ANTI-HEMAGGLUTININ ANTIBODIES AND METHODS OF USE

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 61/725,859, filed on 13 November 2012, which is incorporated by reference herein in its entirety.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on October 31, 2013, is named P4982R1WO_PCTSequenceListing.txt and is 227,598 bytes in size.

FIELD OF THE INVENTION

The present invention provides anti-hemagglutinin antibodies, compositions comprising anti-hemagglutinin antibodies, and methods of using the same.

BACKGROUND

Influenza virus infection causes between three and five million cases of severe illness and between 250,000 and 500,000 deaths every year around the world. In the United States alone, 5% to 20% of the population becomes infected with influenza virus each year, with the majority of these infections caused by the influenza A virus. (See, *e.g.*, Dushoff *et al.*, (2006) Am J Epidemiology 163:181-187; Thompson *et al.*, (2004) JAMA 292:1333-1340; Thompson *et al.*, (2003) JAMA 289:179-186.) Approximately 200,000 people in the United States become hospitalized with influenza-related complications every year, resulting in 7,000 to 30,000 deaths annually. The burden associated with influenza virus infection on health care costs and lost productivity is extensive. Hospitalization and deaths mainly occur in high-risk groups, such as the elderly, children, and chronically ill.

Influenza viruses are segmented membrane-enveloped negative-strand RNA viruses belonging to the *Orthomyxoviridae* family. Influenza A virus consists of 9 structural proteins and 1 non-structural protein, which include three virus surface proteins: hemagglutinin (HA or H), neuraminidase (NA or N), and matrix protein 2 (M2). The segmented nature of the influenza viral genome allows the mechanism of genetic reassortment (*i.e.*, exchange of genome

segments) to take place during mixed infection of a cell with different influenza viral strains. Annual epidemics of influenza occur when the antigenic properties of the viral surface proteins hemagglutinin and neuraminidase are altered. The mechanism of altered antigenicity is twofold: antigenic shift, caused by genetic rearrangement between human and animal viruses after co- infection of host cells with at least two viral subtypes, which can cause a pandemic; and antigenic drift, caused by small changes in the hemagglutinin and neuraminidase proteins on the virus surface, which can cause influenza epidemics.

Influenza A viruses may be further classified into various subtypes depending on the different hemagglutinin and neuraminidase viral proteins displayed on their surface. Each influenza A virus subtype is identified by the combination of its hemagglutinin and neuraminidase proteins. There are 16 known HA subtypes (H1 – H16) and 9 known NA subtypes (N1 – N9). The 16 hemagglutinin subtypes are further classified into two phylogenetic groups: Group1 includes hemagglutinin H1, H2, H5, H6, H8, H9, H11, H12, H13, and H16 subtypes; Group2 includes hemagglutinin H3, H4, H7, H10, H14, and H15 subtypes.

Hemagglutinin promotes viral attachment and entry into the host cell; neuraminidase is required for viral budding from the infected cell. The hemagglutinin of influenza A virus comprises two structurally distinct regions - a globular head region and a stalk or stem region. The globular head region contains a receptor binding site which is responsible for virus attachment to a target cell. The stalk (or stem) region of hemagglutinin contains a fusion peptide which is necessary for membrane fusion between the viral envelope and an endosomal membrane of the infected cell. (See, *e.g.*, Bouvier and Palese (2008) Vaccine 26 Suppl 4: D49-53; Wiley *et al.*, (1987) Ann Rev Biochem 556:365-394.)

Current treatment for influenza virus infection includes neuraminidase inhibitors, such as oseltamivir and zanamivir. Oseltamivir is a widely used prophylactic and early therapeutic treatment option for influenza A virus infection. (See, *e.g.*, Kandel and Hartshorn (2001) BioDrugs: Clinical Immunotherapy, Biopharmaceuticals and Gene Therapy 15:303-323; Nicholson *et al.*, (2000) Lancet 355:1845-1850; Treanor *et al.*, (2000) JAMA 283:1016-1024; and Welliver *et al.*, (2001) JAMA 285:748-754.) However, oseltamivir treatment must begin within 48 hours of symptom onset to provide a significant clinical benefit. (See, *e.g.*, Aoki et al (2003) J Antimicrobial Chemotherapy 51:123-129.) This liability compromises oseltamivir's ability to treat severely ill patients, who are typically beyond the optimal 48-hour

treatment window at the time of seeking treatment. Therefore, significant focus has recently been placed on identifying influenza virus therapeutics to treat hospitalized influenza virus infected patients. One strategy has focused on development of human monoclonal antibodies (mAbs) that target a highly conserved epitope on the stalk of influenza A virus hemagglutinin. (See, *e.g.*, Corti et al., (2011) Science 333:850-856; Ekiert *et al.*, (2009) Science 324:246-251; Ekiert *et al.*, (2011) Science 333:843-850; Sui *et al.*, (2009) Nature Structural & Molecular Biology 16:265-273; Dreyfus *et al.*, (2012) Science 337:1343-1348; Wu *et al.*, (2012) J Virology 2012.09.034; Clementi *et al.*, (2011) PLoS One 6:1-10. See also International Patent Application Publication Nos: WO2009/115972, WO2011/117848, WO2008/110937, WO2010/010466, WO2008/028946, WO2010/130636, WO2012/021786, WO2010/073647, WO2011/160083, WO2011/111966, WO2002/46235, and WO2009/053604; U.S. Patent Nos: 5,631,350 and 5,589,174.)

Several reports have described monoclonal antibodies (mAb) that bind hemagglutinin and broadly neutralize influenza A virus. For example, Corti *et al.* (*supra*) described antibody FI6v3, which was cloned from a human plasma cell and shown to neutralize human influenza A viruses belonging to both Group1 and Group2 hemagglutinin subtypes. The FI6v3 mAb was discovered as a result of a heroic effort of analyzing approximately 104,000 human plasma cells. Additionally, Dreyfus *et al.* (*supra*) recently described the identification of antibody CR9114 by phage display panning; antibody CR9114 was shown to bind to a highly conserved stalk epitope shared between influenza A virus and influenza B virus hemagglutinin.

Despite these reports, a need still exists in the art for novel influenza A virus therapies effective against Group1 and Group2 influenza A virus subtypes. The present invention meets this need and provides other benefits for the treatment of influenza A virus infection.

SUMMARY OF THE INVENTION

The present invention provides anti-hemagglutinin antibodies, compositions comprising anti-hemagglutinin antibodies, and methods of using the same.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises three heavy chain hypervariable regions (HVR-H1, HVR-H2, and HVR-H3) and three light chain hypervariable regions (HVR-L1, HVR-L2, and HVR-L3), wherein:

(a) HVR-H1 comprises the amino acid sequence of SEQ ID NO:178;

(b) HVR-H2 comprises the amino acid sequence of SEQ ID NO:179;

(c) HVR-H3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:180 and 181;

(d) HVR-L1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:182, 183, 184, 185, and 186;

(e) HVR-L2 comprises the amino acid sequence of SEQ ID NO:187; and

(f) HVR-L3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:188, 189, and 190.

In some embodiments, the invention provides an isolated anti-hemagglutinin antibody comprising: at least one, two, three, four, five and/or six hypervariable region (HVR) sequences, wherein:

(a) HVR-H1 comprises the amino acid sequence of SEQ ID NO:178;

(b) HVR-H2 comprises the amino acid sequence of SEQ ID NO:179;

(c) HVR-H3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:180 and 181;

(d) HVR-L1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:182, 183, 184, 185, and 186;

(e) HVR-L2 comprises the amino acid sequence of SEQ ID NO:187; and

(f) HVR-L3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:188, 189, and 190.

In some embodiments, the invention provides an isolated anti-hemagglutinin antibody comprising three light chain hypervariable regions (HVR-L1, HVR-L2, and LVR-L3), wherein:

(a) HVR-L1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:182, 183, 184, 185, and 186;

(b) HVR-L2 comprises the amino acid sequence of SEQ ID NO:187; and

(c) HVR-L3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:188, 189, and 190.

In some embodiments, the invention provides an isolated anti-hemagglutinin antibody comprising three heavy chain hypervariable regions (HVR-H1, HVR-H2, and HVR-H3), wherein:

(a) HVR-H1 comprises the amino acid sequence of SEQ ID NO:178;

(b) HVR-H2 comprises the amino acid sequence of SEQ ID NO:179; and

(c) HVR-H3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:180 and 181.

In some embodiments, the invention provides an isolated anti-hemagglutinin antibody comprising: at least one, two, and/or three light chain hypervariable region (HVR) sequences, wherein:

(a) HVR-L1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:182, 183, 184, 185, and 186;

(b) HVR-L2 comprises the amino acid sequence of SEQ ID NO:187; and

(c) HVR-L3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:188, 189, and 190.

In some embodiments, the invention provides an isolated anti-hemagglutinin antibody comprising: at least one, two, and/or three heavy chain hypervariable region (HVR) sequences, wherein:

(a) HVR-H1 comprises the amino acid sequence of SEQ ID NO:178;

(b) HVR-H2 comprises the amino acid sequence of SEQ ID NO:179; and

(c) HVR-H3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:180 and 181.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:111 and 115, and the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:113, 117, 119, 122, 124, 126, 128, 130, and 132.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:113, 117, 119, 122, 124, 126, 128, 130, and 132.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises a heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:111 and 115.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises a heavy chain and a light chain, wherein the heavy chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:110, 114, and 120, and the light chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:112, 116, 118, 121, 123, 125, 127, 129, and 131.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises a light chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:112, 116, 118, 121, 123, 125, 127, 129, and 131.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:110, 114, and 120.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises three heavy chain hypervariable regions (HVR-H1, HVR-H2, and HVR-H3) and three light chain hypervariable regions (HVR-L1, HVR-L2, and HVR-L3), wherein:

(a) HVR-H1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:191 and 192;

(b) HVR-H2 comprises the amino acid sequence of SEQ ID NO:193;

(c) HVR-H3 comprises the amino acid sequence of SEQ ID NO:194;

(d) HVR-L1 comprises the amino acid sequence of SEQ ID NO:195;

(e) HVR-L2 comprises the amino acid sequence of SEQ ID NO:196; and

(f) HVR-L3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:197, 198, and 199.

In some embodiments, the invention provides an isolated anti-hemagglutinin antibody comprising: at least one, two, three, four, five and/or six hypervariable region (HVR) sequences, wherein:

(a) HVR-H1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:191 and 192;

(b) HVR-H2 comprises the amino acid sequence of SEQ ID NO:193;

(c) HVR-H3 comprises the amino acid sequence of SEQ ID NO:194;

(d) HVR-L1 comprises the amino acid sequence of SEQ ID NO:195;

(e) HVR-L2 comprises the amino acid sequence of SEQ ID NO:196; and

(f) HVR-L3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:197, 198, and 199.

In some embodiments, the invention provides an isolated anti-hemagglutinin antibody comprising three light chain hypervariable regions (HVR-L1, HVR-L2, and LVR-L3), wherein:

(a) HVR-L1 comprises the amino acid sequence of SEQ ID NO:195;

(b) HVR-L2 comprises the amino acid sequence of SEQ ID NO:196; and

(c) HVR-L3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:197, 198, and 199.

In some embodiments, the invention provides an isolated anti-hemagglutinin antibody comprising three heavy chain hypervariable regions (HVR-H1, HVR-H2, and HVR-H3), wherein:

(a) HVR-H1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:191 and 192;

(b) HVR-H2 comprises the amino acid sequence of SEQ ID NO:193; and

(c) HVR-H3 comprises the amino acid sequence of SEQ ID NO:194.

In some embodiments, the invention provides an isolated anti-hemagglutinin antibody comprising: at least one, two, and/or three light chain hypervariable region (HVR) sequences, wherein:

(a) HVR-L1 comprises the amino acid sequence of SEQ ID NO:195;

(b) HVR-L2 comprises the amino acid sequence of SEQ ID NO:196; and

(c) HVR-L3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:197, 198, and 199.

In some embodiments, the invention provides an isolated anti-hemagglutinin antibody comprising: at least one, two, and/or three heavy chain hypervariable region (HVR) sequences, wherein:

(a) HVR-H1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:191 and 192;

(b) HVR-H2 comprises the amino acid sequence of SEQ ID NO:193; and

(c) HVR-H3 comprises the amino acid sequence of SEQ ID NO:194.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:134, 138, 142, 148, and 234, and the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:136, 140, 144, 146, 150, 152, and 235.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 136, 140, 144, 146, 150, 152, and 235.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises a heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 134, 138, 142, 148, and 234.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises a heavy chain and a light chain, wherein the heavy chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:133, 137, 141, and 147, and the light chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:135, 139, 143, 145, 149, and 151.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises a light chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 135, 139, 143, 145, 149, and 151.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 133, 137, 141, and 147.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises three heavy chain hypervariable regions (HVR-H1, HVR-H2, and HVR-H3) and three light chain hypervariable regions (HVR-L1, HVR-L2, and HVR-L3), wherein:

(a) HVR-H1 comprises the amino acid sequence of SEQ ID NO:200;

(b) HVR-H2 comprises the amino acid sequence of SEQ ID NO:201;

(c) HVR-H3 comprises the amino acid sequence of SEQ ID NO:202;

(d) HVR-L1 comprises the amino acid sequence of SEQ ID NO:203;

(e) HVR-L2 comprises the amino acid sequence of SEQ ID NO:204; and

(f) HVR-L3 comprises the amino acid sequence of SEQ ID NO:205.

In some embodiments, the invention provides an isolated anti-hemagglutinin antibody comprising: at least one, two, three, four, five and/or six hypervariable region (HVR) sequences, wherein:

(a) HVR-H1 comprises the amino acid sequence of SEQ ID NO:200;

(b) HVR-H2 comprises the amino acid sequence of SEQ ID NO:201;

(c) HVR-H3 comprises the amino acid sequence of SEQ ID NO:202;

(d) HVR-L1 comprises the amino acid sequence of SEQ ID NO:203;

(e) HVR-L2 comprises the amino acid sequence of SEQ ID NO:204; and

(f) HVR-L3 comprises the amino acid sequence of SEQ ID NO:205.

In some embodiments, the invention provides an isolated anti-hemagglutinin antibody comprising three light chain hypervariable regions (HVR-L1, HVR-L2, and LVR-L3), wherein:

(a) HVR-L1 comprises the amino acid sequence of SEQ ID NO:203;

(b) HVR-L2 comprises the amino acid sequence of SEQ ID NO:204; and

(c) HVR-L3 comprises the amino acid sequence of SEQ ID NO:205.

In some embodiments, the invention provides an isolated anti-hemagglutinin antibody comprising three heavy chain hypervariable regions (HVR-H1, HVR-H2, and HVR-H3), wherein:

(a) HVR-H1 comprises the amino acid sequence of SEQ ID NO:200;

(b) HVR-H2 comprises the amino acid sequence of SEQ ID NO:201; and

(c) HVR-H3 comprises the amino acid sequence of SEQ ID NO:202.

In some embodiments, the invention provides an isolated anti-hemagglutinin antibody comprising: at least one, two, and/or three light chain hypervariable region (HVR) sequences, wherein:

(a) HVR-L1 comprises the amino acid sequence of SEQ ID NO:203;

(b) HVR-L2 comprises the amino acid sequence of SEQ ID NO:204; and

(c) HVR-L3 comprises the amino acid sequence of SEQ ID NO:205.

In some embodiments, the invention provides an isolated anti-hemagglutinin antibody comprising: at least one, two, and/or three heavy chain hypervariable region (HVR) sequences, wherein:

(a) HVR-H1 comprises the amino acid sequence of SEQ ID NO:200;

(b) HVR-H2 comprises the amino acid sequence of SEQ ID NO:201; and

(c) HVR-H3 comprises the amino acid sequence of SEQ ID NO:202.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:154 and 158, and the light chain variable region comprises the amino acid sequence of SEQ ID NO:156.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO:156.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises a heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 154 and 158.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises a heavy chain and a light chain, wherein the heavy chain comprises an amino acid

sequence selected from the group consisting of SEQ ID NOs:153 and 157, and the light chain comprises the amino acid sequence of SEQ ID NO:155.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises a light chain comprising the amino acid sequence of SEQ ID NO:155.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:153 and 157.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises three heavy chain hypervariable regions (HVR-H1, HVR-H2, and HVR-H3) and three light chain hypervariable regions (HVR-L1, HVR-L2, and HVR-L3), wherein:

(a) HVR-H1 comprises the amino acid sequence of SEQ ID NO:206;

(b) HVR-H2 comprises the amino acid sequence of SEQ ID NO:207;

(c) HVR-H3 comprises the amino acid sequence of SEQ ID NO:208;

(d) HVR-L1 comprises the amino acid sequence of SEQ ID NO:209;

(e) HVR-L2 comprises the amino acid sequence of SEQ ID NO:210; and

(f) HVR-L3 comprises the amino acid sequence of SEQ ID NO:211.

In some embodiments, the invention provides an isolated anti-hemagglutinin antibody comprising: at least one, two, three, four, five and/or six hypervariable region (HVR) sequences, wherein:

(a) HVR-H1 comprises the amino acid sequence of SEQ ID NO:206;

(b) HVR-H2 comprises the amino acid sequence of SEQ ID NO:207;

(c) HVR-H3 comprises the amino acid sequence of SEQ ID NO:208;

(d) HVR-L1 comprises the amino acid sequence of SEQ ID NO:209;

(e) HVR-L2 comprises the amino acid sequence of SEQ ID NO:210; and

(f) HVR-L3 comprises the amino acid sequence of SEQ ID NO:211.

In some embodiments, the invention provides an isolated anti-hemagglutinin antibody comprising three light chain hypervariable regions (HVR-L1, HVR-L2, and LVR-L3), wherein:

(a) HVR-L1 comprises the amino acid sequence of SEQ ID NO:209;

(b) HVR-L2 comprises the amino acid sequence of SEQ ID NO:210; and

(c) HVR-L3 comprises the amino acid sequence of SEQ ID NO:211.

In some embodiments, the invention provides an isolated anti-hemagglutinin antibody comprising three heavy chain hypervariable regions (HVR-H1, HVR-H2, and HVR-H3), wherein:

(a) HVR-H1 comprises the amino acid sequence of SEQ ID NO:206;

(b) HVR-H2 comprises the amino acid sequence of SEQ ID NO:207; and

(c) HVR-H3 comprises the amino acid sequence of SEQ ID NO:208.

In some embodiments, the invention provides an isolated anti-hemagglutinin antibody comprising: at least one, two, and/or three light chain hypervariable region (HVR) sequences, wherein:

(a) HVR-L1 comprises the amino acid sequence of SEQ ID NO:209;

(b) HVR-L2 comprises the amino acid sequence of SEQ ID NO:210; and

(c) HVR-L3 comprises the amino acid sequence of SEQ ID NO:211.

In some embodiments, the invention provides an isolated anti-hemagglutinin antibody comprising: at least one, two, and/or three heavy chain hypervariable region (HVR) sequences, wherein:

(a) HVR-H1 comprises the amino acid sequence of SEQ ID NO:206;

(b) HVR-H2 comprises the amino acid sequence of SEQ ID NO:207; and

(c) HVR-H3 comprises the amino acid sequence of SEQ ID NO:208.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:160, and the light chain variable region comprises the amino acid sequence of SEQ ID NO:162.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO:162.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises a heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 160.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises a heavy chain and a light chain, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO:159, and the light chain comprises the amino acid sequence of SEQ ID NO:161.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises a light chain comprising the amino acid sequence of SEQ ID NO:161.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:159.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises three heavy chain hypervariable regions (HVR-H1, HVR-H2, and HVR-H3) and three light chain hypervariable regions (HVR-L1, HVR-L2, and HVR-L3), wherein:

(a) HVR-H1 comprises the amino acid sequence of SEQ ID NO:212;

(b) HVR-H2 comprises the amino acid sequence of SEQ ID NO:213;

(c) HVR-H3 comprises the amino acid sequence of SEQ ID NO:214;

(d) HVR-L1 comprises the amino acid sequence of SEQ ID NO:215;

(e) HVR-L2 comprises the amino acid sequence of SEQ ID NO:216; and

(f) HVR-L3 comprises the amino acid sequence of SEQ ID NO:217.

In some embodiments, the invention provides an isolated anti-hemagglutinin antibody comprising: at least one, two, three, four, five and/or six hypervariable region (HVR) sequences, wherein:

(a) HVR-H1 comprises the amino acid sequence of SEQ ID NO:212;

(b) HVR-H2 comprises the amino acid sequence of SEQ ID NO:213;

(c) HVR-H3 comprises the amino acid sequence of SEQ ID NO:214;

(d) HVR-L1 comprises the amino acid sequence of SEQ ID NO:215;

(e) HVR-L2 comprises the amino acid sequence of SEQ ID NO:216; and

(f) HVR-L3 comprises the amino acid sequence of SEQ ID NO:217.

In some embodiments, the invention provides an isolated anti-hemagglutinin antibody comprising three light chain hypervariable regions (HVR-L1, HVR-L2, and LVR-L3), wherein:

(a) HVR-L1 comprises the amino acid sequence of SEQ ID NO:215;

(b) HVR-L2 comprises the amino acid sequence of SEQ ID NO:216; and

(c) HVR-L3 comprises the amino acid sequence of SEQ ID NO:217.

In some embodiments, the invention provides an isolated anti-hemagglutinin antibody comprising three heavy chain hypervariable regions (HVR-H1, HVR-H2, and HVR-H3), wherein:

(a) HVR-H1 comprises the amino acid sequence of SEQ ID NO:212;

(b) HVR-H2 comprises the amino acid sequence of SEQ ID NO:213; and

(c) HVR-H3 comprises the amino acid sequence of SEQ ID NO:214.

In some embodiments, the invention provides an isolated anti-hemagglutinin antibody comprising: at least one, two, and/or three light chain hypervariable region (HVR) sequences, wherein:

(a) HVR-L1 comprises the amino acid sequence of SEQ ID NO:215;

(b) HVR-L2 comprises the amino acid sequence of SEQ ID NO:216; and

(c) HVR-L3 comprises the amino acid sequence of SEQ ID NO:217.

In some embodiments, the invention provides an isolated anti-hemagglutinin antibody comprising: at least one, two, and/or three heavy chain hypervariable region (HVR) sequences, wherein:

(a) HVR-H1 comprises the amino acid sequence of SEQ ID NO:212;

(b) HVR-H2 comprises the amino acid sequence of SEQ ID NO:213; and

(c) HVR-H3 comprises the amino acid sequence of SEQ ID NO:214.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:164, and the light chain variable region comprises the amino acid sequence of SEQ ID NO:166.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO:166.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises a heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 164.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises a heavy chain and a light chain, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO:163, and the light chain comprises the amino acid sequence of SEQ ID NO:165.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises a light chain comprising the amino acid sequence of SEQ ID NO:165.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:163.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises three heavy chain hypervariable regions (HVR-H1, HVR-H2, and HVR-H3) and three light chain hypervariable regions (HVR-L1, HVR-L2, and HVR-L3), wherein:

(a) HVR-H1 comprises the amino acid sequence of SEQ ID NO:218;

(b) HVR-H2 comprises the amino acid sequence of SEQ ID NO:219;

(c) HVR-H3 comprises the amino acid sequence of SEQ ID NO:220;

(d) HVR-L1 comprises the amino acid sequence of SEQ ID NO:221;

(e) HVR-L2 comprises the amino acid sequence of SEQ ID NO:222; and

(f) HVR-L3 comprises the amino acid sequence of SEQ ID NO:223.

In some embodiments, the invention provides an isolated anti-hemagglutinin antibody comprising: at least one, two, three, four, five and/or six hypervariable region (HVR) sequences, wherein:

(a) HVR-H1 comprises the amino acid sequence of SEQ ID NO:218;

(b) HVR-H2 comprises the amino acid sequence of SEQ ID NO:219;

(c) HVR-H3 comprises the amino acid sequence of SEQ ID NO:220;

(d) HVR-L1 comprises the amino acid sequence of SEQ ID NO:221;

(e) HVR-L2 comprises the amino acid sequence of SEQ ID NO:222; and

(f) HVR-L3 comprises the amino acid sequence of SEQ ID NO:223.

In some embodiments, the invention provides an isolated anti-hemagglutinin antibody comprising three light chain hypervariable regions (HVR-L1, HVR-L2, and LVR-L3), wherein:

(a) HVR-L1 comprises the amino acid sequence of SEQ ID NO:221;

(b) HVR-L2 comprises the amino acid sequence of SEQ ID NO:222; and

(c) HVR-L3 comprises the amino acid sequence of SEQ ID NO:223.

In some embodiments, the invention provides an isolated anti-hemagglutinin antibody comprising three heavy chain hypervariable regions (HVR-H1, HVR-H2, and HVR-H3), wherein:

(a) HVR-H1 comprises the amino acid sequence of SEQ ID NO:218;

(b) HVR-H2 comprises the amino acid sequence of SEQ ID NO:219; and

(c) HVR-H3 comprises the amino acid sequence of SEQ ID NO:220.

In some embodiments, the invention provides an isolated anti-hemagglutinin antibody comprising: at least one, two, and/or three light chain hypervariable region (HVR) sequences, wherein:

(a) HVR-L1 comprises the amino acid sequence of SEQ ID NO:221;

(b) HVR-L2 comprises the amino acid sequence of SEQ ID NO:222; and

(c) HVR-L3 comprises the amino acid sequence of SEQ ID NO:223.

In some embodiments, the invention provides an isolated anti-hemagglutinin antibody comprising: at least one, two, and/or three heavy chain hypervariable region (HVR) sequences, wherein:

(a) HVR-H1 comprises the amino acid sequence of SEQ ID NO:218;

(b) HVR-H2 comprises the amino acid sequence of SEQ ID NO:219; and

(c) HVR-H3 comprises the amino acid sequence of SEQ ID NO:220.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises a heavy chain variable region and a light chain variable region, wherein the heavy

chain variable region comprises the amino acid sequence of SEQ ID NO:168, and the light chain variable region comprises the amino acid sequence of SEQ ID NO:170.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO:170.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises a heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 168.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises a heavy chain and a light chain, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO:167, and the light chain comprises the amino acid sequence of SEQ ID NO:169.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises a light chain comprising the amino acid sequence of SEQ ID NO:169.

In some embodiments, an isolated anti-hemagglutinin antibody of the present invention comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:167.

The invention also provides isolated nucleic acids encoding an anti-hemagglutinin antibody of the present invention. The invention also provides vectors comprising a nucleic acid encoding an anti-hemagglutinin antibody of the present invention. The invention also provides host cells comprising a nucleic acid or a vector of the present invention. A vector can be of any type, for example, a recombinant vector such as an expression vector. Any of a variety of host cells can be used. In one embodiment, a host cell is a prokaryotic cell, for example, *E. coli*. In another embodiment, a host cell is a eukaryotic cell, for example, a mammalian cell, such as a Chinese Hamster Ovary (CHO) cell.

The invention further provides a method of producing an anti-hemagglutinin antibody of the present invention. For example, the invention provides methods for making an anti-hemagglutinin antibody (which, as defined herein, includes full length antibody and fragments

thereof), the method comprising expressing in a suitable host cell a recombinant vector of the invention encoding the anti-hemagglutinin antibody or fragments thereof so that the antibody or fragments thereof are produced. In some embodiments, the method comprises culturing a host cell comprising nucleic acid encoding an anti-hemagglutinin antibody of the present invention (or fragments thereof) so that the nucleic acid is expressed. The method may further comprise recovering the anti-hemagglutinin antibody or fragments thereof from the host cell culture or the host cell culture medium.

The invention also provides a pharmaceutical formulation comprising an anti-hemagglutinin antibody of the present invention and a pharmaceutically acceptable carrier. The pharmaceutical formulation may further comprise an additional therapeutic agent (*e.g.*, a neuraminidase inhibitor, such as oseltamivir or zanamivir; another antibody, such as another anti-hemagglutinin antibody or an anti-M2 antibody; etc).

The invention also provides compositions comprising an anti-hemagglutinin antibody of the present invention. The composition may further comprise an additional therapeutic agent (*e.g.*, a neuraminidase inhibitor, such as oseltamivir or zanamivir; another antibody, such as another anti-hemagglutinin antibody or an anti-M2 antibody; etc).

The invention also provides a composition comprising an anti-hemagglutinin antibody of the present invention for use in preventing influenza A virus infection. In some embodiments, the invention provides a pharmaceutical composition comprising an anti-hemagglutinin antibody of the present invention for use in preventing influenza A virus infection. The invention further provides a composition comprising an anti-hemagglutinin antibody of the present invention for use in treating influenza A virus infection. In some embodiments, the invention provides a pharmaceutical composition comprising an anti-hemagglutinin antibody of the present invention for use in treating influenza A virus infection. The invention further provides a composition comprising an anti-hemagglutinin antibody of the present invention for use in inhibiting influenza A virus infection. In some embodiments, the invention provides a pharmaceutical composition comprising an anti-hemagglutinin antibody of the present invention for use in inhibiting influenza A virus infection.

Compositions comprising an anti-hemagglutinin antibody of the present invention may also be used in the manufacture of a medicament. The medicament may be for use in the inhibition,

treatment, or prevention of influenza A virus infection. In certain embodiments, the medicament may further comprise an additional therapeutic agent (*e.g.*, a neuraminidase inhibitor, such as oseltamivir or zanamivir; another antibody, such as another anti-hemagglutinin antibody or an anti-M2 antibody; etc).

The invention also provides a method for inhibiting influenza A virus infection, the method comprising administering to a patient in need thereof an effective amount of a composition comprising an anti-hemagglutinin antibody of the present invention, thereby inhibiting influenza A virus infection. The invention also provides a method for treating influenza A virus infection, the method comprising administering to a patient in need thereof an effective amount of a composition comprising an anti-hemagglutinin antibody of the present invention, thereby treating influenza A virus infection. The invention also provides a method for preventing influenza A virus infection, the method comprising administering to a patient in need thereof an effective amount of a composition comprising an anti-hemagglutinin antibody of the present invention, thereby preventing influenza A virus infection.

The invention also provides a method for inhibiting, treating, or preventing influenza A virus infection, the method comprising administering to a patient in need thereof an effective amount of a composition comprising an anti-hemagglutinin antibody of the present invention, and administering to the patient an effective amount of an additional therapeutic agent, thereby inhibiting, treating, or preventing influenza A virus infection. In some embodiments, the additional therapeutic agent is a neuraminidase inhibitor, such as oseltamivir or zanamivir. In other embodiments, the additional therapeutic agent is another anti-hemagglutinin antibody. In yet other embodiments, the additional therapeutic agent is an anti-M2 antibody. In various aspects of such combination treatments, the therapeutic agents are administered at about the same time, are administered together, or are administered sequentially or consecutively. In particular embodiments, an anti-neuraminidase inhibitor is administered prior to the administration of an anti-hemagglutinin antibody of the present invention.

In another aspect, the invention provides use of an anti-hemagglutinin antibody of the present invention in the manufacture of a medicament. The medicament may be for use in the inhibition, treatment, or prevention of influenza A virus infection. In certain embodiments, the medicament may further comprise an additional therapeutic agent (*e.g.*, a neuraminidase

inhibitor, such as oseltamivir or zanamivir; another antibody, such as another anti-hemagglutinin antibody or an anti-M2 antibody; etc).

In another aspect, the invention provides use of a nucleic acid of the invention in the manufacture of a medicament. The medicament may be for use in the inhibition, treatment, or prevention of influenza A virus infection. In certain embodiments, the medicament may further comprise an additional therapeutic agent (*e.g.*, a neuraminidase inhibitor, such as oseltamivir or zanamivir; another antibody, such as another anti-hemagglutinin antibody or an anti-M2 antibody; etc).

In another aspect, the invention provides use of an expression vector of the invention in the manufacture of a medicament. The medicament may be for use in the inhibition, treatment, or prevention of influenza A virus infection. In certain embodiments, the medicament may further comprise an additional therapeutic agent (*e.g.*, a neuraminidase inhibitor, such as oseltamivir or zanamivir; another antibody, such as another anti-hemagglutinin antibody or an anti-M2 antibody; etc).

In another aspect, the invention provides use of a host cell of the invention in the manufacture of a medicament. The medicament may be for use in the inhibition, treatment, or prevention of influenza A virus infection. In certain embodiments, the medicament may further comprise an additional therapeutic agent (*e.g.*, a neuraminidase inhibitor, such as oseltamivir or zanamivir; another antibody, such as another anti-hemagglutinin antibody or an anti-M2 antibody; etc).

In another aspect, the invention provides use of an article of manufacture of the invention in the manufacture of a medicament. The medicament may be for use in the inhibition, treatment, or prevention of influenza A virus infection. In certain embodiments, the medicament may further comprise an additional therapeutic agent (*e.g.*, a neuraminidase inhibitor, such as oseltamivir or zanamivir; another antibody, such as another anti-hemagglutinin antibody or an anti-M2 antibody; etc).

In another aspect, the invention provides use of a kit of the invention in the manufacture of a medicament. The medicament may be for use in the inhibition, treatment, or prevention of influenza A virus infection. In certain embodiments, the medicament may further comprise an

additional therapeutic agent (*e.g.*, a neuraminidase inhibitor, such as oseltamivir or zanamivir; another antibody, such as another anti-hemagglutinin antibody or an anti-M2 antibody; etc).

In various aspects, an anti-hemagglutinin antibody of the present invention binds hemagglutinin. In some aspects, an anti-hemagglutinin antibody of the present invention binds Group1 hemagglutinin, binds Group2 hemagglutinin, or binds Group1 and Group2 hemagglutinin. In other aspects, an anti-hemagglutinin antibody of the present invention binds hemagglutinin and neutralizes influenza A virus. In some embodiments, an anti-hemagglutinin antibody of the present invention neutralizes influenza A virus *in vitro*, *in vivo*, or *in vitro* and *in vivo*.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A and1B sets forth data showing FACS analysis of anti-hemagglutinin-positive (hemagglutinin H3+ and hemagglutinin H1+) plasmablasts from day 7 post-vaccinated human peripheral blood mononuclear cells (PBMCs) prior to SCID/beige mouse enrichment (Figure 1A) and day 8 post-intrasplenic implantation after SCID/beige mouse enrichment with and without antigen premix (Figure 1B) in the upper and lower panels, respectively.

Figure 2 sets forth data showing analysis of splenocytes obtained from day 8 post-intrasplenic implantation of PBMCs from individual SCID/beige mice with no PBMC/antigen premix (circles) and with PBMC/antigen premix (squares), as percent hemagglutinin $(H1)^{+}/CD38^{high}$ plasmablasts. The rectangle indicates mice that presented hemagglutinin $H1^{+}$ plasmablasts.

Figure 3 sets for data showing *in vitro* neutralization of various influenza A Group1 and Group2 virus strains by anti-hemagglutinin antibodies of the present invention.

Figures 4A and 4B set forth data showing *in vitro* neutralization of various influenza A Group1 (Figure 4A) and Group2 (Figure 4B) virus strains by monoclonal antibody 39.29 NWPP ("NWPP" disclosed as SEQ ID NO: 177).

Figures 5A and 5B set forth data showing *in vitro* neutralization of various influenza A Group1 (Figure 5A) and Group2 (Figure 5B) virus strains by monoclonal antibody 81.39 SVSH-NYP ("SVSH" disclosed as SEQ ID NO: 171).

Figure 6 sets forth data showing *in vitro* neutralization of various influenza A Group1 virus strains by monoclonal antibody 39.18 B11.

Figure 7 sets forth data showing *in vitro* neutralization of various influenza A Group1 and Group2 virus strains by monoclonal antibody 36.89.

Figure 8 sets forth data showing *in vitro* neutralization of various influenza A Group1 and Group2 virus strains by monoclonal antibody mAb9 01F3.

Figure 9 sets forth data showing *in vitro* neutralization of various influenza A Group 1 and Group2 virus strains by monoclonal antibody mAb23 06C2.

Figure 10 sets forth data showing *in vitro* neutralization of an hemagglutinin H5-expressing pseudovirus by monoclonal antibody 39.29 NCv1.

Figure 11 sets forth data showing *in vitro* neutralization of an H7N7 equine influenza virus by monoclonal antibody 39.29 NWPP ("NWPP" disclosed as SEQ ID NO: 177).

Figures 12A, 12B, 12C, and 12D set forth data showing percent survival of mice infected with various influenza A virus strains (A/PR/8/1934 (PR8), Figure 12A; A/Port Chalmers/1/1973 (PC73), Figure 12B; A/Hong Kong/1/1968 (HK68), Figure 12C); and A/Aichi/2/1968 (Aichi68), Figure 12D) and administered various amounts of monoclonal antibody 39.29 NWPP ("NWPP" disclosed as SEQ ID NO: 177).

Figure 13 sets forth data showing percent survival of mice infected with A/PR/8/1934 influenza A virus and administered various amounts of monoclonal antibody 39.29 NCv1.

Figure 14 sets forth data showing percent survival of mice infected with A/Hong Kong/1/1968 influenza A virus (an influenza A virus having a high IC50) and administered various amounts of monoclonal antibody 39.29 NCv1.

Figure 15 sets forth data showing percent survival of mice infected with A/Port Chalmers/1/1973 influenza A virus and administered various amounts of monoclonal antibody 39.29 NCv1.

Figure 16 sets forth data showing percent survival of mice infected with A/Aichi/2/1968 influenza A virus and administered various amounts of monoclonal antibody 39.29 NCv1.

Figure 17 sets forth data comparing percent survival of mice infected with influenza A virus strain A/PR/8/1934 and administered a 50:50 mixture of monoclonal antibody 39.29 D8C2 and monoclonal antibody 39.29 NWPP ("NWPP" disclosed as SEQ ID NO: 177) or oseltamivir (Tamiflu®).

Figure 18 sets forth data showing comparing percent survival of mice infected with influenza A virus strain A/PR/8/1934 and administered monoclonal antibody 39.29 NWPP ("NWPP" disclosed as SEQ ID NO: 177), oseltamivir (Tamiflu®), or a combination of monoclonal antibody 39.29 NWPP ("NWPP" disclosed as SEQ ID NO: 177) and oseltamivir.

Figures 19A and 19B set for data comparing percent survival of ferrets infected with influenza A virus strain A/Vietnam/1203/04 (H5N1) and administered monoclonal antibody 39.29 D8C2 (Figure 19A), monoclonal antibody 81.39 B1C1 (Figure 19B), or oseltamivir (Tamiflu®) at 48 hours or 72 hours post-infection.

Figure 20 shows an amino acid sequence alignment of hemagglutinin amino acid sequences from hemagglutinin H1, H2, H3, H5 and H7, showing hemagglutinin contact residues (shaded) of monoclonal antibody 39.29NCv1 and the hemagglutinin binding epitope.

Figures 21A and 21B set forth data from competition ELISA experiments of various monoclonal antibodies of the present invention competing with binding of biotin-labeled monoclonal antibody 39.29 to hemagglutinin H1 from A/NWS/1933 (Figure 21A) and hemagglutinin H3 from A/HK/8/1968 (Figure 21B).

Figures 22A and 22B show an amino acid sequence alignment of the light chain variable region and the heavy chain variable region of monoclonal antibody 81.39 B1C1 (SEQ ID NOs:113 and 111, respectively) with the immunoglobulin kappa variable 3-15*01 germ-line (IGKV3-15*01) and the immunoglobulin heavy chain variable 3-30*01 germ-line (IGHV3-30*01) (SEQ ID NOs:236 and 237, respectively). The amino acids are numbers according to Kabat numbering. The Kabat, Chothia, and Contact CDRs are indicated.

Figures 23A and 23B show an amino acid sequence alignment of the light chain variable region and the heavy chain variable region of monoclonal antibody 81.39 SVSH-NYP ("SVSH" disclosed as SEQ ID NO: 171) (SEQ ID NOs:117 and 115, respectively) with immunoglobulin kappa variable 3-15*01 germ-line (IGKV3-15*01) and the immunoglobulin heavy chain variable 3-30*01 germ-line (IGHV3-30*01) (SEQ ID NOs:236 and 237, respectively). The amino acids are numbers according to Kabat numbering. The Kabat, Chothia, and Contact CDRs are indicated.

Figures 24A and 24B show an amino acid sequence alignment of the light chain variable region and the heavy chain variable region of monoclonal antibody 81.39 B1F1 (SEQ ID NOs:119 and 111, respectively) with the immunoglobulin kappa variable 3-15*01 germ-line (IGKV3-15*01) and the immunoglobulin heavy chain variable 3-30*01 germ-line (IGHV3-30*01) (SEQ ID NOs:236 and 237, respectively). The amino acids are numbers according to Kabat numbering. The Kabat, Chothia, and Contact CDRs are indicated.

Figures 25A and 25B show an amino acid sequence alignment of the light chain variable region and the heavy chain variable region of monoclonal antibody 81.39 SVDS ("SVDS" disclosed as SEQ ID NO: 172) (SEQ ID NOs:113 and 115, respectively) with the immunoglobulin kappa variable 3-15*01 germ-line (IGKV3-15*01) and the immunoglobulin heavy chain variable 3-30*01 germ-line (IGHV3-30*01) (SEQ ID NOs:236 and 237, respectively). The amino acids are numbers according to Kabat numbering. The Kabat, Chothia, and Contact CDRs are indicated.

Figures 26A and 26B show an amino acid sequence alignment of the light chain variable region and the heavy chain variable region of monoclonal antibody 81.39 SVSS ("SVSS" disclosed as SEQ ID NO: 173) (SEQ ID NOs:122 and 115, respectively) with the immunoglobulin kappa variable 3-15*01 germ-line (IGKV3-15*01) and the immunoglobulin heavy chain variable 3-30*01 germ-line (IGHV3-30*01) (SEQ ID NOs:236 and 237, respectively). The amino acids are numbers according to Kabat numbering. The Kabat, Chothia, and Contact CDRs are indicated.

Figures 27A and 27B show an amino acid sequence alignment of the light chain variable region and the heavy chain variable region of monoclonal antibody 81.39 SVDH ("SVDH" disclosed

as SEQ ID NO: 174) (SEQ ID NOs:124 and 115, respectively) with the immunoglobulin kappa variable 3-15*01 germ-line (IGKV3-15*01) and the immunoglobulin heavy chain variable 3-30*01 germ-line (IGHV3-30*01) (SEQ ID NOs:236 and 237, respectively). The amino acids are numbers according to Kabat numbering. The Kabat, Chothia, and Contact CDRs are indicated.

Figures 28A and 28B show an amino acid sequence alignment of the light chain variable region and the heavy chain variable region of mAb 81.39 SVSH ("SVSH" disclosed as SEQ ID NO: 171) (SEQ ID NOs:126 and 115, respectively) with the immunoglobulin kappa variable 3-15*01 germ-line (IGKV3-15*01) and the immunoglobulin heavy chain variable 3-30*01 germ-line (IGHV3-30*01) (SEQ ID NOs:236 and 237, respectively). The amino acids are numbers according to Kabat numbering. The Kabat, Chothia, and Contact CDRs are indicated.

Figures 29A and 29B show an amino acid sequence alignment of the light chain variable region and the heavy chain variable region of monoclonal antibody 81.39 SVSH.NFP ("SVSH" disclosed as SEQ ID NO: 171) (SEQ ID NOs:128 and 115, respectively) with the immunoglobulin kappa variable 3-15*01 germ-line (IGKV3-15*01) and the immunoglobulin heavy chain variable 3-30*01 germ-line (IGHV3-30*01) (SEQ ID NOs:236 and 237, respectively). The amino acids are numbers according to Kabat numbering. The Kabat, Chothia, and Contact CDRs are indicated.

Figures 30A and 30B show an amino acid sequence alignment of the light chain variable region and the heavy chain variable region of monoclonal antibody 81.39 SVDS.F ("SVDS" disclosed as SEQ ID NO: 172) (SEQ ID NOs:130 and 115, respectively) with the immunoglobulin kappa variable 3-15*01 germ-line (IGKV3-15*01) and the immunoglobulin heavy chain variable 3-30*01 germ-line (IGHV3-30*01) (SEQ ID NOs:236 and 237, respectively). The amino acids are numbers according to Kabat numbering. The Kabat, Chothia, and Contact CDRs are indicated.

Figures 31A and 31B show an amino acid sequence alignment of the light chain variable region and the heavy chain variable region of monoclonal antibody 81.39 SVDS.Y ("SVDS" disclosed as SEQ ID NO: 172) (SEQ ID NOs:132 and 115, respectively) with the immunoglobulin kappa variable 3-15*01 germ-line (IGKV3-15*01) and the immunoglobulin heavy chain variable 3-30*01 germ-line (IGHV3-30*01) (SEQ ID NOs:236 and 237, respectively). The amino acids

are numbers according to Kabat numbering. The Kabat, Chothia, and Contact CDRs are indicated.

Figures 32A and 32B show an amino acid sequence alignment of the light chain variable region and the heavy chain variable region of monoclonal antibody 39.29 D2C4 (SEQ ID NOs:136 and 134, respectively) with the immunoglobulin kappa variable 3-15*01 germ-line (IGKV3-15*01) and the immunoglobulin heavy chain variable 3-30*01 germ-line (IGHV3-30*01) (SEQ ID NOs:236 and 245, respectively). The amino acids are numbers according to Kabat numbering. The Kabat, Chothia, and Contact CDRs are indicated.

Figures 33A and 33B show an amino acid sequence alignment of the light chain variable region and the heavy chain variable region of monoclonal antibody 39.29 D8C2 (SEQ ID NOs:140 and 138, respectively) with the immunoglobulin kappa variable 3-15*01 germ-line (IGKV3-15*01) and the immunoglobulin heavy chain variable 3-30*01 germ-line (IGHV3-30*01) (SEQ ID NOs:236 and 245, respectively). The amino acids are numbers according to Kabat numbering. The Kabat, Chothia, and Contact CDRs are indicated.

Figures 34A and 34B show an amino acid sequence alignment of the light chain variable region and the heavy chain variable region of monoclonal antibody 39.29 NCv1 (SEQ ID NOs:144 and 142, respectively) with the immunoglobulin kappa variable 3-15*01 germ-line (IGKV3-15*01) and the immunoglobulin heavy chain variable 3-30*01 germ-line (IGHV3-30*01) (SEQ ID NOs:236 and 245, respectively). The amino acids are numbers according to Kabat numbering. The Kabat, Chothia, and Contact CDRs are indicated.

Figures 35A and 35B show an amino acid sequence alignment of the light chain variable region and the heavy chain variable region of monoclonal antibody 39.29 D8E7 (SEQ ID NOs:146 and 138, respectively) with the immunoglobulin kappa variable 3-15*01 germ-line (IGKV3-15*01) and the immunoglobulin heavy chain variable 3-30*01 germ-line (IGHV3-30*01) (SEQ ID NOs:236 and 245, respectively). The amino acids are numbers according to Kabat numbering. The Kabat, Chothia, and Contact CDRs are indicated.

Figures 36A and 36B show an amino acid sequence alignment of the light chain variable region and the heavy chain variable region of monoclonal antibody 39.29 NFPP ("NFPP" disclosed as SEQ ID NO: 175) (SEQ ID NOs:150 and 148, respectively) with the immunoglobulin kappa

variable 3-15*01 germ-line (IGKV3-15*01) and the immunoglobulin heavy chain variable 3-30*01 germ-line (IGHV3-30*01) (SEQ ID NOs:236 and 245, respectively). The amino acids are numbers according to Kabat numbering. The Kabat, Chothia, and Contact CDRs are indicated.

Figures 37A and 37B show an amino acid sequence alignment of the light chain variable region and the heavy chain variable region of monoclonal antibody 39.29 NYPP ("NYPP" disclosed as SEQ ID NO: 176) (SEQ ID NOs:152 and 148, respectively) with the immunoglobulin kappa variable 3-15*01 germ-line (IGKV3-15*01) and the immunoglobulin heavy chain variable 3-30*01 germ-line (IGHV3-30*01) (SEQ ID NOs:236 and 245, respectively). The amino acids are numbers according to Kabat numbering. The Kabat, Chothia, and Contact CDRs are indicated.

Figures 38A and 38B show an amino acid sequence alignment of the light chain variable region and the heavy chain variable region of monoclonal antibody 39.29 NWPP ("NWPP" disclosed as SEQ ID NO: 177) (SEQ ID NOs:235 and 234, respectively) with the immunoglobulin kappa variable 3-15*01 germ-line (IGKV3-15*01) and the immunoglobulin heavy chain variable 3-30*01 germ-line (IGHV3-30*01) (SEQ ID NOs:236 and 245, respectively). The amino acids are numbers according to Kabat numbering. The Kabat, Chothia, and Contact CDRs are indicated.

Figures 39A and 39B show an amino acid sequence alignment of the light chain variable region and the heavy chain variable region of monoclonal antibody 39.18 B11 (SEQ ID NOs:156 and 154, respectively) with the immunoglobulin kappa variable 3-15*01 germ-line (IGKV3-15*01) and the immunoglobulin heavy chain variable 1-69*01 germ-line (IGHV1-69*01) (SEQ ID NOs:236 and 238, respectively). The amino acids are numbers according to Kabat numbering. The Kabat, Chothia, and Contact CDRs are indicated.

Figures 40A and 40B show an amino acid sequence alignment of the light chain variable region and the heavy chain variable region of monoclonal antibody 39.18 E12 (SEQ ID NOs:156 and 158, respectively) with the immunoglobulin kappa variable 3-15*01 germ-line (IGKV3-15*01) and the immunoglobulin heavy chain variable 1-69*01 germ-line (IGHV1-69*01) (SEQ ID NOs:236 and 238, respectively). The amino acids are numbers according to Kabat numbering. The Kabat, Chothia, and Contact CDRs are indicated.

Figures 41A and 41B show an amino acid sequence alignment of the light chain variable region and the heavy chain variable region of monoclonal antibody 36.89 (SEQ ID NOs:162 and 160, respectively) with the immunoglobulin kappa variable 1-5*03 germ-line (IGKV1-5*03) and the immunoglobulin heavy chain variable 1-18*01 germ-line (IGHV1-18*01) (SEQ ID NOs:239 and 240, respectively). The amino acids are numbers according to Kabat numbering. The Kabat, Chothia, and Contact CDRs are indicated.

Figures 42A and 42B show an amino acid sequence alignment of the light chain variable region and the heavy chain variable region of monoclonal antibody 9.01F3 (SEQ ID NOs:166 and 164, respectively) with the immunoglobulin light variable 1-44*01 germ-line (IGKV1-44*01) and the immunoglobulin heavy chain variable 1-2*02*01 germ-line (IGHV1-2*02) (SEQ ID NOs:241 and 242, respectively). The amino acids are numbers according to Kabat numbering. The Kabat, Chothia, and Contact CDRs are indicated.

Figures 43A and 43B show an amino acid sequence alignment of the light chain variable region and the heavy chain variable region of monoclonal antibody 23.06C2 (SEQ ID NOs:170 and 168, respectively) with the immunoglobulin kappa variable 2-30*01 germ-line (IGKV2-30*01) and the immunoglobulin heavy chain variable 4-39*01 germ-line (IGHV4-39*01) (SEQ ID NOs:243 and 244, respectively). The amino acids are numbers according to Kabat numbering. The Kabat, Chothia, and Contact CDRs are indicated.

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

I. DEFINITIONS

An "acceptor human framework" for the purposes herein is a framework comprising the amino acid sequence of a light chain variable domain (VL) framework or a heavy chain variable domain (VH) framework derived from a human immunoglobulin framework or a human consensus framework, as defined below. An acceptor human framework "derived from" a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain amino acid sequence changes. In some embodiments, the number of amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. In some embodiments, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

"Affinity" refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (*e.g.*, an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (*e.g.*, antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (Kd). Affinity can be measured by common methods known in the art, including those described herein. Specific illustrative and exemplary embodiments for measuring binding affinity are described in the following.

An "affinity matured" antibody refers to an antibody with one or more alterations in one or more hypervariable regions (HVRs), compared to a parent antibody which does not possess such alterations, such alterations resulting in an improvement in the affinity of the antibody for antigen.

The terms "anti-hemagglutinin antibody" and "an antibody that binds to hemagglutinin" refer to an antibody that binds hemagglutinin with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting hemagglutinin, including targeting hemagglutinin of influenza virus. In one embodiment, the extent of binding of an anti-hemagglutinin antibody to an unrelated, non-hemagglutinin protein is less than about 10% of the binding of the antibody to hemagglutinin as measured, *e.g.*, by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to hemagglutinin has a dissociation constant (Kd) of $\leq$ 1μM, $\leq$ 100 nM, $\leq$ 10 nM, $\leq$ 1 nM, $\leq$ 0.1 nM, $\leq$ 0.01 nM, or $\leq$ 0.001 nM (*e.g.*, 10^{-8} M or less, *e.g.*, from 10^{-8} M to 10^{-13} M, *e.g.*, from 10^{-9} M to 10^{-13} M). In certain embodiments, an anti-hemagglutinin antibody binds to an epitope of hemagglutinin that is conserved among hemagglutinin from different strains, subtypes, and isolates of influenza A viruses.

The term "antibody" herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity.

An "antibody fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. An

antibody fragment also refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds hemagglutinin and neutralizes influenza A virus. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, $F(ab')_2$; diabodies; linear antibodies; single-chain antibody molecules (*e.g.*, scFv); and multispecific antibodies formed from antibody fragments.

An "antibody that binds to the same epitope" as a reference antibody refers to an antibody that blocks binding of the reference antibody to its antigen in a competition assay by 50% or more, and conversely, the reference antibody blocks binding of the antibody to its antigen in a competition assay by 50% or more. An exemplary competition assay is provided herein.

The term "chimeric" antibody refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species.

The "class" of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ε, γ, and μ, respectively.

The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents a cellular function and/or causes cell death or destruction. Cytotoxic agents include, but are not limited to, radioactive isotopes (*e.g.*, At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu); chemotherapeutic agents or drugs (e.g., methotrexate, adriamicin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents); growth inhibitory agents; enzymes and fragments thereof such as nucleolytic enzymes; antibiotics; toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof; and the various antitumor or anticancer agents disclosed below.

"Effector functions" refer to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (*e.g.*, B cell receptor); and B cell activation.

An "effective amount" of an agent, *e.g.*, a pharmaceutical formulation, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result.

The term "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. In one embodiment, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991.

"Framework" or "FR" refers to variable domain residues other than hypervariable region (HVR) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following sequence in VH (or VL): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

The terms "full length antibody," "intact antibody," and "whole antibody" are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or having heavy chains that contain an Fc region as defined herein.

The terms "host cell," "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may

contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

A "human antibody" is an antibody which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

A "human consensus framework" is a framework which represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, Fifth Edition, NIH Publication 91-3242, Bethesda MD (1991), vols. 1-3. In one embodiment, for the VL, the subgroup is subgroup kappa I as in Kabat et al., *supra.* In one embodiment, for the VH, the subgroup is subgroup III as in Kabat *et al., supra*.

A "humanized" antibody refers to a chimeric antibody comprising amino acid residues from non-human HVRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (*e.g.*, CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A "humanized form" of an antibody, *e.g.*, a non-human antibody, refers to an antibody that has undergone humanization.

The term "hypervariable region" or "HVR" as used herein refers to each of the regions of an antibody variable domain which are hypervariable in sequence ("complementarity determining regions" or "CDRs") and/or form structurally defined loops ("hypervariable loops") and/or contain the antigen-contacting residues ("antigen contacts"). Generally, antibodies comprise six HVRs: three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). Exemplary HVRs herein include:

(a) hypervariable loops occurring at amino acid residues 26-32 (L1), 50-52 (L2), 91-96 (L3), 26-32 (H1), 53-55 (H2), and 96-101 (H3) (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987));

(b) CDRs occurring at amino acid residues 24-34 (L1), 50-56 (L2), 89-97 (L3), 31-35b (H1), 50-65 (H2), and 95-102 (H3) (Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991));

(c) antigen contacts occurring at amino acid residues 27c-36 (L1), 46-55 (L2), 89-96 (L3), 30-35b (H1), 47-58 (H2), and 93-101 (H3) (MacCallum et al. *J. Mol. Biol.* 262: 732-745 (1996)); and

(d) combinations of (a), (b), and/or (c), including HVR amino acid residues 46-56 (L2), 47-56 (L2), 48-56 (L2), 49-56 (L2), 26-35 (H1), 26-35b (H1), 49-65 (H2), 93-102 (H3), and 94-102 (H3).

Unless otherwise indicated, HVR residues and other residues in the variable domain (*e.g.*, FR residues) are numbered herein according to Kabat *et al., supra*.

An "immunoconjugate" is an antibody conjugated to one or more heterologous molecule(s), including but not limited to a cytotoxic agent.

An "individual" or "subject" is a mammal. Mammals include, but are not limited to, domesticated animals (*e.g.*, cows, sheep, cats, dogs, and horses), primates (*e.g.*, humans and non-human primates such as monkeys), rabbits, and rodents (*e.g.*, mice and rats). In certain embodiments, the individual or subject is a human.

An "isolated" antibody is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (*e.g.*, SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (e.g., ion exchange or reverse phase HPLC). For review of methods for assessment of antibody purity, see, *e.g.*, Flatman et al., *J. Chromatogr. B* 848:79-87 (2007).

An "isolated" nucleic acid refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic

acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

"Isolated nucleic acid encoding an anti-hemagglutinin antibody" refers to one or more nucleic acid molecules encoding antibody heavy and light chains (or fragments thereof), including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, *e.g.*, containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, the modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein.

A "naked antibody" refers to an antibody that is not conjugated to a heterologous moiety (*e.g.*, a cytotoxic moiety) or radiolabel. The naked antibody may be present in a pharmaceutical formulation.

"Native antibodies" refer to naturally occurring immunoglobulin molecules with varying structures. For example, native IgG antibodies are heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light chains and two identical heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant

domains (CH1, CH2, and CH3). Similarly, from N- to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a light chain variable domain, followed by a constant light (CL) domain. The light chain of an antibody may be assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain.

The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

"Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, California, or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or

comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

100 times the fraction X/Y

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject., A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

The term "hemagglutinin," as used herein, refers to any native hemagglutinin from any influenza virus source, unless otherwise indicated. The term encompasses "full-length," unprocessed hemagglutinin as well as any form of hemagglutinin that results from processing in an influenza virus or an influenza virus-infected cell. The term also encompasses naturally occurring variants of hemagglutinin, *e.g.*, splice variants or allelic variants. The amino acid sequences of exemplary hemagglutinin proteins from various influenza A virus strains are shown in SEQ ID NOs:225 (H2 from A/Japan/305/1957), 226 (H3 from A/Perth/16/2009), 227 (H5 from A/Vietnam/1203/2004), 228 (H7 from A/chicken/NSW/1/1997), 229 (H1 from A/California/07/2009), 230 (H1 from A/NSW/1933), 231 (H3 from A/Hong Kong/8/1968), 232 (H7 from A/Netherlands/219/2003), and 233 (A/South Carolina/1918).

As used herein, "treatment" (and grammatical variations thereof such as "treat" or "treating") refers to clinical intervention in an attempt to alter the natural course of the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease (e.g., preventing occurrence or recurrence of influenza A virus infection), reduction (e.g., reducing) or alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, antibodies of the invention are used to delay development of a disease or to slow the progression of a disease.

The term "variable region" or "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). (See, e.g., Kindt et al. *Kuby Immunology*, 6th ed., W.H. Freeman and Co., page 91 (2007).) A single VH or VL domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a VH or VL domain from an antibody that binds the antigen to screen a library of complementary VL or VH domains, respectively. See, *e.g.*, Portolano et al., *J. Immunol.* 150:880-887 (1993); Clarkson et al., *Nature* 352:624-628 (1991).

The term "vector," as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors."

II. COMPOSITIONS AND METHODS

In one aspect, the invention is based, in part, on anti-hemagglutinin antibodies and uses thereof. In certain embodiments, antibodies that bind to hemagglutinin are provided. Antibodies of the

invention are useful, *e.g.*, for the diagnosis, treatment, or prevention of influenza A virus infection.

A. Exemplary Anti-Hemagglutinin Antibodies

In one aspect, the invention provides isolated antibodies that bind to hemagglutinin. In certain embodiments, an anti-hemagglutinin antibody of the present invention binds hemagglutinin, binds Group1 hemagglutinins, binds Group2 hemagglutinins, or binds Group1 and Group2 hemagglutinins. In other embodiments, an anti-hemagglutinin antibody of the present invention neutralizes influenza A virus *in vitro*. In other embodiments, an anti-hemagglutinin antibody of the present invention neutralizes influenza A virus *in vivo*. In yet other embodiments, an anti-hemagglutinin antibody of the present invention reduces influenza A virus infection, prevents influenza A virus infection, inhibits influenza A virus infection, or treats influenza A virus infection. In some embodiments, an anti-hemagglutinin antibody of the present invention prevents, inhibits, or reduces hemagglutinin-mediated fusion between influenza virus membrane and infected cell endosomal membranes (thus preventing, inhibiting, or reducing viral RNA entry into the infected cell cytoplasm, thus preventing, inhibiting, or reducing further propagation of influenza virus infection.)

In one aspect, the invention provides an anti-hemagglutinin antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:178; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:179; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:180; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:182; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:187; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:188.

In one aspect, the invention provides an anti-hemagglutinin antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:178; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:179; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:181; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:183; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:187; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:189.

In one aspect, the invention provides an anti-hemagglutinin antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:178; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:179; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:181; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:182; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:187; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:188.

In one aspect, the invention provides an anti-hemagglutinin antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:178; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:179; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:181; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:184; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:187; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:188.

In one aspect, the invention provides an anti-hemagglutinin antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:178; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:179; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:181; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:185; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:187; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:188.

In one aspect, the invention provides an anti-hemagglutinin antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:178; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:179; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:181; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:183; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:187; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:188.

In one aspect, the invention provides an anti-hemagglutinin antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid

sequence of SEQ ID NO:178; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:179; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:181; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:183; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:187; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:190.

In one aspect, the invention provides an anti-hemagglutinin antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:178; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:179; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:181; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:182; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:187; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:190.

In one aspect, the invention provides an anti-hemagglutinin antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:178; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:179; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:181; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:186; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:187; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:189.

In one aspect, the invention provides an anti-hemagglutinin antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:178; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:179; (c) HVR-H3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:180 and 181; (d) HVR-L1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:182, 183, 184, 185, and 186; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:187; and (f) HVR-L3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:188, 189, and 190.

In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:178; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:179; and

(c) HVR-H3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:180 and 181.

In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:182, 183, 184, 185, and 186; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:187; and (c) HVR-L3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:188, 189, and 190.

In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:178; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:179; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:180; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:182; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:187; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:188.

In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:178; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:179; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:181; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:183; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:187; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:189.

In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:178; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:179; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:181; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:182; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:187; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:188.

In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:178; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:179; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:181; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:184; (e) HVR-L2 comprising the

amino acid sequence of SEQ ID NO:187; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:188.

In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:178; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:179; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:181; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:185; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:187; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:188.

In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:178; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:179; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:181; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:183; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:187; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:188.

In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:178; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:179; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:181; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:183; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:187; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:190.

In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:178; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:179; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:181; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:182; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:187; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:190.

In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:178; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:179; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:181; (d)

HVR-L1 comprising the amino acid sequence of SEQ ID NO:186; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:187; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:189.

In another aspect, the invention provides an antibody comprising a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:111 and 115.

In another aspect, the invention provides an antibody comprising a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:113, 117, 119, 122, 124, 126, 128, 130, and 132.

In another aspect, the invention provides an antibody comprising a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:111 and 115 and a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:113, 117, 119, 122, 124, 126, 128, 130, and 132.

In one embodiment, the invention provides an antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:111 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:113.

In one embodiment, the invention provides an antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:115 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:117.

In one embodiment, the invention provides an antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:111 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:119.

In one embodiment, the invention provides an antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:115 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:113.

In one embodiment, the invention provides an antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:115 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:122.

In one embodiment, the invention provides an antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:115 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:124.

In one embodiment, the invention provides an antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:115 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:126.

In one embodiment, the invention provides an antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:115 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:128.

In one embodiment, the invention provides an antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:115 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:130.

In one embodiment, the invention provides an antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:115 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:132.

In another aspect, the invention provides an antibody comprising a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:110, 114, and 120.

In another aspect, the invention provides an antibody comprising a light chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:112, 116, 118, 121, 123, 125, 127, 129, and 131.

In another aspect, the invention provides an antibody comprising a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:110, 114, and 120,

and a light chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:112, 116, 118, 121, 123, 125, 127, 129, and 131.

In one embodiment, the invention provides an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:110, and a light chain comprising the amino acid sequence of SEQ ID NO:112.

In one embodiment, the invention provides an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:114, and a light chain comprising the amino acid sequence of SEQ ID NO:116.

In one embodiment, the invention provides an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:110, and a light chain comprising the amino acid sequence of SEQ ID NO:118.

In one embodiment, the invention provides an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:114, and a light chain comprising the amino acid sequence of SEQ ID NO:112.

In one embodiment, the invention provides an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:120, and a light chain comprising the amino acid sequence of SEQ ID NO:121.

In one embodiment, the invention provides an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:114, and a light chain comprising the amino acid sequence of SEQ ID NO:123.

In one embodiment, the invention provides an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:114, and a light chain comprising the amino acid sequence of SEQ ID NO:125.

In one embodiment, the invention provides an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:114, and a light chain comprising the amino acid sequence of SEQ ID NO:127.

In one embodiment, the invention provides an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:114, and a light chain comprising the amino acid sequence of SEQ ID NO:129.

In one embodiment, the invention provides an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:114, and a light chain comprising the amino acid sequence of SEQ ID NO:131.

In one aspect, the invention provides an anti-hemagglutinin antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:191; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:193; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:194; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:195; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:196; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:197.

In one aspect, the invention provides an anti-hemagglutinin antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:192; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:193; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:194; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:195; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:196; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:197.

In one aspect, the invention provides an anti-hemagglutinin antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:191; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:193; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:194; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:195; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:196; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:198.

In one aspect, the invention provides an anti-hemagglutinin antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:191; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:193; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:194; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:195; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:196; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:199.

In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:191 and 192; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:193; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:194.

In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:195; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:196; and (c) HVR-L3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:197, 198, and 199.

In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:191; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:193; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:194; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:195; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:196; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:197.

In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:192; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:193; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:194; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:195; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:196; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:197.

In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:191; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:193; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:194; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:195; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:196; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:198.

In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:191; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:193; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:194; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:195; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:196; and (f) HVR-L3 comprising an amino acid sequence selected from SEQ ID NO:199.

In another aspect, the invention provides an antibody comprising a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:134, 138, 142, 148, and 234.

In another aspect, the invention provides an antibody comprising a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:136, 140, 144, 146, 150, 152, and 235.

In another aspect, the invention provides an antibody comprising a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:134, 138, 142, 148, and 234, and a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:136, 140, 144, 146, 150, 152, and 235.

In one embodiment, the invention provides an antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:134 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:136.

In one embodiment, the invention provides an antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:138 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:140.

In one embodiment, the invention provides an antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:142 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:144.

In one embodiment, the invention provides an antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:138 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:146.

In one embodiment, the invention provides an antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:148 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:150.

In one embodiment, the invention provides an antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:148 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:152.

In one embodiment, the invention provides an antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:148 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:140.

In one embodiment, the invention provides an antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:234 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:235.

In another aspect, the invention provides an antibody comprising a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:133, 137, 141, and 147.

In another aspect, the invention provides an antibody comprising a light chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:135, 139, 143, 145, 149, and 151.

In another aspect, the invention provides an antibody comprising a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:133, 137, 141, and 147, and a light chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:135, 139, 143, 145, 149, and 151.

In one embodiment, the invention provides an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:133, and a light chain comprising the amino acid sequence of SEQ ID NO:135.

In one embodiment, the invention provides an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:137, and a light chain comprising the amino acid sequence of SEQ ID NO:139.

In one embodiment, the invention provides an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:141, and a light chain comprising the amino acid sequence of SEQ ID NO:143.

In one embodiment, the invention provides an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:137, and a light chain comprising the amino acid sequence of SEQ ID NO:145.

In one embodiment, the invention provides an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:147, and a light chain comprising the amino acid sequence of SEQ ID NO:149.

In one embodiment, the invention provides an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:147, and a light chain comprising the amino acid sequence of SEQ ID NO:151.

In one embodiment, the invention provides an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:147, and a light chain comprising the amino acid sequence of SEQ ID NO:139.

In one aspect, the invention provides an anti-hemagglutinin antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:200; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:201; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:202; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:203; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:204; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:205.

In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:200; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:201; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:202.

In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:203; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:204; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:205.

In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:200; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:201; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:202; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:203; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:204; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:205.

In another aspect, the invention provides an antibody comprising a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:154 and 158.

In another aspect, the invention provides an antibody comprising a light chain variable region comprising the amino acid sequence of SEQ ID NO:156.

In another aspect, the invention provides an antibody comprising a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:154

and 158, and a light chain variable region comprising the amino acid sequence of SEQ ID NO:156.

In one embodiment, the invention provides an antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:154 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:156.

In one embodiment, the invention provides an antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:158 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:156.

In another aspect, the invention provides an antibody comprising a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:153 and 157.

In another aspect, the invention provides an antibody comprising a light chain comprising the amino acid sequence of SEQ ID NO:155.

In another aspect, the invention provides an antibody comprising a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:153 and 157, and a light chain comprising the amino acid sequence of SEQ ID NO:155.

In one embodiment, the invention provides an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:153, and a light chain comprising the amino acid sequence of SEQ ID NO:155.

In one embodiment, the invention provides an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:157, and a light chain comprising the amino acid sequence of SEQ ID NO:155.

In one aspect, the invention provides an anti-hemagglutinin antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:206; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:207; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:208; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:209; (e) HVR-L2 comprising the amino

acid sequence of SEQ ID NO:210; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:211.

In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:206; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:207; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:208.

In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:209; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:210; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:211.

In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:206; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:207; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:208; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:209; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:210; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:211.

In another aspect, the invention provides an antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:160.

In another aspect, the invention provides an antibody comprising a light chain variable region comprising the amino acid sequence of SEQ ID NO:162.

In one embodiment, the invention provides an antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:160 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:162.

In another aspect, the invention provides an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:159.

In another aspect, the invention provides an antibody comprising a light chain comprising the amino acid sequence of SEQ ID NO:161.

In another aspect, the invention provides an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:159, and a light chain comprising the amino acid sequence of SEQ ID NO:161.

In one aspect, the invention provides an anti-hemagglutinin antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:212; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:213; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:214; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:215; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:216; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:217.

In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:212; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:213; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:214.

In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:215; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:216; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:217.

In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:212; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:213; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:214; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:215; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:216; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:217.

In another aspect, the invention provides an antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:164.

In another aspect, the invention provides an antibody comprising a light chain variable region comprising the amino acid sequence of SEQ ID NO:166.

In one embodiment, the invention provides an antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:164 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:166.

In another aspect, the invention provides an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:163.

In another aspect, the invention provides an antibody comprising a light chain comprising the amino acid sequence of SEQ ID NO:165.

In another aspect, the invention provides an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:163, and a light chain comprising the amino acid sequence of SEQ ID NO:165.

In one aspect, the invention provides an anti-hemagglutinin antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:218; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:219; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:220; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:221; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:222; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:223.

In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:218; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:219; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:220.

In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of

SEQ ID NO:221; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:222; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:223.

In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:218; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:219; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:220; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:221; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:222; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:223.

In another aspect, the invention provides an antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:168.

In another aspect, the invention provides an antibody comprising a light chain variable region comprising the amino acid sequence of SEQ ID NO:170.

In one embodiment, the invention provides an antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:168 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:170.

In another aspect, the invention provides an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:167.

In another aspect, the invention provides an antibody comprising a light chain comprising the amino acid sequence of SEQ ID NO:169.

In another aspect, the invention provides an antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO:167, and a light chain comprising the amino acid sequence of SEQ ID NO:169.

In any of the above embodiments, an anti-hemagglutinin antibody of the present invention is humanized. In one embodiment, an anti-hemagglutinin antibody comprises HVRs as in any of the above embodiments, and further comprises an acceptor human framework, *e.g.*, a human immunoglobulin framework or a human consensus framework.

In another aspect, an anti-hemagglutinin antibody of the present comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs:111, 115, 134, 138, 142, 148, 154, 158, 160, 164, 168, and 234. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-hemagglutinin antibody comprising that sequence retains the ability to bind to hemagglutinin. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NOs: 111, 115, 134, 138, 142, 148, 154, 158, 160, 164, 168, or 234. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (*i.e.*, in the FRs). Optionally, the anti hemagglutinin antibody comprises the VH sequence in SEQ ID NO: 111, 115, 134, 138, 142, 148, 154, 158, 160, 164, 168, or 234, including post-translational modifications of that sequence.

In another aspect, an anti-hemagglutinin antibody is provided, wherein the antibody comprises a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs:113, 117, 119, 122,, 124, 126, 128, 130, 132, 136, 140, 144, 146, 150, 152, 156, 162, 166, 170, and 235. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-hemagglutinin antibody comprising that sequence retains the ability to bind to hemagglutinin. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NOs: 113, 117, 119, 122, 124, 126, 128, 130, 132, 136, 140, 144, 146, 150, 152, 156, 162, 166, 170, or 235. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (*i.e.*, in the FRs). Optionally, the anti-hemagglutinin antibody comprises the VL sequence in SEQ ID NOs: 113, 117, 119, 122,, 124, 126, 128, 130, 132, 136, 140, 144, 146, 150, 152, 156, 162, 166, 170, or 235, including post-translational modifications of that sequence.

In another aspect, an anti-hemagglutinin antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments

provided above. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NOs: 111, 115, 134, 138, 142, 148, 154, 158, 160, 164, 168, or 234, and SEQ ID NOs: 113, 117, 119, 122,, 124, 126, 128, 130, 132, 136, 140, 144, 146, 150, 152, 156, 162, 166, 170, or 235, respectively, including post-translational modifications of those sequences.

In a further aspect, the invention provides an antibody that binds to the same epitope as an anti-hemagglutinin antibody provided herein. For example, in certain embodiments, an antibody is provided that binds to the same epitope as an anti-hemagglutinin antibody comprising a VH sequence of SEQ ID NO:111 and a VL sequence of SEQ ID NO:113; a VH sequence of SEQ ID NO:115 and a VL sequence of SEQ ID NO:117; a VH sequence of SEQ ID NO:111 and a VL sequence of SEQ ID NO:119; a VH sequence of SEQ ID NO:115 and a VL sequence of SEQ ID NO:113; a VH sequence of SEQ ID NO:115 and a VL sequence of SEQ ID NO:122; a VH sequence of SEQ ID NO:115 and a VL sequence of SEQ ID NO:124; a VH sequence of SEQ ID NO:115 and a VL sequence of SEQ ID NO:126; a VH sequence of SEQ ID NO:115 and a VL sequence of SEQ ID NO:128; a VH sequence of SEQ ID NO:115 and a VL sequence of SEQ ID NO:130; a VH sequence of SEQ ID NO:115 and a VL sequence of SEQ ID NO:132; a VH sequence of SEQ ID NO:134 and a VL sequence of SEQ ID NO:136; a VH sequence of SEQ ID NO:138 and a VL sequence of SEQ ID NO:140; a VH sequence of SEQ ID NO:142 and a VL sequence of SEQ ID NO:144; a VH sequence of SEQ ID NO:138 and a VL sequence of SEQ ID NO:146; a VH sequence of SEQ ID NO:148 and a VL sequence of SEQ ID NO:150; a VH sequence of SEQ ID NO:148 and a VL sequence of SEQ ID NO:152; a VH sequence of SEQ ID NO:148 and a VL sequence of SEQ ID NO:140; a VH sequence of SEQ ID NO:234 and a VL sequence of SEQ ID NO:235; a VH sequence of SEQ ID NO:154 and a VL sequence of SEQ ID NO:156; a VH sequence of SEQ ID NO:158 and a VL sequence of SEQ ID NO:156; a VH sequence of SEQ ID NO:160 and a VL sequence of SEQ ID NO:162; a VH sequence of SEQ ID NO:164 and a VL sequence of SEQ ID NO:166; or a VH sequence of SEQ ID NO:168 and a VL sequence of SEQ ID NO:170.

In a further aspect of the invention, an anti-hemagglutinin antibody according to any of the above embodiments is a monoclonal antibody, including a chimeric, humanized, or human antibody. In one embodiment, an anti-hemagglutinin antibody is an antibody fragment, *e.g.*, a Fv, Fab, Fab', scFv, diabody, or $F(ab')_2$ fragment. In another embodiment, the antibody is a full length antibody, *e.g.*, an intact, *e.g.*, IgG1 antibody or other antibody class or isotype as defined herein.

In a further aspect, an anti-hemagglutinin antibody according to any of the above embodiments may incorporate any of the features, singly or in combination, as described in Sections 1-7 below:

1. Antibody Affinity

In certain embodiments, an antibody provided herein has a dissociation constant (Kd) of ≤ 1μM, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM (*e.g.*, 10^{-8} M or less, *e.g.*, from 10^{-8} M to 10^{-13} M, *e.g.*, from 10^{-9} M to 10^{-13} M).

In one embodiment, Kd is measured by a radiolabeled antigen binding assay (RIA). In one embodiment, an RIA is performed with the Fab version of an antibody of interest and its antigen. For example, solution binding affinity of Fabs for antigen is measured by equilibrating Fab with a minimal concentration of (^{125}I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (see, *e.g.*, Chen et al., *J. Mol. Biol.* 293:865-881(1999)). To establish conditions for the assay, MICROTITER® multi-well plates (Thermo Scientific) are coated overnight with 5 μg/ml of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23°C). In a non-adsorbent plate (Nunc #269620), 100 pM or 26 pM [^{125}I]-antigen are mixed with serial dilutions of a Fab of interest (*e.g.*, consistent with assessment of the anti-VEGF antibody, Fab-12, in Presta *et al.*, *Cancer Res.* 57:4593-4599 (1997)). The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period (e.g., about 65 hours) to ensure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature (*e.g.*, for one hour). The solution is then removed and the plate washed eight times with 0.1% polysorbate 20 (TWEEN-20®) in PBS. When the plates have dried, 150 μl/well of scintillant (MICROSCINT-20 ™; Packard) is added, and the plates are counted on a TOPCOUNT ™ gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

According to another embodiment, Kd is measured using a BIACORE® surface plasmon resonance assay. For example, an assay using a BIACORE®-2000 or a BIACORE ®-3000 (BIAcore, Inc., Piscataway, NJ) is performed at 25°C with immobilized antigen CM5 chips at

~10 response units (RU). In one embodiment, carboxymethylated dextran biosensor chips (CM5, BIACORE, Inc.) are activated with *N*-ethyl-*N'*- (3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5 μg/ml (~0.2 μM) before injection at a flow rate of 5 μl/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% polysorbate 20 (TWEEN-20™) surfactant (PBST) at 25°C at a flow rate of approximately 25 μl/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIACORE® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (Kd) is calculated as the ratio k_{off}/k_{on}. See, *e.g.*, Chen et al., *J. Mol. Biol.* 293:865-881 (1999). If the on-rate exceeds 10^6 M^{-1} s^{-1} by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25°C of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophometer (Aviv Instruments) or a 8000-series SLM-AMINCO™ spectrophotometer (ThermoSpectronic) with a stirred cuvette.

2. Antibody Fragments

In certain embodiments, an antibody provided herein is an antibody fragment. Antibody fragments include, but are not limited to, Fab, Fab', Fab'-SH, $F(ab')_2$, Fv, and scFv fragments, and other fragments described below. For a review of certain antibody fragments, see Hudson *et al.*, *Nat. Med.* 9:129-134 (2003). For a review of scFv fragments, see, e.g., Pluckthün, in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., (Springer-Verlag, New York), pp. 269-315 (1994); see also WO 93/16185; and U.S. Patent Nos. 5,571,894 and 5,587,458. For discussion of Fab and $F(ab')_2$ fragments comprising salvage receptor binding epitope residues and having increased *in vivo* half-life, see U.S. Patent No. 5,869,046.

Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific. See, for example, EP 404,097; WO 1993/01161; Hudson *et al.*, *Nat. Med.* 9:129-

134 (2003); and Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson *et al.*, *Nat. Med.* 9:129-134 (2003).

Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; *see, e.g.*, U.S. Patent No. 6,248,516 B1).

Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (*e.g.*, *E. coli* or phage), as described herein.

3. Chimeric and Humanized Antibodies

In certain embodiments, an antibody provided herein is a chimeric antibody. Certain chimeric antibodies are described, *e.g.*, in U.S. Patent No. 4,816,567; and Morrison et al., *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). In one example, a chimeric antibody comprises a non-human variable region (*e.g.*, a variable region derived from a mouse, rat, hamster, rabbit, or non-human primate, such as a monkey) and a human constant region. In a further example, a chimeric antibody is a "class switched" antibody in which the class or subclass has been changed from that of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof.

In certain embodiments, a chimeric antibody is a humanized antibody. Typically, a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which HVRs, *e.g.*, CDRs, (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (*e.g.*, the antibody from which the HVR residues are derived), *e.g.*, to restore or improve antibody specificity or affinity.

Humanized antibodies and methods of making them are reviewed, *e.g.*, in Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008), and are further described, *e.g.*, in Riechmann et al., *Nature* 332:323-329 (1988); Queen et al., *Proc. Nat'l Acad. Sci. USA* 86:10029-10033 (1989); US Patent Nos. 5, 821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri *et al.*, *Methods* 36:25-34 (2005) (describing specificity determining region (SDR) grafting); Padlan, *Mol. Immunol.* 28:489-498 (1991) (describing "resurfacing"); Dall'Acqua *et al.*, *Methods* 36:43-60 (2005) (describing "FR shuffling"); and Osbourn et al., *Methods* 36:61-68 (2005) and Klimka *et al.*, *Br. J. Cancer*, 83:252-260 (2000) (describing the "guided selection" approach to FR shuffling).

Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the "best-fit" method (see, *e.g.*, Sims et al. *J. Immunol.* 151:2296 (1993)); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (see, *e.g.*, Carter et al. *Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); and Presta et al. *J. Immunol.*, 151:2623 (1993)); human mature (somatically mutated) framework regions or human germline framework regions (see, *e.g.*, Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008)); and framework regions derived from screening FR libraries (see, *e.g.*, Baca *et al.*, *J. Biol. Chem.* 272:10678-10684 (1997) and Rosok *et al.*, *J. Biol. Chem.* 271:22611-22618 (1996)).

4. Human Antibodies

In certain embodiments, an antibody provided herein is a human antibody. Human antibodies can be produced using various techniques known in the art or using techniques described herein. Human antibodies are described generally in van Dijk and van de Winkel, *Curr. Opin. Pharmacol.* 5: 368-74 (2001) and Lonberg, *Curr. Opin. Immunol.* 20:450-459 (2008).

Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic mice, the endogenous immunoglobulin loci have generally been inactivated. For review of methods for obtaining human antibodies from transgenic animals, see Lonberg, *Nat. Biotech.* 23:1117-1125 (2005). See also, *e.g.*, U.S. Patent Nos. 6,075,181 and 6,150,584

describing XENOMOUSE™ technology; U.S. Patent No. 5,770,429 describing HUMAB® technology; U.S. Patent No. 7,041,870 describing K-M MOUSE® technology, and U.S. Patent Application Publication No. US 2007/0061900, describing VELOCIMOUSE® technology). Human variable regions from intact antibodies generated by such animals may be further modified, *e.g.*, by combining with a different human constant region.

Human antibodies can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described. (See, *e.g.*, Kozbor *J. Immunol.*, 133: 3001 (1984); Brodeur *et al.*, *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner et al., *J. Immunol.*, 147: 86 (1991).) Human antibodies generated via human B-cell hybridoma technology are also described in Li *et al., Proc. Natl. Acad. Sci. USA*, 103:3557-3562 (2006). Additional methods include those described, for example, in U.S. Patent No. 7,189,826 (describing production of monoclonal human IgM antibodies from hybridoma cell lines) and Ni, *Xiandai Mianyixue*, 26(4):265-268 (2006) (describing human-human hybridomas). Human hybridoma technology (Trioma technology) is also described in Vollmers and Brandlein, *Histology and Histopathology*, 20(3):927-937 (2005) and Vollmers and Brandlein, *Methods and Findings in Experimental and Clinical Pharmacology*, 27(3):185-91 (2005).

Human antibodies may also be generated by isolating Fv clone variable domain sequences selected from human-derived phage display libraries. Such variable domain sequences may then be combined with a desired human constant domain. Techniques for selecting human antibodies from antibody libraries are described below.

5. Library-Derived Antibodies

Antibodies of the invention may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, *e.g.*, in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien *et al.*, ed., Human Press, Totowa, NJ, 2001) and further described, *e.g.*, in the McCafferty *et al.*, *Nature* 348:552-554; Clackson et al., *Nature* 352: 624-628 (1991); Marks *et al.*, *J. Mol. Biol.* 222: 581-597 (1992); Marks and Bradbury, in *Methods in Molecular Biology* 248:161-175 (Lo, ed., Human Press, Totowa, NJ,

2003); Sidhu *et al.*, *J. Mol. Biol.* 338(2): 299-310 (2004); Lee *et al.*, *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee *et al.*, *J. Immunol. Methods* 284(1-2): 119-132(2004).

In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter *et al.*, *Ann. Rev. Immunol.*, 12: 433-455 (1994). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (*e.g.*, from human) to provide a single source of antibodies to a wide range of non-self and also self antigens without any immunization as described by Griffiths *et al.*, *EMBO J,* 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement *in vitro*, as described by Hoogenboom and Winter, *J. Mol. Biol.*, 227: 381-388 (1992). Patent publications describing human antibody phage libraries include, for example: US Patent No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

Antibodies or antibody fragments isolated from human antibody libraries are considered human antibodies or human antibody fragments herein.

6. Multispecific Antibodies

In certain embodiments, an antibody provided herein is a multispecific antibody, *e.g.*, a bispecific antibody. Multispecific antibodies are monoclonal antibodies that have binding specificities for at least two different sites. In certain embodiments, one of the binding specificities is for hemagglutinin and the other is for any other antigen. In certain embodiments, bispecific antibodies may bind to two different epitopes of hemagglutinin. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express hemagglutinin. Bispecific antibodies can be prepared as full length antibodies or antibody fragments.

Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (see Milstein and Cuello, *Nature* 305: 537 (1983)), WO 93/08829, and Traunecker *et al.*, *EMBO J.* 10: 3655 (1991)), and "knob-in-hole" engineering (see, *e.g.*, U.S. Patent No. 5,731,168). Multi-specific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004A1); cross-linking two or more antibodies or fragments (see, *e.g.*, US Patent No. 4,676,980, and Brennan *et al.*, *Science*, 229: 81 (1985)); using leucine zippers to produce bi-specific antibodies (see, *e.g.*, Kostelny et al., *J. Immunol.*, 148(5):1547-1553 (1992)); using "diabody" technology for making bispecific antibody fragments (see, *e.g.*, Hollinger et al., *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993)); and using single-chain Fv (sFv) dimers (see,e.g. Gruber *et al., J. Immunol.*, 152:5368 (1994)); and preparing trispecific antibodies as described, *e.g.*, in Tutt *et al. J. Immunol.* 147: 60 (1991).

Engineered antibodies with three or more functional antigen binding sites, including "Octopus antibodies," are also included herein (see, *e.g.*, US 2006/0025576A1).

The antibody or fragment herein also includes a "Dual Acting FAb" or "DAF" comprising an antigen binding site that binds to hemagglutinin as well as another, different antigen (see, US 2008/0069820, for example).

7. Antibody Variants

In certain embodiments, amino acid sequence variants of the antibodies provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, *e.g.*, antigen-binding.

a) Substitution, Insertion, and Deletion Variants

In certain embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs and FRs.

Conservative substitutions are shown in Table 1 under the heading of "preferred substitutions." More substantial changes are provided in Table 1 under the heading of "exemplary substitutions," and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, *e.g.*, retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

TABLE 1

Original Residue	**Exemplary Substitutions**	**Preferred Substitutions**
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

Amino acids may be grouped according to common side-chain properties:

(1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

(2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

(3) acidic: Asp, Glu;

(4) basic: His, Lys, Arg;

(5) residues that influence chain orientation: Gly, Pro;

(6) aromatic: Trp, Tyr, Phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (*e.g.* a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (*e.g.*, improvements) in certain biological properties (*e.g.*, increased affinity, reduced immunogenicity) relative to the parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, *e.g.*, using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more HVR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (*e.g.*, binding affinity).

Alterations (*e.g.*, substitutions) may be made in HVRs, *e.g.*, to improve antibody affinity. Such alterations may be made in HVR "hotspots," *i.e.*, residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (see, *e.g.*, Chowdhury, *Methods Mol. Biol.* 207:179-196 (2008)), and/or residues that contact antigen, with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, *e.g.*, in Hoogenboom *et al.*, in *Methods in Molecular Biology* 178:1-37 (O'Brien *et al.*, ed., Human Press, Totowa, NJ, (2001).) In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (*e.g.*, error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (*e.g.*, 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, e.g., using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

In certain embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody to bind

antigen. For example, conservative alterations (*e.g.*, conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations may, for example, be outside of antigen contacting residues in the HVRs. In certain embodiments of the variant VH and VL sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) *Science*, 244:1081-1085. In this method, a residue or group of target residues (*e.g.*, charged residues such as arg, asp, his, lys, and glu) are identified and replaced by a neutral or negatively charged amino acid (*e.g.*, alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (*e.g.*, for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

b) Glycosylation variants

In certain embodiments, an antibody provided herein is altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the

Fc region. See, *e.g.*, Wright *et al.*, *TIBTECH* 15:26-32 (1997). The oligosaccharide may include various carbohydrates, *e.g.*, mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an antibody of the invention may be made in order to create antibody variants with certain improved properties.

In one embodiment, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (*e.g.*, complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (Eu numbering of Fc region residues); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. See, *e.g.*, US Patent Publication Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to "defucosylated" or "fucose-deficient" antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki *et al.*, *J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki *et al.*, *Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka *et al.*, *Arch. Biochem. Biophys.* 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams *et al.*, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, *FUT8,* knockout CHO cells (see, *e.g.*, Yamane-Ohnuki *et al.*, *Biotech. Bioeng.* 87: 614 (2004); Kanda, Y. et al., *Biotechnol. Bioeng.*, 94(4):680-688 (2006); and WO2003/085107).

Antibodies variants are further provided with bisected oligosaccharides, *e.g.*, in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc.

Such antibody variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, *e.g.*, in WO 2003/011878 (Jean-Mairet *et al.*); US Patent No. 6,602,684 (Umana *et al.*); and US 2005/0123546 (Umana *et al.*). Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, *e.g.*, in WO 1997/30087 (Patel *et al.*); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

c) Fc region variants

In certain embodiments, one or more amino acid modifications may be introduced into the Fc region of an antibody provided herein, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (*e.g.*, a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (*e.g.* a substitution) at one or more amino acid positions.

In certain embodiments, the invention contemplates an antibody variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half life of the antibody *in vivo* is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991). Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Patent No. 5,500,362 (see, *e.g.* Hellstrom, I. *et al. Proc. Nat'l Acad. Sci. USA* 83:7059-7063 (1986)) and Hellstrom, I et al., *Proc. Nat'l Acad. Sci. USA* 82:1499-1502 (1985); 5,821,337 (see Bruggemann, M. *et al.*, *J. Exp. Med.* 166:1351-1361 (1987)). Alternatively, non-radioactive assays methods may be employed (see, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA; and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, WI). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in*

vivo, *e.g.*, in a animal model such as that disclosed in Clynes *et al. Proc. Nat'l Acad. Sci. USA* 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. See, e.g., C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro *et al.*, *J. Immunol. Methods* 202:163 (1996); Cragg, M.S. *et al.*, *Blood* 101:1045-1052 (2003); and Cragg, M.S. and M.J. Glennie, *Blood* 103:2738-2743 (2004)). FcRn binding and *in vivo* clearance/half life determinations can also be performed using methods known in the art (see, *e.g.*, Petkova, S.B. *et al.*, *Int'l. Immunol.* 18(12):1759-1769 (2006)).

Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581).

Certain antibody variants with improved or diminished binding to FcRs are described. (See, *e.g.*, U.S. Patent No. 6,737,056; WO 2004/056312, and Shields *et al., J. Biol. Chem.* 9(2): 6591-6604 (2001).)

In certain embodiments, an antibody variant comprises an Fc region with one or more amino acid substitutions which improve ADCC, *e.g.*, substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues).

In some embodiments, alterations are made in the Fc region that result in altered (*i.e.*, either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), *e.g.*, as described in US Patent No. 6,194,551, WO 99/51642, and Idusogie *et al. J. Immunol.* 164: 4178-4184 (2000).

Antibodies with increased half lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer *et al.*, *J. Immunol.* 117:587 (1976) and Kim *et al.*, *J. Immunol.* 24:249 (1994)), are described in US2005/0014934A1 (Hinton et al.). Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants

include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, *e.g.*, substitution of Fc region residue 434 (US Patent No. 7,371,826).

See also Duncan & Winter, *Nature* 322:738-40 (1988); U.S. Patent No. 5,648,260; U.S. Patent No. 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

d) Cysteine engineered antibody variants

In certain embodiments, it may be desirable to create cysteine engineered antibodies, e.g., "thioMAbs," in which one or more residues of an antibody are substituted with cysteine residues. In particular embodiments, the substituted residues occur at accessible sites of the antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate, as described further herein. In certain embodiments, any one or more of the following residues may be substituted with cysteine: V205 (Kabat numbering) of the light chain; A118 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc region. Cysteine engineered antibodies may be generated as described, *e.g.*, in U.S. Patent No. 7,521,541.

e) Antibody Derivatives

In certain embodiments, an antibody provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of

polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

In another embodiment, conjugates of an antibody and nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In one embodiment, the nonproteinaceous moiety is a carbon nanotube (Kam *et al.*, *Proc. Natl. Acad. Sci. USA* 102: 11600-11605 (2005)). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a temperature at which cells proximal to the antibody-nonproteinaceous moiety are killed.

B. Recombinant Methods and Compositions

Antibodies may be produced using recombinant methods and compositions, *e.g.*, as described in U.S. Patent No. 4,816,567. In one embodiment, isolated nucleic acid encoding an anti-hemagglutinin antibody described herein is provided. Such nucleic acid may encode an amino acid sequence comprising the VL and/or an amino acid sequence comprising the VH of the antibody (*e.g.*, the light and/or heavy chains of the antibody). In a further embodiment, one or more vectors (e.g., expression vectors) comprising such nucleic acid are provided. In a further embodiment, a host cell comprising such nucleic acid is provided. In one such embodiment, a host cell comprises (*e.g.*, has been transformed with): (1) a vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and an amino acid sequence comprising the VH of the antibody, or (2) a first vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and a second vector comprising a nucleic acid that encodes an amino acid sequence comprising the VH of the antibody. In one embodiment, the host cell is eukaryotic, *e.g.* a Chinese Hamster Ovary (CHO) cell or lymphoid cell (*e.g.*, Y0, NS0, Sp20 cell). In one embodiment, a method of making an anti-hemagglutinin antibody is provided, wherein the method comprises culturing a host cell comprising a nucleic acid encoding the antibody, as provided above, under conditions suitable for expression of the antibody, and optionally recovering the antibody from the host cell (or host cell culture medium).

For recombinant production of an anti-hemagglutinin antibody, nucleic acid encoding an antibody, *e.g.*, as described above, is isolated and inserted into one or more vectors for further

cloning and/or expression in a host cell. Such nucleic acid may be readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody).

Suitable host cells for cloning or expression of antibody-encoding vectors include prokaryotic or eukaryotic cells described herein. For example, antibodies may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of antibody fragments and polypeptides in bacteria, see, *e.g.*, U.S. Patent Nos. 5,648,237, 5,789,199, and 5,840,523. (See also Charlton, *Methods in Molecular Biology, Vol. 248* (B.K.C. Lo, ed., Humana Press, Totowa, NJ, 2003), pp. 245-254, describing expression of antibody fragments in *E. coli*.) After expression, the antibody may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been "humanized," resulting in the production of an antibody with a partially or fully human glycosylation pattern. See Gerngross, *Nat. Biotech.* 22:1409-1414 (2004), and Li *et al.*, *Nat. Biotech.* 24:210-215 (2006).

Suitable host cells for the expression of glycosylated antibody are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of *Spodoptera frugiperda* cells.

Plant cell cultures can also be utilized as hosts. *See, e.g.*, US Patent Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTIBODIES™ technology for producing antibodies in transgenic plants).

Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293 cells as described, *e.g.*, in Graham *et al., J. Gen Virol.* 36:59 (1977)); baby hamster kidney cells (BHK); mouse sertoli cells (TM4 cells as described, *e.g.*, in Mather, *Biol. Reprod.* 23:243-251 (1980)); monkey kidney cells (CV1); African green monkey kidney cells (VERO-76);

human cervical carcinoma cells (HELA); canine kidney cells (MDCK; buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); TRI cells, as described, *e.g.*, in Mather *et al., Annals N.Y. Acad. Sci.* 383:44-68 (1982); MRC 5 cells; and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including $DHFR^-$ CHO cells (Urlaub *et al., Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); and myeloma cell lines such as Y0, NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, see, *e.g.*, Yazaki and Wu, *Methods in Molecular Biology, Vol. 248* (B.K.C. Lo, ed., Humana Press, Totowa, NJ), pp. 255-268 (2003).

C. Assays

Anti-hemagglutinin antibodies provided herein may be identified, screened for, or characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

1. Binding assays and other assays

In one aspect, an antibody of the invention is tested for its antigen binding activity, *e.g.*, by known methods such as ELISA, Western blot, etc.

In another aspect, competition assays may be used to identify an antibody that competes for binding of hemagglutinin with any anti-hemagglutinin antibody described herein. In certain embodiments, such a competing antibody binds to the same epitope (*e.g.*, a linear or a conformational epitope) that is bound by an anti-hemagglutinin antibody described here (e.g., an anti-hemagglutinin antibody comprising a VH sequence of SEQ ID NO:111 and a VL sequence of SEQ ID NO:113; a VH sequence of SEQ ID NO:115 and a VL sequence of SEQ ID NO:117; a VH sequence of SEQ ID NO:111 and a VL sequence of SEQ ID NO:119; a VH sequence of SEQ ID NO:115 and a VL sequence of SEQ ID NO:113; a VH sequence of SEQ ID NO:115 and a VL sequence of SEQ ID NO:122; a VH sequence of SEQ ID NO:115 and a VL sequence of SEQ ID NO:124; a VH sequence of SEQ ID NO:115 and a VL sequence of SEQ ID NO:126; a VH sequence of SEQ ID NO:115 and a VL sequence of SEQ ID NO:128; a VH sequence of SEQ ID NO:115 and a VL sequence of SEQ ID NO:130; a VH sequence of SEQ ID NO:115 and a VL sequence of SEQ ID NO:132; a VH sequence of SEQ ID NO:134 and a VL sequence of SEQ ID NO:136; a VH sequence of SEQ ID NO:138 and a VL sequence of SEQ ID NO:140; a VH sequence of SEQ ID NO:142 and a VL sequence of SEQ ID

NO:144; a VH sequence of SEQ ID NO:138 and a VL sequence of SEQ ID NO:146; a VH sequence of SEQ ID NO:148 and a VL sequence of SEQ ID NO:150; a VH sequence of SEQ ID NO:148 and a VL sequence of SEQ ID NO:152; a VH sequence of SEQ ID NO:148 and a VL sequence of SEQ ID NO:140; a VH sequence of SEQ ID NO:234 and a VL sequence of SEQ ID NO:235; a VH sequence of SEQ ID NO:154 and a VL sequence of SEQ ID NO:156; a VH sequence of SEQ ID NO:158 and a VL sequence of SEQ ID NO:156; a VH sequence of SEQ ID NO:160 and a VL sequence of SEQ ID NO:162; a VH sequence of SEQ ID NO:164 and a VL sequence of SEQ ID NO:166; or a VH sequence of SEQ ID NO:168 and a VL sequence of SEQ ID NO:170. Detailed exemplary methods for mapping an epitope to which an antibody binds are provided in Morris (1996) "Epitope Mapping Protocols," in *Methods in Molecular Biology* vol. 66 (Humana Press, Totowa, NJ).

In an exemplary competition assay, immobilized hemagglutinin is incubated in a solution comprising a first labeled antibody that binds to hemagglutinin and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to hemagglutinin. The second antibody may be present in a hybridoma supernatant. As a control, immobilized hemagglutinin is incubated in a solution comprising the first labeled antibody but not the second unlabeled antibody. After incubation under conditions permissive for binding of the first antibody to hemagglutinin, excess unbound antibody is removed, and the amount of label associated with immobilized hemagglutinin is measured. If the amount of label associated with immobilized hemagglutinin is substantially reduced in the test sample relative to the control sample, then that indicates that the second antibody is competing with the first antibody for binding to hemagglutinin. See Harlow and Lane (1988) *Antibodies: A Laboratory Manual* ch.14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

2. Activity assays

In one aspect, assays are provided for identifying anti-hemagglutinin antibodies and fragments thereof having biological activity. Biological activity may include, *e.g.*, specifically binding to influenza A virus hemagglutinin, neutralizing influenza A virus, etc. Antibodies and compositions comprising antibodies or fragments thereof having such biological activity *in vivo* and/or *in vitro* are also provided.

In certain embodiments, an antibody of the invention is tested for such biological activity. See Examples 4, 5, 6, 7, 8, 9, 10, and 13 for exemplary descriptions of such assays.

D. Immunoconjugates

The invention also provides immunoconjugates comprising an anti-hemagglutinin antibody herein conjugated to one or more cytotoxic agents, such as chemotherapeutic agents or drugs, growth inhibitory agents, toxins (*e.g.*, protein toxins, enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), or radioactive isotopes.

In one embodiment, an immunoconjugate is an antibody-drug conjugate (ADC) in which an antibody is conjugated to one or more drugs, including but not limited to a maytansinoid (see U.S. Patent Nos. 5,208,020, 5,416,064 and European Patent EP 0 425 235 B1); an auristatin such as monomethylauristatin drug moieties DE and DF (MMAE and MMAF) (see U.S. Patent Nos. 5,635,483 and 5,780,588, and 7,498,298); a dolastatin; a calicheamicin or derivative thereof (see U.S. Patent Nos. 5,712,374, 5,714,586, 5,739,116, 5,767,285, 5,770,701, 5,770,710, 5,773,001, and 5,877,296; Hinman *et al.*, *Cancer Res.* 53:3336-3342 (1993); and Lode *et al.*, *Cancer Res.* 58:2925-2928 (1998)); an anthracycline such as daunomycin or doxorubicin (see Kratz et al., *Current Med. Chem.* 13:477-523 (2006); Jeffrey *et al.*, *Bioorganic & Med. Chem. Letters* 16:358-362 (2006); Torgov *et al.*, *Bioconj. Chem.* 16:717-721 (2005); Nagy *et al.*, *Proc. Natl. Acad. Sci. USA* 97:829-834 (2000); Dubowchik *et al.*, *Bioorg. & Med. Chem. Letters* 12:1529-1532 (2002); King *et al.*, *J. Med. Chem.* 45:4336-4343 (2002); and U.S. Patent No. 6,630,579); methotrexate; vindesine; a taxane such as docetaxel, paclitaxel, larotaxel, tesetaxel, and ortataxel; a trichothecene; and CC1065.

In another embodiment, an immunoconjugate comprises an antibody as described herein conjugated to an enzymatically active toxin or fragment thereof, including but not limited to diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes.

In another embodiment, an immunoconjugate comprises an antibody as described herein conjugated to a radioactive atom to form a radioconjugate. A variety of radioactive isotopes are available for the production of radioconjugates. Examples include At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu. When the radioconjugate

is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example tc99m or I123, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, mri), such as iodine-123 again, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron.

Conjugates of an antibody and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta *et al.*, *Science* 238:1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026. The linker may be a "cleavable linker" facilitating release of a cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari *et al.*, *Cancer Res.* 52:127-131 (1992); U.S. Patent No. 5,208,020) may be used.

The immunuoconjugates or ADCs herein expressly contemplate, but are not limited to such conjugates prepared with cross-linker reagents including, but not limited to, BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (*e.g.*, from Pierce Biotechnology, Inc., Rockford, IL., U.S.A).

E. Methods and Compositions for Diagnostics and Detection

In certain embodiments, any of the anti-hemagglutinin antibodies provided herein is useful for detecting the presence of hemagglutinin or influenza A virus in a biological sample. The term "detecting" as used herein encompasses quantitative or qualitative detection. In certain embodiments, a biological sample comprises a cell or tissue, such as, for example, lung, upper

respiratory tract, nasal canal, blood, sputum, or comprises a biological sample obtained by nasal or throat swab.

In one embodiment, an anti-hemagglutinin antibody for use in a method of diagnosis or detection is provided. In a further aspect, a method of detecting the presence of hemagglutinin or influenza A virus in a biological sample is provided. In certain embodiments, the method comprises contacting the biological sample with an anti-hemagglutinin antibody as described herein under conditions permissive for binding of the anti-hemagglutinin antibody to hemagglutinin, and detecting whether a complex is formed between the anti-hemagglutinin antibody and hemagglutinin. Such method may be an *in vitro* or *in vivo* method. In one embodiment, an anti-hemagglutinin antibody is used to select subjects eligible for therapy with an anti-hemagglutinin antibody, *e.g.*, where hemagglutinin is a biomarker for selection of patients.

Exemplary disorders that may be diagnosed using an antibody of the invention include influenza A virus infection, including influenza A virus infection in children, infants, adults, and the elderly.

In certain embodiments, labeled anti-hemagglutinin antibodies are provided. Labels include, but are not limited to, labels or moieties that are detected directly (such as fluorescent, chromophoric, electron-dense, chemiluminescent, and radioactive labels), as well as moieties, such as enzymes or ligands, that are detected indirectly, *e.g.*, through an enzymatic reaction or molecular interaction. Exemplary labels include, but are not limited to, the radioisotopes ^{32}P, ^{14}C, ^{125}I, ^{3}H, and ^{131}I, fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luceriferases, *e.g.*, firefly luciferase and bacterial luciferase (U.S. Patent No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase, β-galactosidase, glucoamylase, lysozyme, saccharide oxidases, *e.g.*, glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals, and the like.

F. Pharmaceutical Formulations

Pharmaceutical formulations of an anti-hemagglutinin antibody as described herein are prepared by mixing such antibody having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include insterstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX®, Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Patent Application Publication Nos. 2005/0260186 and 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

Exemplary lyophilized antibody formulations are described in US Patent No. 6,267,958. Aqueous antibody formulations include those described in US Patent No. 6,171,586 and WO2006/044908, the latter formulations including a histidine-acetate buffer.

The formulation herein may also contain more than one active ingredients as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, it may be desirable to further provide a

neuraminidase inhibitor, an anti-hemagglutinin antibody, an anti-M2 antibody, etc. Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended.

Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.* films, or microcapsules.

The formulations to be used for *in vivo* administration are generally sterile. Sterility may be readily accomplished, *e.g.*, by filtration through sterile filtration membranes.

G. Therapeutic Methods and Compositions

Any of the anti-hemagglutinin antibodies provided herein may be used in therapeutic methods.

In one aspect, an anti-hemagglutinin antibody for use as a medicament is provided. In further aspects, an anti-hemagglutinin antibody for use in treating, preventing, or inhibiting influenza A virus infection is provided. In certain embodiments, an anti-hemagglutinin antibody for use in a method of treatment is provided. In certain embodiments, the invention provides an anti-hemagglutinin antibody for use in a method of treating an individual having influenza A virus infection comprising administering to the individual an effective amount of the anti-hemagglutinin antibody. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, *e.g.*, as described below. In further embodiments, the invention provides an anti-hemagglutinin antibody for use in preventing, inhibiting, or reducing hemagglutinin-mediated fusion between influenza A virus viral membrane and infected cell endosomal membranes, thus preventing viral RNA entry into the infected cell cytoplasm and preventing further propagation of infection. In certain embodiments, the invention provides an anti-hemagglutinin antibody for

use in a method of preventing, inhibiting, or treating influenza A virus infection in an individual comprising administering to the individual an effective amount of the anti-hemagglutinin antibody to prevent, inhibit, or treat influenza A virus infection. An "individual" according to any of the above embodiments is preferably a human.

In a further aspect, the invention provides for the use of an anti-hemagglutinin antibody in the manufacture or preparation of a medicament. In one embodiment, the medicament is for treatment of influenza A virus infection. In a further embodiment, the medicament is for use in a method of treating influenza A virus infection comprising administering to an individual having influenza A virus infection an effective amount of the medicament. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, *e.g.*, as described below. In a further embodiment, the medicament is for preventing, inhibiting, or reducing hemagglutinin-mediated fusion between influenza A virus viral membrane and infected cell endosomal membranes, thus preventing viral RNA entry into the infected cell cytoplasm and preventing further propagation of infection. In a further embodiment, the medicament is for use in a method of preventing, inhibiting, or treating influenza A virus infection in an individual comprising administering to the individual an amount effective of the medicament to prevent, inhibit, or reduce, influenza A virus infection. An "individual" according to any of the above embodiments may be a human.

In a further aspect, the invention provides a method for treating influenza A virus infection. In one embodiment, the method comprises administering to an individual having such influenza A virus infection an effective amount of an anti-hemagglutinin antibody. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, as described herein. An "individual" according to any of the above embodiments may be a human.

The present invention provides anti-hemagglutinin antibodies effective at inhibiting, preventing, or treating influenza A virus infection in an individual (*e.g.*, a subject or a patient). In some aspects, an anti-hemagglutinin antibody of the present invention is effective at prophylactically treating an individual in order to prevent influenza A virus infection of the individual.

In some aspects, an individual suitable for treatment with an anti-hemagglutinin antibody of the present invention is an individual having or suspected having influenza A virus infection. In some embodiments, such individuals include infants, children, adults, and the elderly. In some embodiments, the individual is hospitalized with influenza A virus infection. In other embodiments, the individual having influenza A virus infection has one or more co-morbidities, such as, for example, immunodeficiency, pregnancy, lung disease, heart disease, renal disease, or co-infection (*e.g.*, a bacterial infection or a viral infection, such as bacterial or viral pneumonia).

In some aspects, treatment of an individual with an anti-hemagglutinin antibody of the present invention reduces influenza A virus infection severity, reduces the length of influenza A virus infection, or reduces influenza A virus infectivity. In other aspects, treatment of influenza A virus infection with an anti-hemagglutinin antibody of the present invention provides additional benefit, including a reduction in the length of hospital stay, reduction or prevention of the need for intensive care unit (ICU) use, reduction or prevention of the need for assisted or mechanical ventilation, reduction or prevention of the need for supplemental oxygen use, and reduction of mortality. In some aspects, the reduction in the length of hospital stay is 1 day, 2 days, 3 days, 4 days, 5 days, or longer than 5 days. In some aspects, the reduction in the need for intensive care unit use is 1 day, 2 days, 3 days, 4 days, 5 days, or longer than 5 days. In some aspects, the reduction in need for assisted or mechanical ventilation is 1 day, 2 days, 3 days, 4 days, 5 days, or longer than 5 days. In some aspects, the reduction in the need for supplemental oxygen is 1 day, 2 days, 3 days, 4 days, 5 days, or longer than 5 days. In some aspects, treatment of an individual with an anti-hemagglutinin antibody of the present invention reduces influenza A virus infection disease symptoms, such as, for example, fever, coryza, chills, sore throat, muscle pain, body aches, headache, cough, nasal congestion, weakness or fatigue, irritated or watering eyes, and general discomfort.

In some aspects, treatment of an individual with an anti-hemagglutinin antibody of the present invention reduces the time to normalization of respiratory function, such as a reduction of time to normalization of respiratory rate, or a reduction of time to normalization of oxygen saturation. In some aspects, treatment of an individual with an anti-hemagglutinin antibody of the present invention reduces the time to return to normal oxygen saturation, e.g., to an oxygen saturation of about 92% or greater, as measured over a 24 hour period without supplemental oxygen administration. In other aspects, treatment of an individual with an anti-hemagglutinin

antibody of the present invention reduces the time to normalization of vital signs, such as heart rate, blood pressure, respiratory rate, and temperature.

In some aspects, treatment of an individual with an anti-hemagglutinin antibody of the present invention improves virologic endpoints, such as, for example, influenza virus titer. Virus titer can be measured by various ways known to one of skill in the art, such as, for example, viral area under the curve (AUC), as measured by, for example, qPCR or tissue culture infective does (TCID50). In some aspects, the treatment results in greater than or equal to 50% reduction in viral AUC as measured by qPCR or TCID50.

In various aspects of the present invention, an anti-hemagglutinin antibody provided herein is effective at treating influenza A virus infection when administered at about 12 hours, at about 24 hours, at about 36 hours, at about 48 hours, at about 60 hours, at about 72 hours, at about 84 hours, and at about 96 hours after onset of symptoms (*e.g.*, onset of illness). In other aspects, an anti-hemagglutinin antibody provided herein is effective at treating influenza A virus infection when administered between about 24 hours and 48 hours after onset of symptoms (e.g., the individual has been symptomatic for between 24 and 48 hours), when administered between about 48 hours and 72 hours after onset of symptoms, or when administered between about 72 hours and 96 hours after onset of symptoms. In certain embodiments of the present invention, an anti-hemagglutinin antibody of the present invention is effective at treating or reducing influenza A virus infection and extends the treatment window of current standard of care (*e.g.*, oseltamivir) beyond 48 hours after onset of symptoms.

In a further aspect, the invention provides pharmaceutical formulations comprising any of the anti-hemagglutinin antibodies provided herein, *e.g.*, for use in any of the above therapeutic methods. In one embodiment, a pharmaceutical formulation comprises any of the anti-hemagglutinin antibodies provided herein and a pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical formulation comprises any of the anti-hemagglutinin antibodies provided herein and at least one additional therapeutic agent, *e.g.*, as described below.

Antibodies of the invention can be used either alone or in combination with other agents in a therapy. For instance, an antibody of the invention may be co-administered with at least one

additional therapeutic agent. In certain embodiments, an additional therapeutic agent is a neuraminidase inhibitor (*e.g.*, zanamivir, oseltamivir phosphate, amantadine, rimantadine), an anti-M2 antibody, an anti-hemagglutinin antibody, etc. In some aspects, treatment of an individual having influenza A virus infection with an anti-hemagglutinin antibody of the present invention co-administered with a neuraminidase inhibitor provides a synergistic therapeutic effect compared to treatment with either agent alone.

Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and separate administration, in which case, administration of the antibody of the invention can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent or agents. In one embodiment, administration of the anti-hemagglutinin antibody and administration of an additional therapeutic agent occur within about one month, or within about one, two, or three weeks, within about one, two, three, four, five, or six days, or within about one, two, three, four, five, six, eight, ten, twelve, sixteen, twenty, or twenty-four hours of each other.

An antibody of the invention (and any additional therapeutic agent) can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Dosing can be by any suitable route, *e.g.* by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

Antibodies of the invention would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The antibody need not be, but is optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of antibody present in the formulation, the type of disorder or

treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

For the prevention or treatment of disease, the appropriate dosage of an antibody of the invention (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the type of antibody, the severity and course of the disease, whether the antibody is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The antibody is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 μg/kg to about 45 mg/kg (*e.g.*, about 1.0 mg/kg to about 15 mg/kg) of antibody can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1 μg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. Exemplary dosages of the antibody would be in the range from about 1.0 mg/kg to about 45 mg/kg, from about 1.0 mg/kg to about 30 mg/kg, from about 1.0 mg/kg to about 15 mg/kg, from about 1.0 mg/kg to about 10 mg/kg, or from about 1.0 mg/kg to about 5 mg/kg. Thus, one or more doses of about 1.0 mg/kg, 2.5 mg/kg, 5.0 mg/kg, 10 mg/kg, 15 mg/kg, 30 mg/kg, or 45 mg/kg (or any combination thereof) may be administered to the patient. Such doses may be administered intermittently, *e.g.*, every day, every two days, every three days, etc. An initial higher loading dose, followed by one or more lower doses may be administered. Dosing can also be at a fixed dose, such as, for example, 200 mg, 400 mg, 600 mg, 800 mg, 1000 mg, 1200 mg, 1400 mg, 1500 mg, 1600 mg, 1800 mg, 2000 mg, 2200 mg, 2400 mg, 2500 mg, 2600 mg, 2800 mg, 3000 mg, 3200 mg, 3400 mg, 3600 mg, etc. The progress of this therapy is easily monitored by conventional techniques and assays.

It is understood that any of the above formulations or therapeutic methods may be carried out using an immunoconjugate of the invention in place of or in addition to an anti-hemagglutinin antibody.

H. Articles of Manufacture

In another aspect of the invention, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an antibody of the invention. The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises an antibody of the invention; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

It is understood that any of the above articles of manufacture may include an immunoconjugate of the invention in place of or in addition to an anti-hemagglutinin antibody.

III. EXAMPLES

The following are examples of methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

Example 1. Identification of anti-hemagglutinin antibodies by phage display

Construction of phage libraries from influenza virus vaccinated human donors

Antibodies directed against influenza A virus hemagglutinin were identified using a phage display library constructed from peripheral blood mononuclear cells (PBMCs) isolated from human donors vaccinated with the seasonal influenza virus vaccine as follows.

Leukopacs from normal human donors that received the seasonal influenza Fluvirin® vaccine (Novartis Lot #111796P1) 7 days prior to their blood donation were obtained from Blood Centers of the Pacific (San Francisco, CA). PBMCs were isolated from the leukopacs using standard methodologies. The PBMCs were sorted for $CD19^+/CD20^-$ plasmablast cells by FACS. RNA from the $CD19^+/CD20^-$ sorted plasmablasts was extracted using RNeasy purification kit (Qiagen, USA) and cDNA was generated from the isolated RNA by reverse transcription using SuperScript® III Reverse Transcriptase (Invitrogen, USA). Human variable heavy (VH), variable kappa (VK), and variable light (VL) genes were PCR amplified from the cDNA using the following back and forward DNA primer mixtures.

VH Back

BssHII.HuVH1: ATCGTTTCATAAGCGCGCCAGGTGCAGCTGGTGCAGTC (SEQ ID NO: 1)
BssHII.HuVH2: ATCGTTTCATAAGCGCGCCAGRTCACCTTGAAGGAGTC (SEQ ID NO: 2)
BssHII.HuVH3.1: ATCGTTTCATAAGCGCGCGAGGTGCAGCTGGTGGAGTC (SEQ ID NO: 3)
BssHII.HuVH3.2: ATCGTTTCATAAGCGCGCCAGGTGCAGCTGGTGGAGTC (SEQ ID NO: 4)
BssHII.HuVH3.3: ATCGTTTCATAAGCGCGCGAAGTGCAGCTGGTGGAGTC (SEQ ID NO: 5)
BssHII.HuVH4.1: ATCGTTTCATAAGCGCGCCAGGTGCAGCTGCAGGAGTC (SEQ ID NO: 6)
BssHII.HuVH4.2: ATCGTTTCATAAGCGCGCCAGCTGCAGCTGCAGGAGTC (SEQ ID NO: 7)
BssHII.HuVH5: ATCGTTTCATAAGCGCGCGARGTGCAGCTGGTGCAGTC (SEQ ID NO: 8)
BssHII.HuVH6: ATCGTTTCATAAGCGCGCCAGGTACAGCTGCAGCAGTC (SEQ ID NO: 9)
BssHII.HuVH7: ATCGTTTCATAAGCGCGCCAGGTGCAGCTGGTGCAATC (SEQ ID NO: 10)
BssHII.HuVH1.A: ATCGTTTCATAAGCGCGCCAGGTCCAGCTTGTGCAGTC (SEQ ID NO: 11)
BssHII.HuVH1.B: ATCGTTTCATAAGCGCGCCAGGTTCAGCTGGTGCAGTC (SEQ ID NO: 12)
BssHII.HuVH1.C: ATCGTTTCATAAGCGCGCCAGGTCCAGCTGGTACAGTC (SEQ ID NO: 13)
BssHII.HuVH1.D: ATCGTTTCATAAGCGCGCCAGATGCAGCTGGTGCAGTC (SEQ ID NO: 14)
BssHII.HuVH1.E: ATCGTTTCATAAGCGCGCCAAATCCAGCTGGTGCAGTC (SEQ ID NO: 15)
BssHII.HuVH1.F: ATCGTTTCATAAGCGCGCGAGGTCCAGCTGGTGCAGTC (SEQ ID NO: 16)

BssHII.HuVH3.A: ATCGTTTCATAAGCGCGCGAGGTGCAGCTGTTGGAGTC (SEQ ID NO: 17)
BssHII.HuVH3.B: ATCGTTTCATAAGCGCGCGAGGTGCAGCTGGTGGAGAC (SEQ ID NO: 18)
BssHII.HuVH4.A: ATCGTTTCATAAGCGCGCCAGGTGCAGCTACAGCAGTG (SEQ ID NO: 19)

VH Forward

NheI.JH 2: GACATTCTACGAGCTAGCTGAGGAGACAGTGACCAGGGT (SEQ ID NO: 20)
NheI.JH1/4/5: GACATTCTACGAGCTAGCTGAGGAGACGGTGACCAGGGT (SEQ ID NO: 21)
NheI.JH3: GACATTCTACGAGCTAGCTGAAGAGACGGTGACCATTGTC (SEQ ID NO: 22)
NheI.JH6: GACATTCTACGAGCTAGCTGAGGAGACGGTGACCGTGG (SEQ ID NO: 23)

VK Back

NheI.OL.HuVK 1:
TCTCCTCAGCTAGCGGTGGCGGCGGTTCCGGAGGTGGTGGTTCTGGCGGTGGTGGCAGC

GACATCCAGWTGACCCAGTC (SEQ ID NO: 24)

NheI.OL.HuVK2:
TCTCCTCAGCTAGCGGTGGCGGCGGTTCCGGAGGTGGTGGTTCTGGCGGTGGTGGCAGC
GATGTTGTGATGACTCAGTC (SEQ ID NO: 25)

NheI.OL.HuVK3:
TCTCCTCAGCTAGCGGTGGCGGCGGTTCCGGAGGTGGTGGTTCTGGCGGTGGTGGCAGC
GAAATTGTGWTGACRCAGTC (SEQ ID NO: 26)

NheI.OL.HuVK4:
TCTCCTCAGCTAGCGGTGGCGGCGGTTCCGGAGGTGGTGGTTCTGGCGGTGGTGGCAGC
GATATTGTGATGACCCACAC (SEQ ID NO: 27)

NheI.OL.HuVK5:
TCTCCTCAGCTAGCGGTGGCGGCGGTTCCGGAGGTGGTGGTTCTGGCGGTGGTGGCAGC
GAAACGACACTCACGCAGTC (SEQ ID NO: 28)

NheI.OL.HuVK6:
TCTCCTCAGCTAGCGGTGGCGGCGGTTCCGGAGGTGGTGGTTCTGGCGGTGGTGGCAGC
GAAATTGTGCTGACTCAGTC (SEQ ID NO: 29)

<u>VK Forward</u>
NcoI.JK1-: AGTTCATGCCATGGTTTTGATTTCCACCTTGGTCCCTT (SEQ ID NO: 30)
NcoI.JK2-: AGTTCATGCCATGGTTTTGATCTCCACCTTGGTCCC (SEQ ID NO: 31)
NcoI.JK3-: AGTTCATGCCATGGTTTTGATATCCACTTTGGTCCCAG (SEQ ID NO: 32)
NcoI.JK4-: AGTTCATGCCATGGTTTTGATCTCCAGCTTGGTCCCT (SEQ ID NO: 33)
NcoI.JK5-: AGTTCATGCCATGGTTTTAATCTCCAGTCGTGTCCCTT (SEQ ID NO: 34)

<u>VL Back</u>
NheI.OL.HuVL1.1:
TCTCCTCAGCTAGCGGTGGCGGCGGTTCCGGAGGTGGTGGTTCTGGCGGTGGTGGCAGCCAGTCTGT
G CTGACTCAGCC (SEQ ID NO: 35)

NheI.OL.HuVL1.2:
TCTCCTCAGCTAGCGGTGGCGGCGGTTCCGGAGGTGGTGGTTCTGGCGGTGGTGGCAGCCAGTCTGT
G YTGACGCAGCC (SEQ ID NO: 36)

NheI.OL.HuVL1.3:
TCTCCTCAGCTAGCGGTGGCGGCGGTTCCGGAGGTGGTGGTTCTGGCGGTGGTGGCAGCCAGTCTGT
C GTGACGCAGCC (SEQ ID NO: 37)

NheI.OL.HuVL2:
TCTCCTCAGCTAGCGGTGGCGGCGGTTCCGGAGGTGGTGGTTCTGGCGGTGGTGGCAGCCARTCTGC
C CTGACTCAGCC (SEQ ID NO: 38)

NheI.OL.HuVL3.1:
TCTCCTCAGCTAGCGGTGGCGGCGGTTCCGGAGGTGGTGGTTCTGGCGGTGGTGGCAGCTCCTATGW
G CTGACTCAGCC (SEQ ID NO: 39)

NheI.OL.HuVL3.2:
TCTCCTCAGCTAGCGGTGGCGGCGGTTCCGGAGGTGGTGGTTCTGGCGGTGGTGGCAGCTCTTCTGA
G CTGACTCAGGA (SEQ ID NO: 40)

NheI.OL.HuVL4:
TCTCCTCAGCTAGCGGTGGCGGCGGTTCCGGAGGTGGTGGTTCTGGCGGTGGTGGCAGCCACGTTAT
A CTGACTCAACC (SEQ ID NO: 41)

NheI.OL.HuVL5:
TCTCCTCAGCTAGCGGTGGCGGCGGTTCCGGAGGTGGTGGTTCTGGCGGTGGTGGCAGCCAGGCTGT
G CTGACTCAGCC (SEQ ID NO: 42)

NheI.OL.HuVL6:
TCTCCTCAGCTAGCGGTGGCGGCGGTTCCGGAGGTGGTGGTTCTGGCGGTGGTGGCAGCAATTTTAT
G CTGACTCAGCC (SEQ ID NO: 43)

NheI.OL.HuVL7/8:
TCTCCTCAGCTAGCGGTGGCGGCGGTTCCGGAGGTGGTGGTTCTGGCGGTGGTGGCAGCCAGRCTGT
G GTGACYCAGGA (SEQ ID NO: 44)

NheI.OL.HuVL9:
TCTCCTCAGCTAGCGGTGGCGGCGGTTCCGGAGGTGGTGGTTCTGGCGGTGGTGGCAGCCWGCCTG
TG CTGACTCAGCC (SEQ ID NO: 45)

VL Forward

NcoI.JL1-:	AGTTCATGCCATGGTTAGGACGGTGACCTTGGTCC	(SEQ ID NO: 46)
NcoI.JL2/3-:	AGTTCATGCCATGGTTAGGACGGTCAGCTTGGTCC	(SEQ ID NO: 47)
NcoI.JL7-:	AGTTCATGCCATGGTGAGGACGGTCAGCTGGGTG	(SEQ ID NO: 48)

The resulting amplified cDNA products were assembled to scFv using overlap PCR with the following overlap primers.

BssHII.VH.OL+:	ATCGTTTCATAAGCGCGCSA	(SEQ ID NO: 49)
NotI.JK.OL-:	AGTTCATGCCATGGTTTTGAT	(SEQ ID NO: 50)
NotI.JL.OL-:	AGTTCATGCCATGGTKAGGAC	(SEQ ID NO: 51)

Purified scFv cDNA fragments (1 μg) and phagemid vector p2056BNN (2 μg) were digested with BssHII and NcoI restriction endonuclease (New England Biolabs, USA). Phagemid vector p2056BNN is a modified version of pS2025e (Sidhu *et al.*, (2004) J Mol Biol 338:299-310), engineered to contain BssHII, NheI, and NcoI restriction sites. The scFv cDNA fragments were then ligated into the p2056BNN vector (6:1 M ratio) using T4 DNA ligase enzyme (New England Biolabs). The resulting cDNA/phage ligation products were purified using a PCR purification kit (Qiagen, USA) and transformed into electro-competent SS320 *E. coli* cells. The size of the phage library was estimated by plating 10 μl of 1:10 diluted library culture onto LB/Carbenicillin plates. The library culture was then further amplified and propagated in a total volume of 60 ml 2YT medium, and phage-scFv expression was induced by co-infection with M13KO7 helper phage. Kanamycin was later added to the library culture, and incubated with shaking for 30 hours at 30 °C. The library culture was then centrifuged to pellet the cells. The phage-scFv-containing supernatant was precipitated with 5× PEG/2.5 M NaCl and resuspended in PBS.

Phage library sorting and screening to identify anti-hemagglutinin antibodies

Influenza A virus hemagglutinin H1 and H3 proteins (produced as described below in Example 2) were used as antigens for phage library sorting. Hemagglutinin H1 and H3 antigens were coated onto a high-binding 96-well maxisorp plate. The plates and phage libraries were pre-blocked with phage blocking buffer (phosphate-buffered saline (PBS), 1% (w/v) bovine serum albumin (BSA), and 0.05% (v/v) tween-20 (PBS-T)) and incubated for 2 hours at room temperature. The blocked phage library (100 µl) was added to the hemagglutinin-coated wells and incubated for 3 hours. The unbound phage were washed off the plates using 0.05% PBS–Tween, and bound phage were eluted with 100 µL 50 mM HCl and 500 mM NaCl for 30 minutes followed by neutralization with 100 µL of 1 M Tris base (pH 7.5). Recovered phage were amplified in *E. coli* XL-1 Blue cells. The resulting phage were precipitated and subjected another round of panning/selection against the hemagglutinin proteins. During subsequent panning/selection rounds, antibody phages were incubated with same or different hemagglutinin antigens. The stringency of plate washing was gradually increased from washing 15x to washing 40x.

After 2-3 rounds of panning and selection, significant enrichment of hemagglutinin-specific phage was observed. 96 phage clones were picked from the library sorting to determine whether they specifically bound to hemagglutinin H1 and/or H3. The variable regions of the phage clones displaying specific binding to the hemagglutinin proteins were sequenced to identify phage clones containing unique immunoglobulin nucleic acid sequences. Unique phage antibodies that bound hemagglutinin H1 and/or H3 with at least 5x above background were further characterized. Phage-derived clones of interest were reformatted into IgGs by cloning V_L and V_H regions of individual clones into the LPG3 and LPG4 expression vectors, respectively, transiently expressed in mammalian 293 cells, and purified using a protein A column. Two antibodies (mAb9 and mAb23) were identified for further analysis. (See Example 5 below.)

Example 2. Plasmablast enrichment and expansion

To discover and identify rare antibodies against influenza A virus hemagglutinin, the following plasmablast enrichment and expansion technique was developed. (See co-pending patent application U.S. patent application serial number 61/725,764, which is incorporated by reference herein in its entirety.)

Leukopacs from normal human donors that received the seasonal influenza Fluvirin® vaccine (Novartis Lot #111796P1) 7 days prior to their blood donation were obtained from Blood Centers of the Pacific (San Francisco, CA). Peripheral blood mononuclear cells (PBMCs) were isolated from the leukopacs using standard methodologies. Six- to eight-week old female SCID/beige mice were purchased from Charles River Laboratories (Hollister, CA) and housed and maintained at Genentech in accordance with American Association of Laboratory Animal Care guidelines. All experimental studies were conducted under the approval of the Institutional Animal Care and Use Committees of Genentech Lab Animal Research in an AAALACi-accredited facility in accordance with the Guide for the Care and Use of Laboratory Animals and applicable laws and regulations. Leukopac or blood from healthy human donors was obtained after written informed consent was provided and ethical approval granted from the Western Institutional Review Board.

In vivo antigen-driven plasmablast enrichment and expansion was performed using intraspenic transplantation of PBMCs as follows. Isolated PBMCs were resuspended with hemagglutinin antigens (0.1-2 μg for each one million B cells) and incubated for 30 minutes at 37°C (PBMC/antigen pre-mix). Following this incubation, the PBMCs were washed to remove unbound antigens. To enrich for plasmablasts that produced cross-reactive hemagglutinin antibodies, the hemagglutinin antigen variants used for PBMC/antigen pre-mix and single cell sorting were specifically chosen to differ from the hemagglutinin antigen variants contained within the influenza Fluvirin® vaccine. Hemagglutinin antigens used in this study, therefore, included H1 hemagglutinin from influenza A virus isolate A/NWS/1933 (a Group1 influenza A virus hemagglutinin), H3 hemagglutinin from influenza A virus isolate A/Hong Kong/8/1968 (a Group2 influenza A virus hemagglutinin), and H7 hemagglutinin from influenza A virus isolate A/Netherlands/219/2003 (a Group2 influenza A virus hemagglutinin). The hemagglutinin antigens were produced at Genentech using standard molecular biology techniques.

6-8 week old female SCID/beige mice (Charles River Laboratories, Hollister, CA) were sub-lethally irradiated with 350 rads using a Cesium-137 source. Polymyxin B (110 mg/L) and neomycin (1.1 g/L) were added to the drinking water for 7 days following irradiation. Four hours after irradiation, the left flank of each mouse was shaved and prepped with Betadine® (Purdue Pharma, Stamford, CT) and 70% alcohol. Surgical procedures were performed under

anesthesia using aseptic surgical procedures. A 1-cm skin incision was made just below the costal border of each mouse, followed by an incision of the abdominal wall and the peritoneum. The spleen of each mouse was carefully exposed and injected with 50×10^6 human PBMCs resuspened in 30 μL PBS. The incisions were closed in the muscular layer and in the skin using 5-O Vicryl® sutures (Ethicon, Somerville, NJ) and surgical staples, respectively. For antigen-specific cell sorting experiments, mice were sacrificed at 8 days post-transplantation, and their spleens harvested.

Single cell suspensions of spleen cells obtained from the mice were stained with a cocktail of anti-human monoclonal antibodies CD38 PECy7 (BD Biosciences, San Jose, CA) and IgG Dylight (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) which define human IgG+ plasmablasts as $CD38^{high}$/IgG+ expression. To identify hemagglutinin cross-reactive plasmablasts within the suspension of isolated spleen cells, the cells were stained with hemagglutinin H1 from influenza virus A isolate A/NWS/1933 and hemagglutinin H3 from influenza virus A isolate A/Hong Kong/8/1968, which were previously conjugated with FITC or PE, respectively, using Lightning-Link® labeling kits (Innova Biosciences, Cambridge, UK).

Figure 1A shows representative FACS data analysis of anti-hemagglutinin-positive plasmablasts from day 7 post-vaccinated PBMCs prior to SCID/beige mice enrichment (*i.e.*, prior to PBMC/antigen pre-mix). Figure 1B shows representative FACS data analysis of hemagglutinin-positive plasmablasts from day 8 post-transplant after SCID/beige mice enrichment, comparing no pre-mix and antigen pre-mix in the upper and lower panels, respectively. As shown in Figures 1A and 1B, PBMC/antigen pre-mix prior to intrasplenic injection resulted in higher frequency of $H3^+/H1^+$ anti-hemagglutinin plasmablasts.

Table 2 below shows a comparison of anti-$H1^+$/anti-$H3^+$ plasmablast frequencies before and after SCID enrichment as described herein. As shown in Table 2, the frequency of anti-$H1^+$/anti-$H3^+$ plasmablasts was greatly increased using the SCID/beige mouse enrichment methods of the present invention compared to that observed without SCID/beige mouse enrichment.

Table 2

Condition	Anti-H1$^+$/Anti-H3$^+$ Plasmablast Frequency (%)
Vaccinated PBMC	0.00028±0.00008
SCID + Antigen Premix	0.011±0.007

Samples were then analyzed in the presence of propidium iodide dead cell exclusion on Aria high-speed cell sorter (BD Biosciences, San Jose, CA) and anti-hemagglutinin-specific plasmablasts were sorted in a single cell manner into 96-well tissue culture plates containing 50 µl RPMI cell cutlute media supplemented with 5% Low IgG fetal bovine serum. (Gibco, Grand Island, NY). Five million live cells were recorded for all analysis profiles. Profiles were analyzed by Flowjo version 9.4.11 software.

Figure 2 shows analysis of splenocytes obtained from day-8 post-transplant from individual SCID/beige mice showing stochastic response, comparing no pre-mix (circles) and antigen-pre-mix (squares). Data is presented as percent anti-H1$^+$/CD38^{high} plasmablasts. The rectangle indicates mice that presented anti-H1$^+$ plasmablasts.

These results showed that broad hemagglutinin cross-reactive plasmablasts were detected if influenza virus A Group1 (*e.g.*, hemagglutinin H1) and Group2 (*e.g.*, hemagglutinin H3, hemagglutinin H7) hemagglutinin antigens were incubated with PBMCs prior to intrasplenic transplant. These results further indicated that *in vitro* stimulation of hemagglutinin antigen-primed PBMCs from influenza-vaccinated donors promoted hemagglutinin antigen-specific enrichment of plasmablasts within the SCID/beige mouse recipients.

Example 3. IgG cloning from single plasmablasts

Hemagglutinin H1 and H3 cross-reactive human plasmablasts (described above) were single-cell sorted, resulting in approximately 950 plasmablasts. Single plasmablasts were sorted directly into U-bottom 96-well micro-well plates containing 50 µl RPMI containing 5% Low IgG fetal bovine serum. The plates were centrifuged for 5 minutes at 600 x g (Beckman Coulter, Brea, CA) and the media was carefully removed by aspiration. The cells were re-suspended and washed twice in 90 µl of PBS following the same procedure.

To generate cDNA encoding the variable heavy chains and light chains, each cell was re-suspended in 6 µl of Reverse Transcriptase (RT) reaction mixture containing 2 units RNaseout

(Invitrogen, Grand Island, NY), 0.5 mM 4dNTP (Perkin Elmer, Waltham, MA), 1.5 mM $MgCl_2$, 37.5 mM KCl, 10 mM DTT (dithiothreitol), 0.25% Nonidet P40 (US Biological, Marblehead, MA), 0.1 mg/ml bovine serum albumin (Sigma-Aldrich), 25 mM Tris pH 8.3, 0.25 pmol of IgG_{1-4} constant, kappa chain constant, and lambda chain constant region specific oligonucleotides (shown below) and 40 U Superscript III (Invitrogen, Grand Island, NY).

IgG_{1-4} constant:	GAAGTAGTCCTTGACCAGGCAG (SEQ ID NO: 52)
Kappa constant:	CTCAGCGTCAGGGTGYTGCTGAG (SEQ ID NO: 53)
Lambda constant:	GGGTKTGGTSGTCTCCAC (SEQ ID NO: 54)

The reaction was incubated for 3 x 30-minute intervals at 45°C, 50°C, and 55° C each. Following the incubation, the reaction mixture was diluted to 15 µl with TE buffer (10 mm Tris HCl, 1 mM EDTA). Initial polymerase chain reactions (PCR) were performed to amplify IgG heavy chains, kappa chains, and lambda chains using 2 µl of the diluted RT cocktail from above and Advantage-GC 2 Polymerase Mix (Clontech, Mountain View, CA), following protocols provided by the manufacturers. The PCR amplifications were performed using degenerate oligonucleotides based on variable heavy chain and light chain germline and constant region sequences shown below.

IGVH1a	CAGGTGCAGCTGGTGCAGTCTGGGGC	(SEQ ID NO: 55)
IGVH1b	CAGGTCCAGCTGGTGCAGTCTGGGGC	(SEQ ID NO: 56)
IGVH2	CAGGTCACCTTGAAGGAGTCTGGTCC	(SEQ ID NO: 57)
IGVH3	GAGGTGCAGCTGGTGGAGTCTGGGGG	(SEQ ID NO: 58)
IGVH4	CAGGTGCAGCTGCAGGAGTCGGGCCC	(SEQ ID NO: 59)
IGVH5	GAGGTGCAGCTGGTGCAGTCTGG	(SEQ ID NO: 60)
IGVH6	CAGGTACAGCTGCAGCAGTCAGGTCC	(SEQ ID NO: 61)
IGVH7	CAGGTGCAGCTGGTGCAATCTGG	(SEQ ID NO: 62)
IGKV1	GHCATCCRGWTGACCCAGTCTC	(SEQ ID NO: 63)
IGKV2	GATRTTGTGATGACYCAGWCTC	(SEQ ID NO: 64)
IGKV3	GAAATWGTRWTGACRCAGTCTC	(SEQ ID NO: 65)
IGKV4	GACATCGTGATGACCCAGTCTCC	(SEQ ID NO: 66)
IGKV5	GAAACGACACTCACGCAGTCTC	(SEQ ID NO: 67)
IGKV6	GAWRTTGTGMTGACWCAGTCTC	(SEQ ID NO: 68)
IGLV1	CAGTCTGTGYTGACKCAGCCRCCCTC	(SEQ ID NO: 69)
IGLV2	CAGTCTGCCCTGACTCAGCCT	(SEQ ID NO: 70)
IGLV3	TCCTATGAGCTGACWCAGSHVCCCKC	(SEQ ID NO: 71)
IGLV4	CAGCCTGTGCTGACTCARTCVCCCTC	(SEQ ID NO: 72)
IGLV5	CAGCCTGTGCTGACTCAGCCAACTTC	(SEQ ID NO: 73)

IGLV6	AATTTTATGCTGACTCAGCCCCAC	(SEQ ID NO: 74)
IGLV7	CAGGCTGTGGTGACTCAGGAGCCC	(SEQ ID NO: 75)
IGLV8	CAGACTGTGGTGACCCAGGAGCC	(SEQ ID NO: 76)
IGLV9	CAGCCTGTGCTGACTCAGCCACC	(SEQ ID NO: 77)
HC301.5constant	GCAGCCCAGGGCSGCTGTGC	(SEQ ID NO: 78)
Kappa102constant	GCACACAACAGAGGCAGTTCCAG	(SEQ ID NO: 79)
Lambda202constant	CTTGRAGCTCCTCAGAGGAG	(SEQ ID NO: 80)

Heavy chain and light chain PCR amplification reactions were each divided into two reactions as follows: heavy chain families VH.1,2,3 (primers IGVH1a, IGVH1b, IGVH2, IGVH3) and VH.4,5,6,7 (primers IGVH4, IGVH5, IGVH6, and IGVH7); kappa chain families VK.1,2,3 (primers IGKV1, IGKV2, and IGKV3) and VK.4,5,6 (primers IGVK4, IGVK5, and IGVK6); and lambda chain families VL.1,2,3,4,5 (IGLV1, IGLV2, IGLV3, IGLV4, and IGLV5) and VL.6,7,8,9 (primers IGLV6, IGLV7, IGLV8, and IGLV9). A touchdown PCR amplification protocol was used for temperature cycling.

Following the reaction, PCR amplification products were treated with Exonuclease1 (Exo) and Shrimp Alkaline Phosphatase (SAP) to remove excess nucleotides and primers from each of the PCR amplification reactions (U.S. Biologicals, Marblehead, MA). Initial PCR amplification products were directly sequenced to determine the variable sequences of both the heavy chains and light chains using Sanger sequencing. Second nested PCR amplifications were performed using germline-matched heavy chain and light chain variable oligonucleotides in order to insert a mammalian signal and constant region cloning sequences using the following oligonucleotide primers.

sVH1a:
CCACCATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACTGGAGTACATTCACAGG (SEQ ID NO: 81)

sVH2:
CCACCATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACTGGAGTACATTCACAGATC ACCT (SEQ ID NO: 82)

sVH3vv:
CCACCATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACTGGAGTACATTCACAG (SEQ ID NO: 83)

sVH3gl:
CCACCATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACTGGAGTACATTCAGAGG (SEQ ID NO: 84)

sVH4:
CCACCATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACTGGAGTACATTCACAGGT GCAGCTGCAGG (SEQ ID NO: 85)

sVH5:
CCACCATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACTGGAGTACATTCAGAGGT
GCA (SEQ ID NO: 86)

sVH6:
CCACCATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACTGGAGTACATTCACAGGT
ACAGC (SEQ ID NO: 87)

sVH7:
CCACCATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACTGGAGTACATTCACAGGT
GCA (SEQ ID NO: 88)

sVK1:
CCACCATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACTGGAGTACATTCAGACATC
CAGATGACCCAGTCTCCATCCTCCCTG (SEQ ID NO: 89)

sVK2:
CCACCATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACTGGAGTACATTCAGATATT
GTGATGACTCAGTCTCACTCTCCCTGC (SEQ ID NO: 90)

sVK3:
CCACCATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACTGGAGTACATTCAGAAATT
GTGTTGACACAGTCTCCAGCCACCCTGTCTTTG (SEQ ID NO: 91)

sVK4:
CCACCATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACTGGAGTACATTCAGACATC
GTGATGACCCAGTCTCCAGACTCCCTGGCTGTG (SEQ ID NO: 92)

sVK5:
CCACCATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACTGGAGTACATTCAGAAAC
GACACTCACGCAGTCTCCAGC (SEQ ID NO: 93)

sVK6:
CCACCATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACTGGAGTACATTCAGAAATT
GTGCTGACTCAGTCTCCAGACTTTCG (SEQ ID NO: 94)

sVL1:
CCACCATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACTGGAGTACATTCACAGTCT
GTGYTGACKCAGCCRCCCTC (SEQ ID NO: 95)
sVL2:
CCACCATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACTGGAGTACATTCACAGTCT
GCCCTGACTCAGCCT (SEQ ID NO: 96)

sVL3:
CCACCATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACTGGAGTACATTCATCCTAT
GAGCTGACWCAGSHVCCCKC (SEQ ID NO: 97)

sVL4:
CCACCATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACTGGAGTACATTCACAGCCT
GTGCTGACTCARTCVCCCTC (SEQ ID NO: 98)

sVL5:
CCACCATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACTGGAGTACATTCACAGCCT
GTGCTGACTCAGCCAACTTC (SEQ ID NO: 99)

sVL6:
CCACCATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACTGGAGTACATTCAAATTTT
ATGCTGACTCAGCCCCAC (SEQ ID NO: 100)

sVL7:

CCACCATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACTGGAGTACATTCACAGGCT GTGGTGACTCAGGAGCCC (SEQ ID NO: 101)

sVL8:
CCACCATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACTGGAGTACATTCACAGACT GTGGTGACCCAGGAGCC (SEQ ID NO: 102)

wVL9:
CCACCATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACTGGAGTACATTCACAGCCT GTGCTGACTCAGCCACC (SEQ ID NO: 103)

Heavy constant: GCCAGGGGGAAGACCGATG (SEQ ID NO: 104)

Kappa constant:
CTGGGATAGAAGTTATTCAGCAGGCACACAACAGAAGCAGTTCCAGATTTCAACTGCTC (SEQ ID NO: 105)

Lambda constant: CTTGRAGCTCCTCAGAGGAG (SEQ ID NO: 80)

PCR amplification reactions were set up using PrimeStar HS DNA Polymerase with GC (Takara Bio, Shiga, Japan) according to the manufacturer's recommendation. Following the PCR amplification reactions, the amplification products were treated with Exo/SAP as described above. Heavy variable chain and light variable chain encoding PCR amplification products were inserted into a mammalian expression vector using restriction endonuclease free procedures. 20 μl of the PCR amplification products were annealed onto single stranded DNA human templates for IgG_1, kappa, and lambda chain using the Kunkel mutagenesis protocol. (See Kunkel (1985) PNAS 82:488-492.) Correctly inserted constructs were confirmed by DNA sequencing. Plasmids containing nucleic acids encoding heavy chains and light chains were co-transfected into 293T human embryonic kidney cells using Fugene transfection reagent (Roche Diagnostic, Indianapolis, IN) for transient expression, and analyzed for expression and binding as described below in Example 4.

Example 4. Hemagglutinin ELISA screening assay

The ability of each monoclonal anti-hemagglutinin antibody obtained as described above to bind various hemagglutinin subtypes was examined by ELISA as follows. Various hemagglutinin-expressing plasmids were transfected into 293T cells as described above. These included hemagglutinin H1 from H1N1/South Carolina/1918, hemagglutinin H3 from H3N2/Perth/2009, hemagglutinin H5 from H5N1/Viet/2004, and hemagglutinin H7 from H7N7/Netherlands/2003 influenza A viruses. After two days, cells were lysed in 50 mM Tris, pH 8, 5 mM EDTA, 150 mM NaCl, 1% Triton X-100 plus protease inhibitor cocktail (Roche). Nuclei were cleared by centrifugation and the resulting lysates were stored at -80°C.

For ELISA screening, 384-well plates (Nunc MaxiSorp) were coated with 5 μg/ml Galanthus nivalis lectin (Sigma) in PBS. The plates were washed and then coated with dilutions of the cell lysates containing various expressed hemagglutinins. The plates were washed and incubated with various dilutions of the anti-hemagglutinin antibodies and subsequently with a goat-anti-human-HRP secondary antibody (Jackson). Plates were washed and processed for TMB (3,3',5,5'-tetramethylbenzidine) substrate detection.

Approximately 950 plasmablasts were obtained from single-cell sorting described above in Example 2. Of this, 840 monoclonal antibodies were transiently expressed in 293T cells and screened by ELISA for binding to hemagglutinin subtypes H1, H3, H5, and H7, resulting in 82 monoclonal antibodies that bound influenza A virus Group1 or Group2 hemagglutinin, and 20 monoclonal antibodies that bound both influenza A virus Group1 and Group2 hemagglutinins.

Example 5. *In vitro* influenza A virus neutralization

The ability of the anti-hemagglutinin antibodies of the present invention to elicit broad hemagglutinin subtype binding and neutralization of a panel of influenza A Group1 and Group2 virus isolates *in vitro* was examined as follows.

MDCK cells were grown in DMEM media supplemented with 10% FBS as a single 25% confluent monolayer in 96-well black with clear bottom imaging plates (Costar 3904). Each influenza A virus subtype/strain was diluted in influenza media (DMEM + 0.2%BSA, 2 μg/ml TPCK treated Trypsin) to an MOI of 1 and incubated for 1 hour at 37°C with varying concentrations (ranging from 0.02 nM to 1,600 nM) of each antibody. Each antibody/influenza virus mixture was allowed to infect MDCK cells for 16 hours at 37°C in a 5% CO_2 incubator prior to fixation of the cells with cold 100% ethanol. The fixed cells were then stained with Hoechst 33342 (Invitrogen, Cat# H3570) to visualize cell nuclei and determine total cell number. The cells were also stained with a broadly reactive monoclonal antibody (Millipore Cat# MAB8258) specific for influenza A virus nucleoprotein in order to determine the number of infected cells.

Cells were imaged using the Image Express Micro (Molecular Devices) and data images were analyzed using MetaXpress 3.1 software. The percentage of infected cells was determined and

plotted on the Y-axis versus the Log 10 antibody concentration on the X-axis. All neutralization assays were completed in triplicate. Data were fit using a nonlinear regression dose-response curve and are presented in Figure 3 as IC_{50} values in nM with 95% confidence intervals (95% CI).

The hemagglutinin (HA) subtype of each influenza A virus strain is provided in the table shown in Figure 3.

In vitro neutralization dose-response curves were generated using various concentrations of the monoclonal antibodies described herein against a broad panel of influenza A Group1 and Group2 virus strains. Figures 4A and 4B show neutralization curves of mAb 39.29 NWPP ("NWPP" disclosed as SEQ ID NO: 177) against a panel of influenza A Group1 and Group2 virus strains, respectively. As shown in Figures 4A and 4B, mAb 39.29 NWPP ("NWPP" disclosed as SEQ ID NO: 177) was effective at *in vitro* neutralization of all influenza A virus strains tested. (See also Figure 3.) Additionally, Figures 5A and5B show neutralization curves of mAb 81.39 SVSH-NYP ("SVSH" disclosed as SEQ ID NO: 171) against a panel of influenza A Group1 and Group2 virus strains, respectively. As shown in Figures 5A and 5B, mAb 81.39 SVSH-NYP ("SVSH" disclosed as SEQ ID NO: 171) was effective at the *in vitro* neutralization of all influenza A virus strains tested. (See also Figure 3.)

Four anti-hemagglutinin antibodies of the present invention (specifically mAb 39.18 B11, mAb 36.89, mAb9.01F3, and mAb23.06C2) were effective *in vitro* at neutralization of either Group1 or Group2 influenza A virus strains, but not both. Specifically, mAb 39.18 B11 was effective at *in vitro* neutralization of the entire Group1 influenza A virus panel examined, but was not able to neutralize Group2 influenza A virus strains. (See Figure 6 and Figure 3.) Conversely, mAb 36.89, mAb9.01F3, and mAb23.06C2 were able to neutralize the entire Group2 influenza A virus panel examined, but were not able to neutralize any Group1 influenza A virus isolate tested. (See Figures 7, 8, and 9, showing *in vitro* neutralization curves for mAb 36.89, mAb9.01F3, and mAb23.06C2, respectively; also see Figure 3.)

Taken together, these results showed that monoclonal antibodies of the present invention were able to neutralize in a dose-dependent manner various influenza A virus isolates/strains *in vitro*. Additionally, these results showed that the plasmablast enrichment methodology described herein resulted in the identification of monoclonal antibodies capable of neutralizing both Group1 and Group2 influenza A virus strains from only 950 isolated plasmablasts.

In vitro neutralization studies were also performed using a pseudotype virus engineered to express hemagglutinin H5 to test the efficacy of an antibody of the present invention at neutralizing H5N1 influenza A virus. In particular, an HIV psueudotype virus bearing the H5 hemaggutinin surface protein was tested for neutralization with mAb 39.29 NCv1 on 293T cells as follows. The H5 pseudotype virus was produced by co-transfection of 293T cells with three plasmids: Δ8.9, FCMV-GFP, and a plasmid expressing hemagglutinin H5 from influenza A virus isolate H5N1/Vietnam/1203/2004. Virus was purified by ultra-centrifugation through 20% sucrose. For infection, pseudotype virus was incubated with various amounts of mAb 39.29 NCv1 before adding to target 293T cells cultured in 96-well plates. After two days, the number of infected cells was determined by counting GFP positive cells. Infection was normalized to the number of infected cells at the lowest antibody concentration used. The results are presented in Figure 10. As shown in Figure 10, mAb 39.29 NCv1 displayed a dose-dependent *in vitro* neutralization against the pseudotype virus expressing hemaggutinin H5 surface protein. These data suggested that antibodies of the present invention would be effective at treatment and prevention of H5N1 influenza A virus strains.

An equine influenza virus was also tested for the ability of antibodies of the present invention to exhibit *in vitro* neutralization activity as follows. H7N7 A/Equine/1/Prague/56 influenza A virus was passed on MDCK cells until it achieved a high degree of infectivity. The resulting H7N7 A/Equine/1/Prague/56 influenza A virus was used in neutralization assays (using methods as described above for mAb 39.29 NCv1) on MDCK cells. The results of these experiments are presented in Figure 11. As shown in Figure 11, mAb 39.29 NWPP ("NWPP" disclosed as SEQ ID NO: 177) displayed a dose-dependent *in vitro* neutralization against the H7N7 A/Equine/1/Prague/56 influenza virus expressing hemagglutinin H7 surface protein.

Taken together, these results showed that anti-hemagglutinin antibodies of the present invention exhibited dose-dependent neutralization activity against a variety of influenza A virus strains. Specifically, two anti-hemagglutinin antibodies (mAb 39.29 NWPP ("NWPP" disclosed as SEQ ID NO: 177) and mAb 81.39 SVSH-NYP ("SVSH" disclosed as SEQ ID NO: 171)) were effective at neutralizing all influenza A virus strains examined, including neutralization of both Group1 influenza A virus strains (A/CA/7/2009, A/Brisbane/59/2007, A/Solomon/3/2006, A/New Caledonia/20/1999, A/PR/8/1934, and A/Japan/305/1957) and Group2 influenza A virus strains (A/Victoria/361/2011, A/Perth/16/2009, A/Brisbane/10/2007,

A/Wisconsin/67/2005, A/Victoria/3/1975, A/Port Chalmers/1/1973, A/HK/8/1968, and A/Aichi/2/1968).

Additionally, these results showed that anti-hemagglutinin antibodies of the present invention (e.g., mAb 39.29 NWPP ("NWPP" disclosed as SEQ ID NO: 177) (Figures 4A and 4B) and mAb 81.39 SVSH-NYP ("SVSH" disclosed as SEQ ID NO: 171) (Figures 5A and 5B)) were effective at neutralization of a variety of different seasonal H1N1 influenza A virus strains, H3N2 influenza A virus strains, a H2N2 influenza A virus strain, and the influenza A virus strain associated with the 1957 Japan pandemic (A/Japan/305/1957). These results indicated that antibodies of the present invention are effective in the treatment and prevention of seasonal influenza A virus infection and influenza A virus strains associated with influenza pandemics.

Example 6. *In vivo* efficacy of mAb 39.29 NWPP ("NWPP" disclosed as SEQ ID NO: 177) in mice

The *in vivo* efficacy of mAb 39.29 NWPP ("NWPP" disclosed as SEQ ID NO: 177) to influenza A virus infection in mice was performed as follows. DBA/2J mice (Jackson Lab, Bar Harbor, ME) were infected intranasally with 50 μl of various influenza A virus strains diluted in influenza media (DMEM, 0.2% BSA, 2 μg/mL TPCK-treated trypsin) at the minimum LD_{100} dose. Four different influenza A virus strains exhibiting a range of *in vitro* IC_{50} values were used in this series of experiments, including: H1N1 A/PR/8/1934 (Genentech; IC_{50} 2.0 nM), used at 40 PFU per mouse; H3N2 A/Hong Kong/1/1968 (ViraPur, San Diego, CA; IC_{50} 45.1 nM), used at 3 PFU per mouse; H3N2 A/Port Chalmers/1/1973 (ViraPur, San Diego, CA; IC_{50} 2.2 nM), used at $1.5x10^4$ PFU per mouse; and H3N2 A/Aichi/2/1968 (ViraPur, San Diego, CA; IC_{50} 35 nM), used at $2x10^2$ PFU per mouse. Influenza virus infection was allowed to progress for 72 hours prior to the intravenous administration of mAb 39.29 NWPP ("NWPP" disclosed as SEQ ID NO: 177).

After 72 hours post influenza virus A infection, various amounts of mAb 39.29 NWPP ("NWPP" disclosed as SEQ ID NO: 177) were administered intravenously to the mice at a dose of 900 μg/mouse (approximately 45 mg/kg), 300 μg/mouse (approximately 15 mg/kg), and 100 μg/mouse (approximately 5 mg/kg) in 200 μl PBS. Control treated animals were administered mAb gD5237 (a monoclonal antibody specific for glycoprotein D of herpes simplex virus (HSV)) at the highest tested equivalent dose of mAb 39.29 NWPP ("NWPP" disclosed as SEQ ID NO: 177) (*i.e.*, approximately 45 mg/kg). Mice were monitored daily for body conditioning

and survival, and also weighed daily, until 21 days after infection. All mAb39.29 NWPP ("NWPP" disclosed as SEQ ID NO: 177) doses vs. control in all four influenza A virus strain infections gave a Log-rank test of $P<0.01$.

Figures 12A, 12B, 12C, and 12D show percent survival (over time, in days) of mice administered various amounts of mAb 39.29 NWPP ("NWPP" disclosed as SEQ ID NO: 177) 72 hours after infection with influenza A virus A/PR/8/1934, A/Port Chalmers/1/1973, A/Hong Kong/1/1968, and A/Aichi/2/1968, respectively. As shown in Figures 12A, 12B, 12C, and 12D, 100% mortality was observed by day 14 in infected mice administered control antibody. However, infected mice administered monoclonal antibody of the present invention showed increased survival. In particular, 100% survival was observed in mice infected with influenza virus A/Port Chalmers/1/1973 or influenza virus A/Aichi/2/1968 at all doses of mAb 39.29 NWPP ("NWPP" disclosed as SEQ ID NO: 177) tested. (See Figures 12B and 12D.)

These results showed that monoclonal antibodies of the present invention are effective at treating various influenza A virus infections. Additionally, these data showed that monoclonal antibodies of the present invention were effective at treating influenza A virus infection when administered up to at least 72 hours post influenza A virus infection.

Example 7. *In vivo* efficacy of mAb 39.29 NCv1 in mice

To test the *in vivo* efficacy of mAb 39.29 NCv1 in mice, the antibody was administered i.v. to mice infected with four different influenza A virus isolates that exhibited a range of *in vitro* IC_{50} values. DBA/2J mice (Jackson Lab , Bar Harbor, ME) were infected intranasally with 50 µl of different influenza A virus strains diluted into influenza media (DMEM, 0.2% BSA, 2 ug/mL TPCK treated trypsin) at the minimum LD100 dose.

In one set of experiments, influenza A virus isolateH1N1 A/PR/8/1934 was used at 40 PFU per mouse. At 72 hours post infection, anti-hemagglutinin mAb 39.29 NCv1 was administered intravenously at approximately 15 mg/kg, approximately 5 mg/kg, approximately 1.7 mg/kg, or approximately 0.56 mg/kg in 200 µl PBS intravenously. Control treated animals were given mAb gD5237, which is specific for glycoprotein D of HSV at the highest tested equivalent dose of mAb 39.29 NCv1. Mice were monitored for body conditioning and survival, and weighed until 21 days after infection.

For the H1N1 A/PR/8/1934 infected mice, a single i.v. dose of mAb 39.29 NCv1 at 15 mg/kg per mouse was efficacious compared to that observed with control IgG antibody. (See Figure 13.) Specifically, 100% mortality was observed in the control treatment group by day 12, while a single dose of 15 mg/kg of mAb 39.29 NCv1 saved 87.5% of the infected mice. A threefold lower dose of 100 μg per mouse (approximately 5 mg/kg) of mAb 39.29 NCv1 exhibited some efficacy, being able to protect 25% of animals from the lethal challenge, while doses of approximately 1.7 mg/kg or approximately 0.56 mg/kg showed minimal efficacy beyond that observed in the control treatment group. (See Figure 13.)

In another set of experiments, *in vivo* efficacy of mAb 39.29 NCv1 was further examined against mouse-adapted H3N2 Hong Kong influenza A virus strain (H3N2 A/Hong Kong/1/1968), which has a tenfold higher *in vitro* IC_{50} than A/PR8/1934. As observed in previous experiments described above, mice treated with control antibody following influenza A virus infection showed 100% mortality by day 12. (See Figure 14.) However, a single dose of mAb 39.29 NCv1 at approximately 45 mg/kg or approximately 15 mg/kg was able to protect 87.5% and 75% of the mice, respectively. The minimum efficacious dose of 15 mg/kg *in vivo* of mAb 39.29 NCv1 in both the A/PR8/1934 and the A/Hong Kong/1/1968 influenza A virus infection models is very similar despite the observed contrast in mAb 39.29 NCv1 *in vitro* IC_{50} values between these two strains. (See Figures 3 and 14.)

To further explore the *in vivo* efficacy of mAb 39.29 NCv1, a dose titration of mAb 39.29 NCv1 was tested against two additional influenza A virus strains, Port Chalmers (H3N2 A/Port Chalmers/1/1973) and Aichi (H3N2 A/Aichi/2/1968). mAb 39.29 NCv1 has an *in vitro* IC_{50} against Port Chalmers of 2.9 nM, which is very similar to that of A/PR8/1934, while Aichi has an *in vitro* IC_{50} of 35.0 nM, a value closer to that of A/Hong Kong/1/1968. As shown in Figure 15 and Figure 16, 100% mortality was observed in the control treated animals by day 12 and day 10 for the Port Chalmers and Aichi models, respectively. Monoclonal antibody 39.29 NCv1 exhibited very efficacious against both influenza A virus strains at all tested doses (*e.g.*, 45 mg/kg, 15 mg/kg, 5 mg/kg, and 1.7 mg/kg).

These data indicated, in part, that little correlation existed between the *in vitro* IC_{50} of mAb 39.29 NCv1 and the *in vivo* minimum efficacious dose. None-the-less, a single dose of 15 mg/kg administered i.v. 72 hours post infection was efficacious in all four influenza A virus mouse models despite the range of *in vitro* IC_{50} values for these influenza A virus strains.

Example 8. *In vivo* efficacy of mAb 39.29 and oseltamivir in severe influenza A virus infection in mice

To compare the efficacy of anti-hemagglutinin antibodies of the present invention to that of oseltamivir phosphate (Tamiflu®) in mice, the following studies were performed. Balb/c mice (Charles River Laboratories, Hollister, CA) at 6-weeks old were infected intranasally with 50 µl H1N1 A/PR/8/1934 at 100x the lethal dose ($5X10^4$ PFU/mouse). At 48 hours post infection, anti-hemagglutinin antibody 39.29 (a 50:50 mixture of mAb 39.29 D8C2 and mAb 39.29 NWPP ("NWPP" disclosed as SEQ ID NO: 177)) was administered as a single dose of approximately 15 mg/kg or control IgG in 200 µl PBS intravenously. In these experiments, an oseltamivir dosing regimen consisting of 2 mg dosed twice daily (BID) for five days was compared with a single 300 µg i.v. dose (~15 mg/kg) of mAb 39.29 NWPP ("NWPP" disclosed as SEQ ID NO: 177). A Log-rank test of mAb 39.29 NWPP ("NWPP" disclosed as SEQ ID NO: 177) or oseltamivir vs. control gave $p<0.01$ and a maximum likelihood test of mAb 39.29 NWPP ("NWPP" disclosed as SEQ ID NO: 177) vs. oseltamivir gave $p<0.05$. (Oseltamivir (*i.e.*, Tamiflu®) was obtained from Toronto Research Chemicals, Cat. No. 0701000.)

As shown in Figure 17, 100% mortality was observed by day 9 in control-IgG (mAb gD5237) treated animals. BID treatment of oseltamivir for 5 days only protected 37.5% of mice from lethality. However, a single 15 mg/kg dose of mAb 39.29 NWPP ("NWPP" disclosed as SEQ ID NO: 177) mixture protected 87.5% of the infected animals from the lethal influenza A virus challenge. (See Figure 17.) The fully efficacious 15 mg/kg dose of mAb 39.29 NWPP ("NWPP" disclosed as SEQ ID NO: 177) mixture performed better than oseltamivir in mice severely infected with influenza A virus.

These results showed that a single dose of a monoclonal antibody of the present invention was more effective at treating influenza A virus infection than a 5-day treatment with oseltamivir.

Example 9. *In vivo* efficacy of mAb 39.29 NWPP ("NWPP" disclosed as SEQ ID NO: 177) in mice with and without co-administration of oseltamivir

Administration of oseltamivir is effective at reducing human influenza A virus infection if given within 48 hours after symptom onset. Unfortunately, oseltamivir shows minimal efficacy in patients who have been symptomatic for more than 48 hours. Therefore, the following experiments were performed to test if co-administration of a monoclonal antibody of the

present invention and oseltamivir showed improved efficacy over either treatment alone. These experiments were performed using the severe mouse influenza infection model described above in Example 8. Briefly, female Balb/C mice (Charles River Laboratories) were infected with 100x the lethal dose ($5x10^4$ pfu) of A/PR/8/1934 72-hours prior to i.v. administration of a single dose of 100 μg mAb 39.29 NWPP ("NWPP" disclosed as SEQ ID NO: 177) (approximately 6 mg/kg, a previously-determined sub-efficacious dose), control IgG, 2 mg BID oseltamivir, or a combination of a single dose of mAb 39.29 NWPP ("NWPP" disclosed as SEQ ID NO: 177) and oseltamivir treatment for 5 days. A Log-rank test of the combination treatment vs. mAb 39.29 NWPP ("NWPP" disclosed as SEQ ID NO: 177) or oseltamivir gives $p<0.01$.

As expected, control IgG treated animals exhibited 100% mortality 9 days post infection. (See Figure 18.) The mortality observed for control-treated animals was very similar to the groups receiving only oseltamivir or a sub-efficacious dose of mAb 39.29 NWPP ("NWPP" disclosed as SEQ ID NO: 177). However, co-administration of a sub-efficacious dose of mAb 39.29 NWPP ("NWPP" disclosed as SEQ ID NO: 177) plus oseltamivir significantly improved survival compared to that observed in either treatment alone, resulting in 87.5% survival. (See Figure 18.)

These results showed that a synergistic effect on the treatment of influenza A virus infection occurred during combination therapy using a monoclonal antibody of the present invention used in combination with oseltamivir, a neuraminidase inhibitor.

Example 10. Anti-hemagglutinin antibodies of the present invention perform better than oseltamivir in a ferret H5N1 influenza A virus infection model

Ferret influenza A virus infection models are often used to examine prophylactic and therapeutic efficacy of anti-influenza therapeutics. Ferrets are considered a clinically relevant animal model for human influenza A virus infection. (See Matsuoka *et al.*, (2009) *Current Protocols in Microbiology*, Chapter 15, Unit 15G 12.)

To examine the *in vivo* efficacy of mAb 39.29 D8C2 and mAb 81.39 B1C1 against a human isolate of H5N1 influenza A virus in ferrets, the following studies were performed. The ferret H5N1 study was completed under contract at the Lovelace Respiratory Research Institute (Albuquerque, NM). Male ferrets (*Mustela putorius furo*) were challenged with an intranasal

dose of $1x10^3$ pfu of the highly virulent H5N1 A/Vietnam/1203/04 influenza A virus strain (LD90 dose). Animals were infected 48 or 72 hours prior to receiving antibody by i.v. or oseltamivir (Tamiflu®) by oral gavage. The control treated animals received a 25 mg/kg i.v. dose of mAB gD5237, a monoclonal antibody specific for glycoprotein D of HSV. The anti-influenza treated animals received a single 25 mg/kg i.v. dose of either mAb 39.29 D8C2 or mAb 81.39 B1C1 at 48 or 72 hours post influenza virus infection. Each antibody treatment group included 10 ferrets. The oseltamivir treated animals received a twice-daily oral dose of 25 mg/kg for 5 days. Animals were monitored daily for weight loss, fever, and, body conditioning.

Consistent with an H5N1 infection, the majority of infected ferrets showed early signs of upper respiratory disease by 48 hours post infection. As expected with a lethal dose of H5N1, the negative control antibody treatment group exhibited 90% mortality by 14 days post inoculation. (See Figures 19A and 19B.)

In contrast, ferrets that received a single dose of mAb 39.29 D8C2 at either 48 or 72 hours post influenza virus infection showed 80% and 90% survival (20% and 10% mortality), respectively. (See Figure 19A.) Likewise, ferrets that received a single dose of mAb 81.39 B1C1 at either 48 or 72 hours post infection showed 100% and 80% survival (0% and 20% mortality), respectively. (See Figure 19B.) Irrespective of treatment initiation time, the oseltamivir treated groups showed 50% mortality.

These results showed that broadly neutralizing anti-hemagglutinin antibodies of the present invention were highly protective in the treatment of severe influenza A virus H5N1 infection in ferrets and performed better than oseltamivir when administered at either 48 and 72 hours post influenza A virus infection.

Example 11. Crystallization and data collection

In order to examine the structural basis for hemagglutinin cross-reactivity of the antibodies of the present invention, mAb 39.29 NCv1 Fab fragment was co-cystallized with recombinant hemagglutinin H3 from the human influenza A virus strain A/Perth/16/2009 as follows.

Protein expression and purification

To better understand the structural basis for hemagglutinin neutralization, the crystal structure of mAb 39.29 NCv1 Fab fragment in complex with hemagglutinin was determined as follows. Nucleic acid encoding the extracellular domain of Perth H3 hemagglutinin (H3HA, A/Perth/16/2009, amino acid residues 25-520 (SEQ ID NO: 226 for full-length hemagglutinin H3 (H3HA) amino acid sequence) was cloned into pACGP67 vector (BD Biosciences) in-frame with a thrombin cleavage site (LVPRGS, SEQ ID NO: 106), trimerization "foldon" sequence (PGSGYIPEAPRDGQAYVRKDGEWVLLSTFLG, SEQ ID NO:107), and a C-terminal 6xHis tag (SEQ ID NO: 108). Recombinant baculovirus was generated by co-transfection of *Sf9* cells with the H3HA-pACGP67 vector and linearized baculovirus DNA (Pharmingen).

To generate recombinant H3HA protein, *Trichoplusia ni* PRO cells were infected with the recombinant baculovirus using an MOI of 1 and grown for 72 hours at 27°C. Cell supernatants were treated with 50 mM Tris-HCl, pH 7.5, 5 mM $CaCl_2$, and 1 mM $NiCl_2$ followed by centrifugation and filtering. Media was then concentrated and buffer exchanged into 10 mM Tris, pH 8.0, and 150 mM NaCl (TBS) containing 20 mM imidazole by tangential flow filtration, and protein captured with Ni-agarose and eluted into TBS containing 200 mM imidazole. The foldon tag was cleaved overnight with thrombin, and H3HA was concentrated and further purified on a Superdex 200 16/60 size exclusion column equilibrated in TBS.

To generate the hemagglutinin-Fab complex, the mAb 39.29 NCv1 Fab (under control of the PhoA promoter) was expressed in *E. coli* overnight at 30°C. The cells were pelleted by centrifugation at 6,000 rpm for 15 minutes and lysed by micro-fluidization in PBS supplemented with 25 mM EDTA and 1 mM PMSF. Cell debris was removed by centrifugation at 10,000 rpm for 1 hour at 4°C. The resulting supernatant was passed through a Protein G column and Fab eluted with 0.58% acetic acid. Further purification of mAb 39.29 NCv1 Fab was achieved by SP sepharose chromatography using a gradient from 0 to 1 M NaCl in 20 mM MES, pH 5.5. To generate the HA/39.29 complex, H3HA was incubated overnight with excess mAb 39.29 NCv1 Fab, followed by concentration and S200 size exclusion chromatography in TBS to isolate the complex. The complex was concentrated to 10 mg/ml for crystallization trials.

Crystallization

Crystal generation for the H3HA/39.29 NCv1 Fab complex were found in 0.1M Phosphate/Citrate buffer, pH 4.2, using 40% PEG 300 as precipitant (condition C6, the JCSG+ sparse matrix screen, Qiagen). Diffraction quality crystals were ultimately grown at 19°C in sitting drops containing 0.1 µl protein and 0.1 µl 0.1M Phosphate/Citrate, pH 4.2, 40% PEG 300, and 0.7% 1-butanol. Crystals were cryoprotected in mother liquor followed by flash freezing and storage in liquid nitrogen. Data was collected under cryo-cooled conditions at the Canadian Light Source beamline CMCF-08ID and processed using MOSFLM and SCALA. The crystal belonged to the I213 space group, with unit cell dimensions of $a=b=c=204.4$ and $\alpha=\beta=\gamma=90^{o}$.

Structure determination

Initial phases were obtained by molecular replacement with PHASER using the structure of a H3HA (PDB 3SDY) as a search model. Subsequently the Fc and Fv portions of the Fab were placed separately using PHASER, and underwent initial rounds of rigid body refinement with Phenix. The model went through several iterative rounds of adjustment with COOT and simulated annealing, coordinate, and b-factor refinement with Phenix. Sugar molecules found at Asn-linked glycosylation sites were added using the Carboload package from Phenix, and final rounds of refinement were carried out using REFMAC5. The final model was refined at 3.1Å with R/Rfree values of 19.9 and 25.9%, respectively. Ramachandran statistics calculated by Molprobity indicate 89.7% of the residues lie in favored regions with 1.1% outliers. Contacts were analyzed using the Protein Interfaces, Surfaces, and Assemblies (PISA) software and structural figures were prepared with PYMOL.

Example 12. Structural characterization of the 39.29 epitope on H3 hemagglutinin

As described above in Example 11, mAb 39.29 NCv1 Fab fragment was co-cystallized with recombinant H3 hemagglutinin from the human influenza A virus strain A/Perth/16/2009. The crystal structure of the antibody/hemagglutinin complex was determined at a resolution of 3.1Å. The overall structure of A/Perth/16/2009 H3 hemagglutinin was similar to previously determined hemagglutinin structures with the exception of slight rearrangements and disorder in the HA2 helix 1/helix 2 linker. Disorder at these locations has been seen previously under low pH crystallization conditions, which is consistent with this complex being crystallized at pH 4.2 (Ekiert *et al.*, (2011) Science 333:843-850). The crystal structure of the antibody/HA complex showed a single mAb 39.29 Fab molecule bound to each monomer of the uncleaved

H3 HA trimer. Both the light chain and heavy chain fragments of mAb 39.29 NCv1 Fab fragments were well resolved throughout, allowing close examination of the Fv interaction with HA.

The epitope for mAb 39.29 NCv1 was determined to be on the stalk region of H3 hemagglutinin, roughly on top of the HA2 helix A. This region of the hemagglutinin stalk was first identified as a broadly neutralizing epitope for influenza A viruses expressing Group1 hemagglutinin subtypes (Ekiert *et al.*, (2009) Science 324:246-251; Sui *et al.*, (2009) Nature Structural & Molecular Biology 16:265-273)), and more recently as a neutralizing epitope for influenza A virus strains carrying Group1 and Group2 hemagglutinin subtypes (Corti *et al.*, (2011) Science 333:850-856). mAb 39.29 NCv1 antibody uses extensive heavy and light chain contacts to bury approximately 1175 $Å^2$ of the hemagglutinin stalk surface area. The heavy chain of mAb 39.29 NCv1 contributes to binding largely through an extended hydrophobic CDRH3 loop that inserts into a shallow nonpolar groove adjacent to HA2 helix A and underneath a conserved Group2 hemagglutinin glycosylation site at Asn54. This CDRH3 loop extends Phe99 side-chain out to interact with H3 hemagglutinin Thr334, Ile390, and Ile393, while making main chain polar contacts with the GlcNAc attached to H3 hemagglutinin Asn54. The CDRH3 loop of mAb 39.29 NCv1 also makes a β-turn at Gly100, which is likely stabilized by inter-loop main chain contacts between Val98 and Ile100A. Ile100A faces downward to interact with a conserved H3 hemagglutinin Trp366, while Val98 and Pro100C also make van der Waals contacts with the H3 hemagglutinin stalk. Residing at the heavy/light chain interface, Pro100D and Trp100E terminate the long CDRH3 loop and act to anchor the loop in place.

The light chain of mAb 39.29 NCv1 also contributes significantly to the interaction with the H3 hemagglutinin stalk, making contacts with the H3 hemagglutinin stalk with all three light chain CDR loops as well as framework residues. Of the approximately 1100 $Å^2$ hemagglutinin buried surface area, ~60% is contributed by the light chain (640$Å^2$ vs 480$Å^2$ for light chain and heavy chain, respectively). The CDRL1 Asn32 makes hydrogen bond with H3 HA2 helix A residues Asp391 and Asn394, while CDRL1 His31 stacks against the H3 hemagglutinin Asn376 sidechain. Ser52 in the CDRL2 loop also makes a polar contact with Asn398. Within the CDRL3 loop, the backbone of Asn93 contacts Asp391 while Trp94 makes a cation-π interaction with Lys384 in the HA2 helix A. Interestingly, mAb 39.29 also makes a number of framework contacts with hemagglutinin, primarily through backbone interactions of the

SGSGSG repeat (SEQ ID NO: 109) in beta-strand 6 of the IgKV3 with amino acid residues 403 to 405 in the H3 hemagglutinin polypeptide. Ser67 of mAb 39.29 NCv1 also makes polar interactions with Asp48 and Thr404 of H3 hemagglutinin.

All three mAb 39.89 NCv1 light chain CDR loops contribute to binding of the H3 HA stalk epitope, accounting for approximately 60% of the total buried surface area. This large dependence of light chain contacts is unique among known hemagglutinin Group1 and Group2 binding and neutralizing antibodies, with antibody F16v3 light chain contributing to only 20% to the buried surface area and antibody CR9114 light chain not making contact with the epitope.

Although structurally conserved, Group1 and Group2 hemagglutinin subtypes diverge significantly at the primary amino acid sequence level. To compare mAb 39.29 NCv1 H3HA contact residues with other hemagglutinin subtypes, we aligned the amino acid sequence of H3 hemagglutinin from influenza virus A/Perth/16/2009 with representative hemagglutinin amino acid sequences from other influenza virus strains: H1HA from A/California/07/2009; H2HA from A/Japan/305/1957; H5HA from A/Vietnam/1203/2004; and H7HA from A/chicken/NSW/1/1997. The amino acid numbering of H3 hemagglutinin from A/Perth/16/2009 in the crystal structure matches the hemagglutinin H3 sequence used in the alignment. The hemagglutinin sequence alignment was generated using clustalW and the amino acid sequences corresponding to hemagglutinin H1 from A/California/07/2009,hemagglutinin H2 from A/Japan/305/1957, hemagglutinin H3 from A/Perth/19/2009, hemagglutinin H5 from A/Vietnam/1203/2004, and hemagglutinin H7 from A/chicken/NSW/1/1997. The crystal structure was used to determine the contact residues between the 39.29 NCv1 Fab fragment and the stalk of hemagglutinin H3.

The alignment is presented in Figure 20. Hemagglutinin contact residues (shaded in grey) are defined as residues within 4.5Å of mAb 39.29 NCv1. Each amino acid residue that had greater than 50% of its available surface area buried by mAb 39.29 NCv1 Fab is marked with an asterisk.

A high degree of sequence conservation is observed among the contact residues that contribute significantly to the binding of mAb 39.29 NCv1 to this epitope. (See Figure 20.) This observation suggests that mAb 39.29 NCv1 binds Group1 and Group2 hemagglutinin

molecules via the same stalk epitope seen in the crystal structure described above. This epitope is similar to a hemagglutinin epitope identified for FI6v3 anti-hemagglutinin antibody (Corti *et al.*, (2011), *supra*). However, mAb 39.29 NCv1 binds in a different orientation with respect to the hemagglutinin stalk than does FI6v3. Comparison of the 39.29 NCv1, F16v3, and CR9114 structures in complex with HA revealed that all three antibodies bind an epitope that includes the HA2 helix A and adjacent non-polar groups. However, each of the three antibodies has a unique binding orientation, with each heavy chain bound to a similar topographical position on HA but with light chain positioning rotated by ~60° (F16v3) or ~120° (CR9114) when compared to 39.29 NCv1. Also unique to mAb 39.29 NCv1, the IgKV3 light chain SGSGSG repeat (SEQ ID NO: 109) in beta-strand 6 frame-work makes contact with H3 HA. Therefore, the 39.29 structure represents a third solution to the binding of this highly conserved epitope and solidifies the importance of engaging the HA2 helix A for broad neutralization of influenza A virus.

The crystallography data of mAb 39.29 in complex with H3 hemagglutinin from the human influenza A virus strain A/Perth/16/2009 revealed the following contact positions: 34, 36, 54, 70, 292, 294, 305, 307, 334, 363, 364, 365, 366, 379, 380, 382, 383, 384, 386, 387, 390, 391, 393, 394, 395, 397, 398, 401, 403, 404, and 405. Antibody FI6v3 showed the following contact positions: 334, 352, 356, 363, 364, 365, 366, 381, 383, 384, 386, 387, 388, 390, 391, 393, 394, 397, 398, 401, and 402. Amino acid residue positions correspond to H3 hemagglutinin from influenza A virus strain A/Perth/16/2009 (SEQ ID NO:226). (See International Application Publication Nos: WO 2010/010466 and WO 2013/011347; Corti et al. (2011) Science 333:850-856.) While some overlap is observed, mAb 39.29 showed a greater number of contact positions within hemagglutinin than FI6v3.

The fact that mAb 39.29 NCv1 and FI6v3 antibody CDRs have no sequence homology and that both antibodies engage a similar but not identical stalk epitope in different ways suggests that there are various ways for antibodies to bind the conserved stalk epitope and broadly neutralize influenza A viruses.

Example 13. Competition ELISA

Competition ELISA assays were developed using hemagglutinin H1 from influenza virus A/WSN/1933 and hemagglutinin H3 from influenza virus A/Hong Kong/8/1968. Hemagglutinin-coated ELISA plates were allowed to bind test antibody at various

concentrations (X-axis) prior to the addition of saturating concentrations of biotin labeled mAb 39.29. If the test antibody competed for the hemagglutinin epitope of mAb 39.29, the biotin ELISA signal (Y-axis) was decreased as a function of increasing test antibody concentration. The binding data were fit with a non-linear dose response curve to determine the EC_{50} value given in nM.

mAb 39.29 IgG was biotinylated through amine coupling according to the manufacturer's recommended protocol (Sulfo-NHS-LC-LC, Pierce, Rockford, IL). Final stock concentration of the biotinylated mAb was 13.2 mM. To determine the optimal concentration for usage, the biotinylated 39.29 was serially titrated against immobilized H1 hemagglutinin from influenza A virus A/WSN/1933 and H3 hemagglutinin from influenza A virus A/Hong Kong/8/1968. Recombinant hemagglutinin H1 and H3 proteins were diluted to 2 μg/ml in phosphate buffered saline (PBS) and dispensed (100 μl) onto 96-well Nunc Maxisorp plates (Nunc, Rochester, NY). The plates were coated overnight at 4°C, rinsed in PBS, and then blocked for 1-hour at room temperature with PBS containing 1% bovine serum albumin (BSA, Sigma-Aldrich, St. Louis, MO).

Each plate then received 100 μl of serially diluted biotinylated mAb 39.29 starting at an initial concentration of 88 nM with 1/3 dilutions in PBS containing 1.0% BSA and 0.05% Polysorbate 20 (Sigma-Aldrich). After one hour incubation, the plates were washed and then incubated with 100 μl of a 1:5000 dilution of streptavidin-conjugated horseradish peroxidase (Caltag Laboratories, Carlsbad, CA) for 30 minutes at room temperature. Following the incubation, the plates were washed and developed with 100 μl of TMB substrate (Kirkegaard and Perry Laboratories, Inc. Gaithersburg, MD). Plates were read on a SpectraMax plate reader (Molecular Devices, Sunnyvale, CA.) at O.D. 450 nM. The optimal concentration of biotinylated mAb was determined to be 1 nM.

Various concentration (x-axis) of monoclonal antibodies 39.18, 36.89, 81.39 39.29, mAb 9 , mAb 23 of the present invention and control IgG were incubated with the hemagglutinin-coated plates for 30 minutes at room temperature. Initial concentration was 200 nM followed by 3 fold serial dilutions. Biotinylated mAb 39.29 was added to a final sub-saturating concentration of 1 nM. Following one hour incubation, the plates were washed and incubated with 100 μl of a 1:5000 dilution of Streptavidin-conjugated horseradish peroxidase for 45-

minutes. Plates were washed and then develop with TMB solution. If the test antibody competed for the HA epitope of mAb 39.29, the biotin ELISA signal (Y-axis) was decreased as a function of increasing test antibody concentration. The binding data were fit with a non-linear dose response curve to determine the EC_{50} value given in nM.

Figures 21A and 21B show results of competition ELISA analysis of the mAbs for binding to H1HA from A/NWS/1933 (Figure 21A) or H3HA from A/HK/8/1968 (Figure 21B). The results showed that mAb 39.29, mAb 81.39, mAb 39.18, and mAb 36.89 all bind to an overlapping hemagglutinin stalk epitope (Figures 21A and 21B). Specifically, mAb 81.39 and mAb 39.18 compete for binding of mAb 39.29 on the stalk of hemagglutinin H1 (Figure 21A), while mAb 81.39 and mAb 36.89 compete for binding with mAb 39.29 for the identified stalk epitope on hemagglutinin H3 (Figure 21B).

By using competition ELISA assays it was established that monoclonal antibodies 81.39, 39.18, 36.89, mAb 9, and mAb 23 bind to the highly conserved stalk epitope of hemagglutinin identified by the structural analysis. Specifically, the mAb 81.39 and mAb 39.18 compete for binding of mAb 39.29 on the stalk of the Group1 H1 hemagglutinin. Additionally, mAb 81.39, mAb 36.89, mAb 9, and mAb 23 compete for binding with mAb 39.29 for the identified stalk epitope on the Group2 H3 hemagglutinin. As predicted, since mAb 39.18 neutralizes only Group1 Influenza A isolates, it does not compete for binding of the mAb 39.29 epitope on Group2 hemagglutinin. Likewise, mAb 36.89, mAb 9, and mAb 23 only neutralize Group2 Influenza A isolates and therefore do not compete for binding of mAb 39.29 on Group1 H1 hemagglutinin. The data from these experiments is further summarized in Table 3 below.

Table 3

Influenza Isolate	HA Subtype	mAb 39.18	mAb 39.29	mAb 81.39	mAb 36.89	mAb 9	mAb 23
A/NWS/1933	Grp1/H1	0.88	2.8	2.15	-	-	-
A/HK/8/1968	Grp2/H3	-	2.54	4.21	1.32	8.42	1.84

EC_{50} given in nM

- Indicates EC50 >200 nM

Example 14: Safety and pharmacokinetics of anti-influenza A virus antibody in healthy volunteers

A phase 1 single-ascending dose study of mAb 39.29-NWPP in healthy human male and female subjects 18 years of age or older was performed. Initial dosing to investigate the safety,

tolerability, and pharmacokinetics in healthy adult subjects was performed by i.v. administration of a single dose (1.5 mg/kg, 5 mg/kg, 15 mg/kg, or 45 mg/kg) of mAb39.29. mAb39.29 was safe and well-tolerated at all dose levels after a follow-up period of at least 58 days for the 45 mg/kg dose level and 120 days for the 1.5 mg/kg dose level. No serious adverse events related to study drug were reported.

Serum concentrations of mAb 39.29 exhibited a biphasic disposition with an initial rapid distribution phase followed by a slow elimination phase. mAb39.29 demonstrated linear pharmacokinetics (PK). The mean C_{max} increased in a dose-proportional manner of 33.5 μg/mL for the 1.5 mg/kg dose group and 1180 μg/mL for the 45 mg/kg dose group. Similarly, the group mean $AUC_{0\text{-infinity}}$ was 518 and 5530 μg /mL*day for the 1.5 mg/kg and 15 mg/kg dose groups, respectively, and is approximately dose proportional. On the basis of the available PK data in healthy male and female subjects, mAb 39.29 appeared to have a PK profile consistent with that of a typical IgG1 human antibody with a mean half-life of approximately 20 days (Mean Range 19.3-22.2).

Example 15. Phase 2 study of anti-influenza A virus hemagglutinin antibody

A phase 2 clinical study of an anti-influenza A virus hemagglutinin antibody of the present invention is performed as follows. Hospitalized individuals having influenza A virus infection are administered an anti-influenza A virus hemagglutinin antibody of the present invention by intravenous administration, at a dose of 1.5 mg/kg, 5 mg/kg, 15 mg/kg, or 45 mg/kg. Alternatively, individuals are administered antibody at a fixed dose of 120 mg, 400 mg, 1200 mg, or 3600 mg. Individuals may also be administered oseltamivir (Tamiflu®) (current standard of care) prior to, at the time of, or subsequent to administration of the anti-influenza A virus hemagglutinin antibody. Generally, a one-time dosing regimen of the antibody is used, although subsequent doses are contemplated.

Administration of an anti-influenza A virus hemagglutinin antibody of the present invention shows efficacy at treating influenza A virus infection, including reduction of influenza A virus infectivity, reduction in the length of hospital stay, reduction or prevention of the need for intensive care unit use, reduction or prevention of the need for assisted or mechanical ventilation, or reduction or prevention of the need for supplemental oxygen use.

Administration of an anti-influenza A virus hemagglutinin antibody of the present invention results shows efficacy at treating influenza A virus infection by reduction of time to normalization of respiratory function (such as a reduction of time to normalization of

respiratory rate, or a reduction of time to normalization of oxygen saturation), reduction of time to return to normal oxygen saturation, *e.g.*, to an oxygen saturation of about 92% or greater, as measured over a 24 hour period without supplemental oxygen administration, or reduction of time to normalization of vital signs, such as heart rate, blood pressure, respiratory rate, and temperature.

Statistical analyses

Statistics were calculated using JMP version 9.0.2 software (SAS Institute). Survival experiments were compared using log-rank test. *P* values<0.05 were considered significant. IC_{50} curves and values were plotted and calculated using Graphpad Prism version 5.0 software.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literature cited herein are expressly incorporated in their entirety by reference.

WHAT IS CLAIMED IS:

1. An isolated anti-hemagglutinin antibody comprising three heavy chain hypervariable regions (HVR-H1, HVR-H2, and HVR-H3) and three light chain hypervariable regions (HVR-L1, HVR-L2, and HVR-L3), wherein:

(a) HVR-H1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:191 and 192;

(b) HVR-H2 comprises the amino acid sequence of SEQ ID NO:193;

(c) HVR-H3 comprises the amino acid sequence of SEQ ID NO:194;

(d) HVR-L1 comprises the amino acid sequence of SEQ ID NO:195;

(e) HVR-L2 comprises the amino acid sequence of SEQ ID NO:196; and

(f) HVR-L3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:197, 198, and 199.

2. The isolated anti-hemagglutinin antibody of claim 1, wherein the antibody comprises a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 136, 140, 144, 146, 150, 152, and 235.

3. The isolated anti-hemagglutinin antibody of claim 1, wherein the antibody comprises a heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 134, 138, 142, 148, and 234.

4. The isolated anti-hemagglutinin antibody of claim 1, wherein the antibody comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:134, 138, 142, 148, and 234, and the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:136, 140, 144, 146, 150, 152, and 235.

5. The isolated anti-hemagglutinin antibody of claim 1, wherein the antibody comprises a light chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 135, 139, 143, 145, 149, and 151.

6. The isolated anti-hemagglutinin antibody of claim 1, wherein the antibody comprises a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 133, 137, 141, and 147.

7. The isolated anti-hemagglutinin antibody of claim 1, wherein the antibody comprises a heavy chain and a light chain, wherein the heavy chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:133, 137, 141, and 147, and the light chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:135, 139, 143, 145, 149, and 151.

8. An isolated anti-hemagglutinin antibody comprising three heavy chain hypervariable regions (HVR-H1, HVR-H2, and HVR-H3) and three light chain hypervariable regions (HVR-L1, HVR-L2, and HVR-L3), wherein:

(a) HVR-H1 comprises the amino acid sequence of SEQ ID NO:178;

(b) HVR-H2 comprises the amino acid sequence of SEQ ID NO:179;

(c) HVR-H3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:180 and 181;

(d) HVR-L1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:182, 183, 184, 185, and 186;

(e) HVR-L2 comprises the amino acid sequence of SEQ ID NO:187; and

(f) HVR-L3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:188, 189, and 190.

9. The isolated anti-hemagglutinin antibody of claim 8, wherein the antibody comprises a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:113, 117, 119, 122, 124, 126, 128, 130, and 132.

10. The isolated anti-hemagglutinin antibody of claim 8, wherein the antibody comprises a heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:111 and 115.

11. The isolated anti-hemagglutinin antibody of claim 8, wherein the antibody comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID

NOs:111 and 115, and the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:113, 117, 119, 122, 124, 126, 128, 130, and 132.

12. The isolated anti-hemagglutinin antibody of claim 8, wherein the antibody comprises a light chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:112, 116, 118, 121, 123, 125, 127, 129, and 131.

13. The isolated anti-hemagglutinin antibody of claim 8, wherein the antibody comprises a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:110, 114, and 120.

14. The isolated anti-hemagglutinin antibody of claim 8, wherein the antibody comprises a heavy chain and a light chain, wherein the heavy chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:110, 114, and 120, and the light chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:112, 116, 118, 121, 123, 125, 127, 129, and 131.

15. An isolated anti-hemagglutinin antibody comprising three heavy chain hypervariable regions (HVR-H1, HVR-H2, and HVR-H3) and three light chain hypervariable regions (HVR-L1, HVR-L2, and HVR-L3), wherein:

(a) HVR-H1 comprises the amino acid sequence of SEQ ID NO:200;

(b) HVR-H2 comprises the amino acid sequence of SEQ ID NO:201;

(c) HVR-H3 comprises the amino acid sequence of SEQ ID NO:202;

(d) HVR-L1 comprises the amino acid sequence of SEQ ID NO:203;

(e) HVR-L2 comprises the amino acid sequence of SEQ ID NO:204; and

(f) HVR-L3 comprises the amino acid sequence of SEQ ID NO:205.

16. The isolated anti-hemagglutinin antibody of claim 15, wherein the antibody comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO:156.

17. The isolated anti-hemagglutinin antibody of claim 15, wherein the antibody comprises a heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 154 and 158.

18. The isolated anti-hemagglutinin antibody of claim 15, wherein the antibody comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:154 and 158, and the light chain variable region comprises the amino acid sequence of SEQ ID NO:156.

19. The isolated anti-hemagglutinin antibody of claim 15, wherein the antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO:155.

20. The isolated anti-hemagglutinin antibody of claim 15, wherein the antibody comprises a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:153 and 157.

21. The isolated anti-hemagglutinin antibody of claim 15, wherein the antibody comprises a heavy chain and a light chain, wherein the heavy chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:153 and 157, and the light chain comprises the amino acid sequence of SEQ ID NO:155.

22. An isolated anti-hemagglutinin antibody comprising three heavy chain hypervariable regions (HVR-H1, HVR-H2, and HVR-H3) and three light chain hypervariable regions (HVR-L1, HVR-L2, and HVR-L3), wherein:

(a) HVR-H1 comprises the amino acid sequence of SEQ ID NO:206;

(b) HVR-H2 comprises the amino acid sequence of SEQ ID NO:207;

(c) HVR-H3 comprises the amino acid sequence of SEQ ID NO:208;

(d) HVR-L1 comprises the amino acid sequence of SEQ ID NO:209;

(e) HVR-L2 comprises the amino acid sequence of SEQ ID NO:210; and

(f) HVR-L3 comprises the amino acid sequence of SEQ ID NO:211.

23. The isolated anti-hemagglutinin antibody of claim 22, wherein the antibody comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO:162.

24. The isolated anti-hemagglutinin antibody of claim 22, wherein the antibody comprises a heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 160.

25. The isolated anti-hemagglutinin antibody of claim 22, wherein the antibody comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:160, and the light chain variable region comprises the amino acid sequence of SEQ ID NO:162.

26. The isolated anti-hemagglutinin antibody of claim 22, wherein the antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO:161.

27. The isolated anti-hemagglutinin antibody of claim 22, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:159.

28. The isolated anti-hemagglutinin antibody of claim 22, wherein the antibody comprises a heavy chain and a light chain, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO:159, and the light chain comprises the amino acid sequence of SEQ ID NO:161.

29. An isolated anti-hemagglutinin antibody comprising three heavy chain hypervariable regions (HVR-H1, HVR-H2, and HVR-H3) and three light chain hypervariable regions (HVR-L1, HVR-L2, and HVR-L3), wherein:

(a) HVR-H1 comprises the amino acid sequence of SEQ ID NO:212;

(b) HVR-H2 comprises the amino acid sequence of SEQ ID NO:213;

(c) HVR-H3 comprises the amino acid sequence of SEQ ID NO:214;

(d) HVR-L1 comprises the amino acid sequence of SEQ ID NO:215;

(e) HVR-L2 comprises the amino acid sequence of SEQ ID NO:216; and

(f) HVR-L3 comprises the amino acid sequence of SEQ ID NO:217.

30. The isolated anti-hemagglutinin antibody of claim 29, wherein the antibody comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO:166.

31. The isolated anti-hemagglutinin antibody of claim 29, wherein the antibody comprises a heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 164.

32. The isolated anti-hemagglutinin antibody of claim 29, wherein the antibody comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable

region comprises the amino acid sequence of SEQ ID NO:164, and the light chain variable region comprises the amino acid sequence of SEQ ID NO:166.

33. The isolated anti-hemagglutinin antibody of claim 29, wherein the antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO:165.

34. The isolated anti-hemagglutinin antibody of claim 29, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:163.

35. The isolated anti-hemagglutinin antibody of claim 29, wherein the antibody comprises a heavy chain and a light chain, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO:163, and the light chain comprises the amino acid sequence of SEQ ID NO:165.

36. An isolated anti-hemagglutinin antibody comprising three heavy chain hypervariable regions (HVR-H1, HVR-H2, and HVR-H3) and three light chain hypervariable regions (HVR-L1, HVR-L2, and HVR-L3), wherein:

(a) HVR-H1 comprises the amino acid sequence of SEQ ID NO:218;

(b) HVR-H2 comprises the amino acid sequence of SEQ ID NO:219;

(c) HVR-H3 comprises the amino acid sequence of SEQ ID NO:220;

(d) HVR-L1 comprises the amino acid sequence of SEQ ID NO:221;

(e) HVR-L2 comprises the amino acid sequence of SEQ ID NO:222; and

(f) HVR-L3 comprises the amino acid sequence of SEQ ID NO:223.

37. The isolated anti-hemagglutinin antibody of claim 36, wherein the antibody comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO:170.

38. The isolated anti-hemagglutinin antibody of claim 36, wherein the antibody comprises a heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 168.

39. The isolated anti-hemagglutinin antibody of claim 36, wherein the antibody comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:168, and the light chain variable region comprises the amino acid sequence of SEQ ID NO:170.

40. The isolated anti-hemagglutinin antibody of claim 36, wherein the antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO:169.

41. The isolated anti-hemagglutinin antibody of claim 36, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:167.

42. The isolated anti-hemagglutinin antibody of claim 36, wherein the antibody comprises a heavy chain and a light chain, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO:167, and the light chain comprises the amino acid sequence of SEQ ID NO:169.

43. The isolated anti-hemagglutinin antibody of claims 1-42, wherein the antibody is a human antibody.

44. A method for treating, inhibiting, or preventing influenza A virus infection in an individual in need thereof, the method comprising administering to the individual an effective amount of a composition comprising the anti-hemagglutinin antibody of any one of claims 1-28, thereby treating, inhibiting, or preventing influenza A virus infection.

45. The method of claim 44, wherein the method further comprises administering to the individual an additional therapeutic agent.

46. The method of claim 45, wherein the additional therapeutic agent is a neuraminidase inhibitor, an anti-hemagglutinin antibody, or an anti-M2 antibody.

47. A composition comprising the antibody of any one of claims 1-28.

48. A pharmaceutical composition comprising the antibody of any one of claims 1-28 and a pharmaceutically acceptable carrier.

49. An isolated nucleic acid encoding the antibody of any one of claims 1-28.

50. A host cell comprising the nucleic acid of claim 49.

51. A method of producing an antibody comprising culturing the host cell of claim 50 so that the antibody is produced.

52. Use of the anti-hemagglutinin antibody of any one of claims 1-28 in the manufacture of a medicament.

53. The use of claim 52, wherein the medicament is for treating, inhibiting, or preventing influenza A virus infection.