 8/34	A/NC/ 20/99	A/SI/ 3/06	A/CA/ 4/09	A/CA/ 7/04	A/WI/ 67/05
FI6	2.2	6.3	8.8	12.5	7.9	12.5
FI28	>100	>100	>100	nd	>100	>100

nd, not done

Example 2. FI6 and FI28 Antigenic Binding Sites

To identify the antigenic sites to which the antibodies FI6 and FI28 bind, we first tested their capacity to inhibit binding of C179, a mouse monoclonal antibody that was mapped to a conserved region of the HA stem region (Y. Okuno, *et al.*, *J Virol* **67**, 2552 (1993)). Both FI6 and FI28 completely inhibited binding of C179 to recombinant H5 VN/1203/04 HA, indicating that they recognize an overlapping epitope. In contrast, FI6 and FI28 did not compete with a panel of H5-specific antibodies isolated from H5N1 immune donors that recognize different epitopes in the globular head of the HA (C. P. Simmons *et al.*, *PLoS Med* **4**, e178 (2007); S. Khurana *et al.*, *PLoS Med* **6**, e1000049 (2009)). Attempts to

map the FI6 epitope by selection of escape mutants failed, suggesting that its epitope cannot be easily mutated without compromising viral fitness.

We next performed peptide-based mapping using libraries of linear and cyclised peptides of HA A/VN/1194/04 as well as helix-scan using the systems of Pepscan Presto BV (Lelystad, The Netherlands). This analysis identified a binding region of FI6 that includes the HA2 fusion peptide FGAIAG (amino acid 3-8, according to H3 numbering; SEQ ID NO: 37), the HA2 Helix A peptide DGVTNKVNS (amino acid 46-54; SEQ ID NO: 38), the HA2 Helix B peptide MENERTLDFHDSNVK (amino acid 102-116; SEQ ID NO: 39) and the HA1 C-terminal peptide LVLATGLRNSP (amino acid 315-325; SEQ ID NO: 40). The binding region of FI28 was different from that of FI6 since this antibody did not react with the HA1 C-terminal peptide and the HA2 Helix B peptide.

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SEQUENCE LISTING

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ccaggcaggg ggctggagtg ggtggcagtt atctcatacg atggaaatta taaatactat 180

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35 40 45

Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asp Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Leu Phe Tyr Cys
85 90 95

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Ser

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Leu Val Leu Ala Thr Gly Leu Arg Asn Ser Pro
1 5 10

What is claimed is:

1. An isolated antibody, or an antigen binding fragment thereof, that neutralizes infection of a group 1 subtype and a group 2 subtype of influenza A virus.

2. The antibody of claim **1**, or an antigen binding fragment thereof, wherein the antibody specifically binds to an epitope in the stem region of hemagglutinin, and wherein the epitope is conserved among two or more influenza A virus subtypes selected from group 1 and group 2.

3. The antibody of claim **1 or 2**, or an antigen binding fragment thereof, wherein the antibody or antibody fragment binds a polypeptide comprising the amino acid sequence of any one of SEQ ID NOs: 37, 38, 39 or 40.

4. The antibody of claim **1, 2 or 3**, wherein the concentration of antibody required for 50% neutralization of influenza A virus is 20 μg/ml or less.

5. The antibody of claim **1, 2 or 3**, wherein the concentration of antibody required for 50% neutralization of influenza A virus is 4 μg/ml or less.

6. The antibody of claim **1, 2 or 3**, or an antigen binding fragment thereof, wherein the group 1 subtype is H1, H2, H5 or H9.

7. The antibody of claim **1, 2 or 3**, or an antigen binding fragment thereof, wherein the group 2 subtype is H3 or H7.

8. The antibody of claim **1, 2 or 3**, or an antigen binding fragment thereof, wherein said antibody or antibody fragment neutralizes infection of two, three or more group 1 subtypes and two or more group 2 subtypes of influenza A virus.

9. The antibody of any one claims **1-7**, or an antigen binding fragment thereof, wherein the antibody neutralizes infection of influenza A virus of subtypes (i) H1 and H3; (ii) H1 and H7; (iii) H3 and H5; (iv) H3 and H9; (v) H5 and H7; (vi) H7 and H9; (vii) H1, H3, H5 and H7; or (viii) H1, H3, H5, H7 and H9.

10. An isolated, or an antigen binding fragment thereof, comprising heavy chain complementarity determining region (CDR) 1, 2 and 3 sequences having at least 95% sequence identity to SEQ ID NOs: 1, 2 and 3 respectively, and light chain CDR 1, 2 and 3 sequences having at least 95% sequence identity to SEQ ID NOs: 4, 5 and 6 respectively, wherein the antibody neutralizes a group 1 subtype and a group 2 subtype of influenza A virus.

11. An isolated, or an antigen binding fragment thereof, comprising heavy chain CDR 1, 2 and 3 sequences as set forth in SEQ ID NOs: 1, 2 and 3 respectively, and light chain CDR 1, 2 and 3 sequences as set forth in SEQ ID NOs: 4, 5 and 6 respectively, wherein the antibody neutralizes a group 1 subtype and a group 2 subtype of influenza A virus.

12. The antibody of any one of claims **1-11**, or an antigen binding fragment thereof, comprising a heavy chain variable region having at least 80% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 13 or 33, and a light chain variable region having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 14.

13. An isolated, or an antigen binding fragment thereof, wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 13 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 14; or a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 33 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 14, and wherein the antibody neutralizes a group 1 subtype and a group 2 subtype of influenza A virus.

14. The antibody of any one of the previous claims, or an antigen binding fragment thereof, wherein the antibody is FI6 variant 1 or FI6 variant 2.

15. The antibody of any one of the previous claims, or an antigen binding fragment thereof, wherein the antibody is a human antibody, a monoclonal antibody, a purified antibody, an isolated antibody, a single chain antibody, Fab, Fab', F(ab')2, Fv or scFv.

16. The antibody of any one of the previous claims, or an antigen binding fragment thereof, for treatment of influenza A virus infection.

17. Nucleic acid molecules comprising polynucleotides encoding the antibody, or an antigen binding fragment thereof, of any one of the previous claims.

18. The nucleic acid molecules of claim **17**, wherein the polynucleotides comprise sequences that are at least 80% identical to the nucleic acid sequences of SEQ ID NOs: 7-12.

19. The nucleic acid molecules of claim **17**, wherein the polynucleotides comprise sequences that are at least 80% identical to the nucleic acid sequences of SEQ ID NOs: 15 and 16; or the sequences of SEQ ID NOs: 34 and 16.

20. A vector comprising the nucleic acid molecules of claim **17, 18** or **19**.

21. A cell expressing the antibody of any one of claims **1-16**, or an antigen binding fragment thereof; or comprising the vector of claim **20**, provided, if the cell is a human cell, it is *ex vivo*.

22. An isolated or purified immunogenic polypeptide comprising an epitope comprising the amino acid sequence of any one of SEQ ID NOs: 37, 38, 39 or 40, that binds to the antibody of any one of claims **1-17**, or an antigen binding fragment thereof.

23. A pharmaceutical composition comprising the antibody of any one of claims **1-16**, or an antigen binding fragment thereof, the nucleic acid of claim **17, 18** or **19**, the vector of claim **20**, the cell of claim **21**, or the immunogenic polypeptide of claim **22**, and a pharmaceutically acceptable diluent or carrier.

24. A pharmaceutical composition comprising a first antibody or an antigen binding fragment thereof, and a second antibody, or an antigen binding fragment thereof, wherein the first antibody is the antibody of any one of claims **1-16**, and the second antibody neutralizes influenza A virus infection.

25. Use of the antibody of any one of claims **1-16**, or an antigen binding fragment thereof, the nucleic acid of claim **17, 18** or **19**, the vector of claim **20**, the cell of claim **21**, or the immunogenic polypeptide of claim **22**, or the pharmaceutical composition of claim **23** or **24** in the manufacture of a medicament (i) for the treatment of influenza A virus infection, (ii) for vaccination against influenza A virus, or (iii) for diagnosis of influenza A virus infection.

26. Use of the antibody of any one of claims **1-16**, or an antigen binding fragment thereof, for monitoring the quality of anti-influenza A virus vaccines by checking that the antigen of said vaccine contains the specific epitope in the correct conformation.

27. Use of the antibody of any one of claims **1-16**, or an antigen binding fragment thereof, in the manufacture of a medicament for reducing influenza A virus infection, or lowering the risk of influenza A virus infection.

28. An epitope comprising the amino acid sequence of any one of SEQ ID NOs: 37, 38, 39 or 40, which specifically binds to the antibody of any one of claims **1-16**, or an antigen binding fragment thereof, for use (i) in therapy, (ii) in the manufacture of a medicament for treating influenza A virus infection, (iii) as a vaccine, or (iv) in screening for ligands able to neutralize influenza A virus infection.