Title: HUMAN MONOCLONAL ANTIBODIES TO INFLUENZA M2 PROTEIN AND METHODS OF MAKING AND USING SAME

Abstract: Human, humanized and chimeric monoclonal antibodies that bind to influenza M2 protein. A human monoclonal antibody that binds to influenza M2 protein having different amino acid sequences. The antibodies are useful for, among other things, treatment, diagnostics, purifying and isolating M2 or influenza virus, and identifying the presence of M2 or influenza virus in a sample or a subject.
For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.
AMENDED CLAIMS

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1. (amended) An isolated antibody that specifically binds to an epitope in influenza virus protein M2 extracellular domain, wherein the antibody binds to an epitope to which the antibody produced by the CHO cell deposited as ATCC PTA-5967 or the hybridoma deposited as ATCC PTA-5968 specifically binds.

2. (canceled)

3. (amended) The antibody of claim 1, wherein the antibody binds to an epitope within the amino acid sequence LLTEVETPIR (SEQ ID NO:1).

4. (amended) The antibody of claim 1, wherein the antibody has the same binding specificity as an antibody produced by the CHO cell deposited as ATCC PTA-5967 or the hybridoma deposited as ATCC PTA-5968 specifically binds.

5. (amended) The antibody of claim 1, wherein the antibody has the same minimal binding sequence as an antibody produced by the CHO cell deposited as ATCC PTA-5967 or the hybridoma deposited as ATCC PTA-5968 specifically binds.

6. The antibody of claim 1, wherein the antibody binds to a minimal binding sequence that is LLTEVETPIR (SEQ ID NO:1).

7. (amended) The antibody of claim 1, wherein the minimal binding sequence for antibody binding is LLTEVETPIR (SEQ ID NO:1).

8. The antibody of claim 1, wherein the antibody comprises the heavy and light chain variable region sequence of the antibody produced by the hybridoma deposited as ATCC PTA-5968.
SLLTEVETPTRNGWECRCNDSSD; SLLTEVETPIRGWECRCNDSSD; SLLTEVETPTRNEWECRCSDSSD; SLLTGVETHTRNGWGCCKCSDSSD and SLLPEVETHTRNGWGCRCSDDSS (SEQ ID NO:2-26 respectively).

24. The antibody of claim 22, wherein the antibody binding to two or more sequences of the M2 extracellular domain is at least 20-50% or more of the amount of binding to M2 peptide SLLTEVETPIRGNEWGCRCNDSSD (SEQ ID NO:2).

25. The antibody of claim 1, wherein the antibody has detectable complement dependent cytotoxicity activity.

26. The antibody of claim 1, wherein the antibody has detectable antibody-dependent cell-mediated cytotoxicity activity.

27. The antibody of claim 1, wherein the antibody is selected from IgG, IgA, IgM, IgE, and IgD isotypes.

28. (amended) The antibody of claim 27, wherein the IgG isotype is selected from IgG1, IgG2, IgG3 and IgG4.

29. The antibody of claim 1, wherein the antibody is produced by a CHO cell line or hybridoma deposited as ATCC Deposit No. PTA-5967 or ATCC Deposit No. PTA 5968.

30. The antibody of claim 1, wherein the antibody has the same or substantially the same binding affinity as an antibody produced by a CHO cell line or hybridoma deposited as ATCC Deposit No. PTA-5967 or ATCC Deposit No. PTA 5968.

31. The antibody of claim 1, wherein the binding affinity is within about 5 to 5000 fold of the antibody produced by a CHO cell line or hybridoma deposited as ATCC Deposit No. PTA-5967 or ATCC Deposit No. PTA 5968.

32. (amended) The antibody of claim 1, wherein the antibody binds to substantially the same minimal binding sequence of M2 peptide as the antibody produced by a CHO cell line or hybridoma deposited as ATCC Deposit No. PTA-5967 or ATCC Deposit No. PTA 5968 binds.
59. (amended) The amino acid subsequence of claim 57, wherein the subsequence is selected from heavy and light chain variable regions (V_H and V_L), Fab, Fab', F(ab')_2, Fv, Fd, scFv, and sdFv.

60. The antibody of claim 1, wherein the antibody comprises an antibody multimer.

61. (amended) The antibody of claim 1 or the amino acid subsequence of claim 57, further comprising one or more heterologous domains.

62. The antibody or the subsequence of claim 61, wherein the heterologous domain comprises an amino acid sequence.

63. The antibody or the subsequence of claim 61, wherein the heterologous domain comprises a binding protein, an enzyme activity, a drug, an antiviral, a toxin, an immune-modulator, a detectable moiety or a tag.

64. The antibody of claim 1, wherein the antibody is a bispecific or bifunctional antibody.

65. The antibody of claim 1, wherein the antibody is produced by a non-human animal having a gene locus encoding human immunoglobulin lambda light chain.

66. The antibody of claim 65, wherein the non-human animal comprises a mammal.

67. The antibody of claim 66, wherein the mammal does not express endogeneous immunoglobulins.

68. The antibody of claim 65, wherein the non-human animal comprises a mouse.

69. The antibody of claim 68, wherein the mouse does not express endogeneous immunoglobulins.

70. The antibody of claim 65, wherein the antibody binds to an epitope comprised within amino acid residues 1 to 24 of the N-terminal side of M2 protein sequence.

71. The antibody of claim 65, wherein the antibody binds to an epitope comprised within amino acids residues 2 to 12 of the N-terminal side of M2 protein sequence.
72. (amended) The antibody of claim 65, wherein the antibody binds to a minimal binding sequence of M2 peptide to which the antibody produced by the hybridoma deposited as ATCC PTA-5968 or the CHO cell deposited as ATCC PTA-5967 binds.

73. (amended) A host cell that expresses an antibody or subsequence of any of claims 1-72.

74. The host cell of claim 73, wherein the antibody has the binding specificity or the same or substantially the same binding affinity of an antibody produced by a CHO cell line or hybridoma deposited as ATCC Deposit No. PTA-5967 or ATCC Deposit No. PTA 5968.

75. The host cell of claim 73, wherein the cell is bacteria, yeast, plant or animal.

76. (amended) A non-human transgenic animal or a plant that expresses an antibody or subsequence of any of claims 1-72.

77. A nucleic acid encoding an antibody produced by a CHO cell line or hybridoma deposited as ATCC Deposit No. PTA-5967 or ATCC Deposit No. PTA 5968.

78. The nucleic acid of claim 77, further comprising a vector.

79. (amended) A composition comprising the antibody or subsequence of any of claims 1-72, and an antiviral agent.

80. (amended) A composition comprising the antibody or subsequence of any of claims 1-72, and an agent that inhibits one or more symptoms or complications associated with influenza virus infection.

81. The composition of claim 80, wherein a symptom or complication is selected from chills, fever, cough, sore throat, nasal congestion, sinus congestion, nasal infection, sinus infection, body ache, head ache, fatigue, pneumonia, bronchitis, ear infection, ear ache and death.

82. (amended) A pharmaceutical composition comprising the antibody or subsequence of any of claims 1-72, and a pharmaceutically acceptable carrier or excipient.

83. (amended) A kit comprising the antibody or subsequence of any of claims 1-72, and instructions for treating, inhibiting, preventing or decreasing susceptibility of infection of a subject by one or more influenza virus strains or isolates.
84. The kit of claim 83, further comprising an article of manufacture for delivery of the antibody into a mucosal tissue.

85. The kit of claim 83, wherein the article of manufacture comprises an inhaler, aerosol, spray or squeeze bottle suitable for inhalation or nasal administration to a subject.

86. The kit of claim 83, wherein the mucosal tissue comprises nasal passages, sinuses, mouth, throat, larynx or lungs.

87. The kit of claim 83, further comprising an antiviral agent.

88. The kit of claim 83, further comprising an agent that inhibits one or more symptoms or complications associated with influenza virus infection.

89. (amended) A method for treating influenza virus infection of a subject, comprising administering to the subject an antibody or subsequence of any of claims 1-72 in an amount effective to treat influenza virus infection of the subject.

90. The method of claim 89, wherein the antibody is administered prior to, substantially contemporaneously with or following influenza virus infection of the subject.

91. The method of claim 89, wherein the subject is immunocompromised.

92. The method of claim 89, wherein the administration provides a therapeutic benefit.

93. The method of claim 92, wherein the therapeutic benefit comprises inhibiting increases in influenza virus titer, decreasing influenza virus titer, inhibiting increases in influenza virus replication, decreasing influenza virus replication, inhibiting increases in influenza virus proliferation or decreasing influenza virus proliferation, or decreasing progression, severity, frequency, duration or probability one or more symptoms or complications associated with influenza virus infection in a subject.

94. The method of claim 93, wherein a symptom or complication is selected from chills, fever, cough, sore throat, nasal congestion, sinus congestion, nasal infection, sinus
infection, body ache, head ache, fatigue, pneumonia, bronchitis, ear infection, ear ache and death.

95. The method of claim 92, wherein the therapeutic benefit comprises hastening a subject’s recovery from influenza virus infection.

96. (canceled)

97. (canceled)

98. (canceled)

99. (canceled)

100. The method of claim 89, wherein the influenza virus is human or avian.

101. The method of claim 89, wherein the influenza virus is a strain or isolate selected from A/PR/8/34, A/HK/8/68, A/HK/1/68, A/HK/156/97, A/Turkey/VA/158512/02, A/Viet Nam/1196/04, or a H1N1, H2N2, H3N2, H5N1, H9N2, H2N1, H4N6, H6N2, H7N2, H7N3, H4N8, H5N2, H2N3, H11N9, H3N8, H1N2, H11N2, H11N9, H7N7, H2N3, H6N1, H13N6, H7N1, H11N1, H7N2 and H5N3 subtype.

102. The method of claim 89, wherein the antibody binds to a minimal binding sequence that is LLTEVETPIR (SEQ ID NO: 1).

103. (canceled)
104. (canceled)

105. (canceled)

106. (amended) A method for inhibiting infection of a subject by one or more influenza virus strains or isolates comprising administering to the subject an antibody or subsequence of any of claims 1-72 in an amount effective to inhibit infection of the subject by one or more influenza virus strains or isolates.

107. The method of claim 106, wherein the subject is not currently infected with influenza virus.

108. The method of claim 106, wherein the subject does not exhibit one or more symptoms or complications associated with influenza virus infection.

109. The method of claim 106, wherein the subject has been exposed to or contacted with influenza but does not exhibit one or more symptoms or complications associated with influenza virus infection.

110. The method of claim 106, wherein the antibody is administered prior to, substantially contemporaneously with or following influenza virus infection of the subject.

111. The method of claim 106, wherein the subject is immunocompromised.

112. The method of claim 106, wherein the administration provides a therapeutic or prophylactic benefit.

113. The method of claim 112, wherein the benefit comprises protecting the subject from influenza virus infection or decreasing susceptibility of the subject from influenza virus infection.
114. (canceled)

115. (canceled)

116. (canceled)

117. (canceled)

118. The method of claim 106, wherein the influenza virus strain or isolate is human or avian.

119. The method of claim 106, wherein the influenza virus strain or isolate is selected from A/PR/8/34, A/HK/8/68, A/HK/1/68, A/HK/156/97, A/Turkey/VA/158512/02, A/Viet Nam/1196/04, or a H1N1, H2N2, H3N2, H5N1, H9N2, H2N1, H4N6, H6N2, H7N2, H7N3, H4N8, H5N2, H2N3, H11N9, H3N8, H1N2, H11N2, H11N9, H7N7, H2N3, H6N1, H13N6, H7N1, H11N1, H7N2 and H5N3 subtype.

120. (canceled)

121. (canceled)

122. (canceled)
123. (canceled)

124. (canceled)

125. The method of claim 106, wherein the antibody comprises a heavy-chain variable sequence or a light-chain variable sequence encoded by the nucleic acid sequences set SEQ ID NOS:32, 33 and 36, or a nucleic acid sequence degenerate with respect to the nucleic acids set forth in SEQ ID NOS:32, 33 and 36.

126. The method of claim 106, wherein the antibody comprises a heavy-chain variable sequence or a light-chain variable sequence as set forth in SEQ ID NOS:34, 35 and 37.

127. (canceled)

128. A nucleic acid that encodes a heavy-chain variable sequence or a light-chain variable sequence as set forth in SEQ ID NOS:34, 35 and 37.

129. A nucleic acid that encodes a heavy-chain variable and constant region sequence or a light-chain variable and constant region sequence as set forth in SEQ ID NOS:32, 33 and 36.

130. (amended) A method of producing a human M2 antibody of any of claims 1-56, 60-72, comprising:

a) administering at least two M2 peptides each peptide having a different amino acid sequence, or an immunogenic fragment of two M2 peptides each immunogenic fragment having a different amino acid sequence, to an animal capable of expressing human immunoglobulin;

b) screening the animal for expression of human M2 antibody;

c) selecting an animal that produces a human M2 antibody;

d) isolating an antibody from the animal that produces human M2 antibody; and
e) determining whether the human M2 antibody binds to M2 protein.

131. (amended) A method of producing a human M2 antibody of any of claims 1-56, 60-72, comprising:
   a) administering at least two M2 peptides each peptide having a different amino acid sequence, or an immunogenic fragment of two M2 peptides each immunogenic fragment having a different amino acid sequence, to an animal capable of expressing human immunoglobulin;
   b) screening the animal for expression of human M2 antibody;
   c) selecting an animal that produces an human M2 antibody;
   d) isolating spleen cells from the animal that produces human M2 antibody;
   e) fusing the spleen cells with a myeloma cell to produce a hybridoma; and
   f) screening the hybridoma for expression of a human M2 antibody.

132. (amended) A method of producing a human M2 antibody of any of claims 1-56, 60-72, comprising:
   a) administering a peptide sequence comprised within an M2 protein to a non-human animal having a gene locus encoding human immunoglobulin lambda light chain;
   b) screening the non-human animal for expression of human M2 antibody;
   c) selecting an non-human animal that produces an human M2 antibody;
   d) isolating spleen cells from the non-human animal that produces human M2 antibody;
   e) fusing the spleen cells with a myeloma cell to produce a hybridoma; and
   f) screening the hybridoma for expression of a human M2 antibody.

133. The method of any of claims 130 to 132, wherein the M2 peptides comprise an M2 extracellular domain.

134. The method of any of claims 130 to 132, wherein a minimal binding sequence for the human M2 antibody is the same or substantially the same as LLTEVETPIR (SEQ ID NO:1).

135. The method of any of claims 130 to 132, wherein the human M2 antibody binds to a minimal binding sequence that is LLTEVETPIR (SEQ ID NO:1).

136. The method of claim 130 or 131, wherein the animal is a non-human animal.
137. (amended) A method of producing a human M2 antibody of any of claims 1-56, 60-72, comprising:
   a) providing a CHO cell line or hybridoma deposited as ATCC Deposit No. PTA-5967 or ATCC Deposit No. PTA 5968 that produces a human M2 antibody; and
   b) isolating an antibody from the CHO cell line or hybridoma.

138. (amended) A method of producing a human M2 antibody of any of claims 1-56, 60-72, comprising:
   a) providing an animal that produces a human M2 antibody having the binding specificity of the antibody produced by a CHO cell line or hybridoma deposited as ATCC Deposit No. PTA-5967 or ATCC Deposit No. PTA 5968;
   b) isolating spleen cells from the animal that produces the human M2 antibody;
   c) fusing the spleen cells with a myeloma cell to produce a hybridoma; and
   d) screening the hybridoma for expression of human M2 antibody having the binding specificity of the antibody produced by a CHO cell line or hybridoma deposited as ATCC Deposit No. PTA-5967 or ATCC Deposit No. PTA 5968.

139. The method of claim 137 or 138, wherein the animal or cell is non-human.

140. The method of claim 137 or 138, wherein the animal or cell expresses an antibody having the same or substantially the same binding affinity of antibody produced by a CHO cell line or hybridoma deposited as ATCC Deposit No. PTA-5967 or ATCC Deposit No. PTA 5968.

141. The method of claim 137 or 138, wherein a minimal binding sequence for the human M2 antibody is the same or substantially the same as LLTEVETPIR (SEQ ID NO:1).

142. The method of claim 137 or 138, wherein the human M2 antibody binds to a minimal binding sequence that is LLTEVETPIR (SEQ ID NO:1).