A broadly neutralizing human monoclonal antibody against the hemagglutinin of avian influenza virus H7N9

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Abstract

Background: The new emerging avian influenza A H7N9 virus, causing severe human infection with a mortality rate of around 41%. This study aims to provide a novel treatment option for the prevention and control of H7N9.

Methods: H7 hemagglutinin (HA)-specific B cells were isolated from peripheral blood plasma cells of the patients previously infected by H7N9 in Jiangsu Province, China. The human monoclonal antibodies (mAbs) were generated by amplification and cloning of these HA-specific B cells. First, all human mAbs were screened for binding activity by enzyme-linked immunosorbent assay. Then, those mAbs, exhibiting potent affinity to recognize H7 HAs were further evaluated by hemagglutination-inhibiting (HAI) and microneutralization *in vitro* assays. Finally, the lead mAb candidate was selected and tested against the lethal challenge of the H7N9 virus using murine models.

Results: The mAb 6-137 was able to recognize a panel of H7 HAs with high affinity but not HA of other subtypes, including H1N1 and H3N2. The mAb 6-137 can efficiently inhibit the HA activity in the inactivated H7N9 virus and neutralize 100 tissue culture infectious dose 50 ($TCID_{50}$) of H7N9 virus (influenza A/Nanjing/1/2013) in *vitro*, with neutralizing activity as low as 78 ng/mL. In addition, the mAb 6-137 protected the mice against the lethal challenge of H7N9 prophylactically and therapeutically.

Conclusion: The mAb 6-137 could be an effective antibody as a prophylactic or therapeutic biological treatment for the H7N9 exposure or infection.

Keywords: Avian influenza; H7N9; Monoclonal antibody; Neutralizing activity

Introduction

The avian influenza A (H7N9) virus was first reported in March 2013, which caused sporadic human infections after exposure from poultry.[1,2] From March 2013 to February 24, 2017, a total of 1258 laboratory-confirmed cases of the H7N9 virus, human infection, were reported in China, with a mortality of 41%.[3,4] This A/H7N9 influenza virus is antigenically distinct from the other circulating seasonal influenza viruses; thus, standard seasonal vaccines are not protective against this infection.[5] Although H7N9 viruses are typically sensitive to neuraminidase inhibitors or M2 ion channel blockers, H7N9 resistant variants to treat these antiviral agents have been reported.[6,7] Thus, there is an urgent need for developing a passive immunization with antibodies to combat H7N9 influenza A and protect those high-risk populations, including the immunocompromised or elderly individuals.

Generally, the influenza hemagglutinin (HA) is a primary antigenic target for the humoral immune responses.[8] The antibodies that possess hemagglutination-inhibiting (HAI) activity for the globular domain during natural infection or vaccination are widely accepted as an indispensable component in the immune response of the influenza A

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virus.[9,10] However, most neutralizing antibodies elicited during influenza virus infection or vaccination target immunodominant, variable epitopes on the globular head region of HA, which eventually leads to narrow strain protection due to the hypervariability of this region.[11,12]

In the current paper, we reported the characteristics of a novel human monoclonal antibody (mAb) that can broadly recognize a panel of circulating H7N9 strains isolated from either patient, avian, or environment. More importantly, this human mAb was highly effective in inhibiting the H7N9 virus in *in vitro* and *in vivo* studies, revealing a conservative epitope located around the globular head of the H7 HA.

Materials and methods

Ethics approval

This study was reviewed and approved by Research Ethics Committee of Jiangsu Provincial Center for Disease Control and Prevention (Approval No. JSJK2013-A02-02), and the written informed consent was obtained from each patient before taking blood samples. The animal experiments were conducted in adherence to the Chinese national guidelines for the care of laboratory animals. The protocol was approved by the Institutional Animal Care and Use Committee of the Institute of Laboratory Animal Science at Peking Union Medical College (No.ILAS-PC-2013-008).

Study design

From March to December 2013, patients ($n=9$) confirmed with H7N9 infection in Jiangsu Province were followed up as potential donors with a potent neutralizing serum for the H7N9 virus. At 6 months post-infection, we collected blood samples from three patients who had recovered and had strong neutralizing antibody response for the H7N9 virus. The written informed consent was obtained from each donor. We isolated the H7 HA-specific B cells from the peripheral blood plasma cells of the donors. We then generated human mAbs by amplification and cloning of these HA-specific B cells. All expressed human mAbs were first screened for the binding activity using enzyme-linked immunosorbent assay (ELISA). Then, those exhibiting potent recognition for H7 HAs were further evaluated for the HAI and microneutralization assays *in vitro*. Finally, the lead mAb candidate was selected and tested against the lethal challenge of the H7N9 virus in the murine model. The experiments were performed in an animal biosafety level 3 facility using high-efficiency particulate air-filtered isolators.

Isolation, amplification, and cloning of HA-specific B cells

The H7 HA-specific B cells were sorted from the peripheral blood plasma cells using flow cytometry (BD Aria IIu, Becton, Dickinson and Company, Franklin, New Jersey, USA) at the Institution of Nutrition Sciences, Chinese Academy of Sciences. The immunoglobulin G (IgG) memory cells were stained with allophycocyanin (APC)-labeled recombinant soluble H7 HA (Anhui/1/2013; Immune Technology, Suzhou, China), coupled with the following antibodies: BV421-labeled anti-human IgG, fluorescein isothiocyanate-labeled CD14, PE-Texas-Red-labeled CD19, peridinin chlorophyll protein-Cyanine 5.5-labeled immunoglobulin M (IgM), Apc-Cyanine7 labeled CD3, and P-phycoerythrin (PE)-Cyanine7 labeled CD20. The dead cells were stained with Aqua stain (All mAbs used for labeling were purchased from eBioscience™, San Diego, California, USA).[7] All reagents were titrated in advance and used at optimal concentrations for the flow cytometry. The $CD19^{+}CD3^{-}CD14^{-}CD20^{+}IgM^{-}IgG^{+}$ H^{7} HA-specific memory B cells were sorted and plated at a single cell per well. The variable regions of the heavy chain (V_H) and light chain (V_L) gene transcripts were retrieved by the single-cell reverse transcription-polymerase chain reaction (RT-PCR) and amplified separately using nested PCR in 96-well PCR plates.[13] The paired V_H and V_L genes were constructed into the eukaryotic expression vector using cloning. Then, recombinant plasmids were transfected into 29 F cells by the polyetherimide. After 5 days, IgG was purified from the supernatant using Protein A chromatography (GE Healthcare, Pittsburgh, Pennsylvania, USA) and was desalted against phosphate buffer saline (PBS).[14]

ELISA binding assays

The purified human mAbs were tested initially for the binding activity against HA H7N9/A/Shanghai/1/2013 (GenBank: KF609511.1) using ELISA. Then, the binding breadth of selected mAbs was tested with a panel of HAs, including H7N9/A/Netherlands/219/03, H7N3/A/wild bird/Korea/A4/2011, H7N3/A/environment/California/7451/2010, H1N1/A/California/06/2009, and H3N2/A/Brisbane/299/2011. Recombinant HAs with 200 ng/well were coated on the 96-well microtiter plates at 37°C for 4 h. After incubation, the plates were washed twice and then blocked with 5% non-fat milk in 1× phosphate-buffered saline with Tween-20 at room temperature for 30 min. Serially diluted human H7N9 mAbs with an initial 1000 ng/mL concentration were added into the wells and then mixed with the horseradish-peroxidase-conjugated goat anti-human IgG (1:2000, Sigma Aldrich, St. Louis, MO, USA). The absorbance at 450 nm with a spectramax plate reader (Autobio Diagnostics Co., Zhengzhou, China). The serum from the H7N9 patient donors was used as a positive control, while the serum from an H7N9 infection-free donor was used for negative controls. All samples were run in experimental triplicates. Analogous studies were also performed to evaluate the binding affinity of the germline gene-encoded H7N9 human mAb with the H7 HA.[15]

HAI assay

The inhibition of hemagglutination activities of the selected human mAb was measured using a modified horse red blood cell HAI assay, as recommended by the World Health Organization (WHO).[16] The inactivated H7N9 viruses (influenza A/Nanjing/1/2013) with a standardized quantity of four HA units were mixed with the serially diluted receptor destroying enzyme-treated human mAb with an initial concentration of 1000 ng/mL

or donor's serum as controls (1:5 initial dilution) in 96-well V bottom microtiter plates, followed by incubation at room temperature for 30 min. Then, 50 μL of 1% horse red blood cells were added to each well, and then the plates were incubated at room temperature for another 30 min before the final reading for HAI titers. The highest dilution of the mAb causing complete hemagglutination inhibition was considered as the HAI titration end-point.

Microneutralization assay

The neutralizing ability of selected human mAb against influenza virus was tested using the microneutralization assay, adapted from the WHO manual for the laboratory diagnosis and virological surveillance of influenza in a Biosafety level 3 laboratory.[17] Briefly, 100 tissue culture infectious dose 50 ($TCID_{50}$) of H7N9 virus (influenza A/Nanjing/1/2013) was mixed with the two-fold serial dilutions of human mAb, with an initial concentration of 0.2 mg/mL or donor's serum as controls (1:10 initial dilution) in 96-well tissue culture plates and incubated for 1 h at 37°C. Next, Madin-Daby canine kidney cells were added to the plates (4.5×10^4 cells/well), followed by 18 h incubation at 37°C with 5% CO_2. To establish the endpoint, cell monolayers were washed with PBS and fixed in acetone. Next, indirect ELISA was used to detect the viral antigen with a mouse mAb against influenza A Nucleo-protein (NP, Millipore, mixed A1, A3) and a horseradish-peroxidase-conjugated sheep anti-mice IgG (KPL, Milford, Massachusetts, USA). After that, plates were visualized by O-phenylenediamine, and absorbance was taken at 492 nm. The highest dilution of the mAb neutralizing the 100 $TCID_{50}$ of the H7N9 virus was recorded.

Prophylactic and therapeutic studies

Specific-pathogen-free, 6-week-old, female BALB/c mice were randomly assigned to the experimental or control groups. Each group had eight mice, with four mice per cage. The staff monitoring these mice were blinded to the group allocation. To examine the therapeutic efficacy, BALB/c mice were infected intranasally with 50 μL of five median lethal doses (MLD_{50})of H7N9 (A/Anhui/1-YK_RG25/2013, GenBank: CY191843.1) 2 h before being passively immunized with either 5, 10, or 20 mg/kg of the candidate human mAb 6-137 or 20 mg/kg of a control human mAb 3-2 using intraperitoneal injection. In addition, for prophylactic efficacy against H7N9, candidate human mAbs at 5 or 10 mg/kg were administered into mice 2 h before intranasal challenge with five MLD_{50} of H7N9 virus (A/Anhui/1-YK_RG25/2013), whereas the irrelevant human mAb, 3-2 was administered to the control group mice. The body weight and mortality of the mice were monitored for up to 14 days post-challenge. Mice that lost 30% of the initial body weight were euthanized.

For a better understanding of the candidate's potential protective ability, 6 or 24 h after the challenge, the human mAb 6-137 (20 mg/kg) was administered to the mice. In addition, the animals were observed for survival and weight loss post-challenge.

Histological examination

Three of eight mice per group received the candidate human mAbs, 2 h post-challenge therapeutically or 1 day before the challenge prophylactically. The test and the control animals were necropsied according to a standard protocol on days 2 and 4, respectively. The lungs were fixed into 10% neutral buffered formaldehyde and embedded in the paraffin. Tissue sections (5 mm) were analyzed microscopically after staining with hematoxylin & eosin. A blinded pathologist graded scoring of lung pathology.

Results

Isolation and characterization of H7N9 HA-specific mAb in vitro

Blood samples were collected from the convalescent patients previously infected by the H7N9 A/Nanjing/1/2013 strain (GenBank: KC896774.1). First, H7 HA-specific B cells were isolated, and then human mAbs were generated by the amplification and cloning of these HA-specific B cells. As a result, we identified one secreted mAb 6-137, isolated from the H7 HA-specific human B cells, potent enough to bind with an entire panel of expressed H7 HAs with high affinity, including those from avian and environment [Figure 1A and Table 1]. However, the mAb 6-137 had no detectable binding with the 2009 H1N1 or 2011 H3N2 strain HAs. In addition, the cross-reactivity of the mAb 6-137 with a broad range of H7 viruses indicated conserved antigenic sites on H7 HA.

Further, mAb 6-137 inhibited the HA activity in the inactivated H7N9 influenza A/Nanjing/1/2013 by using the even lowest mAb HAI concentration of 6.25 μg/mL. However, no inhibition of HA activity was noticed in either serum from an H7N9-negative donor or in H7 irrelevant human mAb 3-2 [Figure 1B]. The mAb 3-2 was also isolated from the convalescent H7N9 patients, using the same procedure for isolating mAb 6-137. However, mAb 3-2 did not inhibit H7 HA activity and therefore was used as a negative human mAb control. Based on the inhibition ability in HAI, we speculated that the epitope responsible for getting recognized by the mAb 6-137 was likely to be located on the globular head region of H7 HA.[18]

The highest dilution of the mAb 6-137 with an initial 0.1 mg/mL concentration for neutralizing 100 median $TCID_{50}$ of H7N9 virus (A/Nanjing/1/2013) was 1:1280. Of note, it also showed neutralizing potential to H7N9, at a minimum concentration of 78 ng/mL.

Prophylactic efficacy of the human mAb in vivo

The protective efficacy of the candidate human mAb 6-137 was analyzed in lethally challenged H7N9 influenza A/Anhui/1-YK_RG25/2013 (GenBank: CY191843.1) infected mice [Figure 2A]. A significant prophylactic potential of mAb 6-137 with 100% survival in mice was also evident in both 5 and 10 mg/kg treatment groups. On the other hand, injected with an irrelevant human mAb

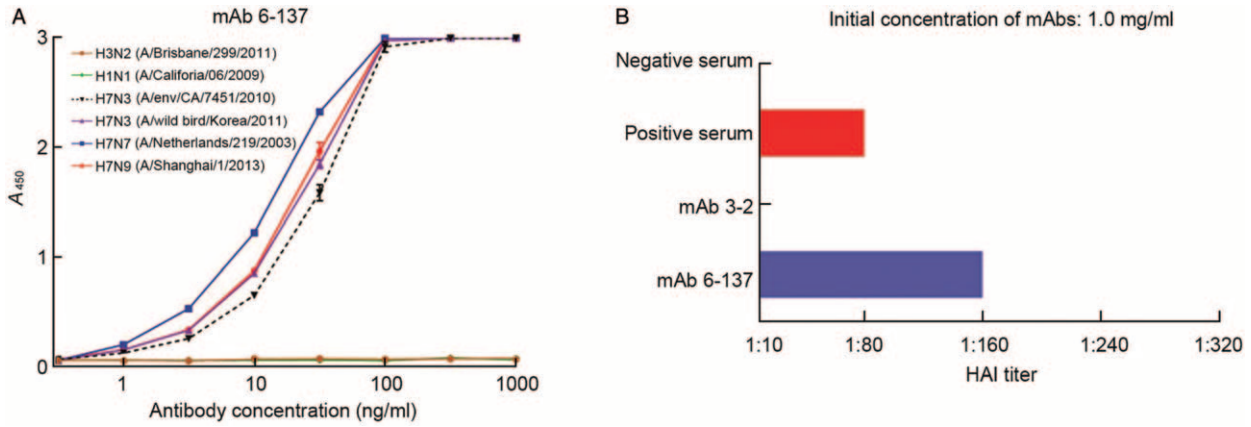

Figure 1: *In vitro* binding and inhibition of HA for the human mAb. (A) The binding of mAb 6-137 with a panel of HAs was determined by ELISA. (B) The highest dilution Inhibited four HA unit of the inactivated H7N9 A/Nanjing/1/2013 was measured by the modified horse red blood cell haemagglutination-inhibition assay. The mAb 3-2 is an H7N9-irrelevant human mAb and is used as a human mAb control. The initial human mAb concentration (6-137 and 3-2) is 1.0 mg/mL. The initial dilution of the donor's serum is 1:10. ELISA: Enzyme-linked immunosorbent assay; HA: Hemagglutinin; HAI: Hemagglutinin-inhibiting; mAb: Monoclonal antibody.

Table 1: Human mAb ELISA end-point titer against recombinant HA.

Recombinant HA	Human mAb 6-137 ELISA end-point titer (mg/mL)
H7N9 A/Shanghai/1/2013	1.0×10^{-3}
H7N7 A/Netherlands/219/2003	1.0×10^{-3}
H7N3 A/wild bird/Korea/2011	1.0×10^{-3}
H7N3 A/env/CA/7451/2010	1.0×10^{-3}
H1N1 A/California/06/2009	>1.0
H3N2 A/Brisbane/299/2011	>1.0

The end-point titer for ELISA was defined as the lowest mAb concentration with an optional density (OD) value >0.1 with the tested HAs. The OD at >1.0 mg/mL indicated that the end-point titer for ELISA was not achieved up to the tested concentration. ELISA: Enzyme-linked immunosorbent assay; HA: Hemagglutinin; mAb: Monoclonal antibody.

3-2, none of the mice survived in the control group. However, after the challenge, moderate but transient weight loss was observed in the mice of the prophylactic groups of mAb 6-137 at both doses, especially in the 10 mg/kg group. Furthermore, post-challenge, virus titers in the mice lungs were substantially reduced in the prophylactic groups of mAb 6-137 at 10 mg/kg. The above-mentioned results indicated that the candidate human mAb 6-137 had the potential to be used prophylactically [Supplementary Figure 1, http://links.lww.com/CM9/A9481].

Therapeutic efficacy of the human mAbs in vivo

The therapeutic efficacy of mAb 6-137 to protect mice from lethal infection with H7N9 influenza A/Anhui/1-YK_RG25/2013 was tested at 2 h post-challenge [Figure 2B]. mAb 6-137 provided complete survival protection when administrated at 10 or 20 mg/kg. However, no specific protection was observed in animals who received 5 mg/kg of the mAb 6-137. The control mice received an irrelevant mAb 3-2, died, or were euthanized because of 30% body weight loss within the 11 days after the challenge. All mice uniformly showed significant weight losses within 5 days after the virus challenge. Still, those who received 20 mg/kg of mAb 6-137 gradually recovered their body weight later during the observation period. Pulmonary virus titers in the lungs from the group treated with 20 mg/kg of mAb 6-137 were substantially inhibited and hardly detected at day 4 post-challenge. In contrast, those from the control group constantly had high titers of the virus [Supplementary Figure 1, http://links.lww.com/CM9/A9481]. However, less therapeutic efficacy was observed in the mice, who received mAb 6-137 at 6 h post-challenge, but no detectable therapeutic efficacy was demonstrated at 24 h post-challenge [Figure 3].

Characterization of the germline antibody of the human mAb

For investigating the changes in binding affinity with the H7 HA, we produced the mAb 6-137 germline version (H-GL or/and L-GL) [Figure 4]. Although the germline gene-encoded mAb 6-137 recognized the H7 HA but with much lower efficiency. Compared with the replacement of germline gene-encoded V_L, the replacement germline gene-encoded V_H resulted in a significant loss of binding. Besides, more somatic mutations were found in germline gene-encoded V_H than those in the V_L [Supplementary Table 1, http://links.lww.com/CM9/A948]. The contribution of somatic mutations to the V_H gene was more significant for the high binding affinity of mAb 6-137, demonstrating an optimal evolution and maturation of viral sequences in nature. Additionally, a similar binding affinity was found with the H7 HA1 and the trimeric HA. This finding indicated that the mAb 6-137 could only recognize the receptor region on H7 HA1.

Discussion

H7N9 viruses are antigenically distinct from the rest of the circulating seasonal influenza virus. Therefore, the human population at large is immunologically naïve to the

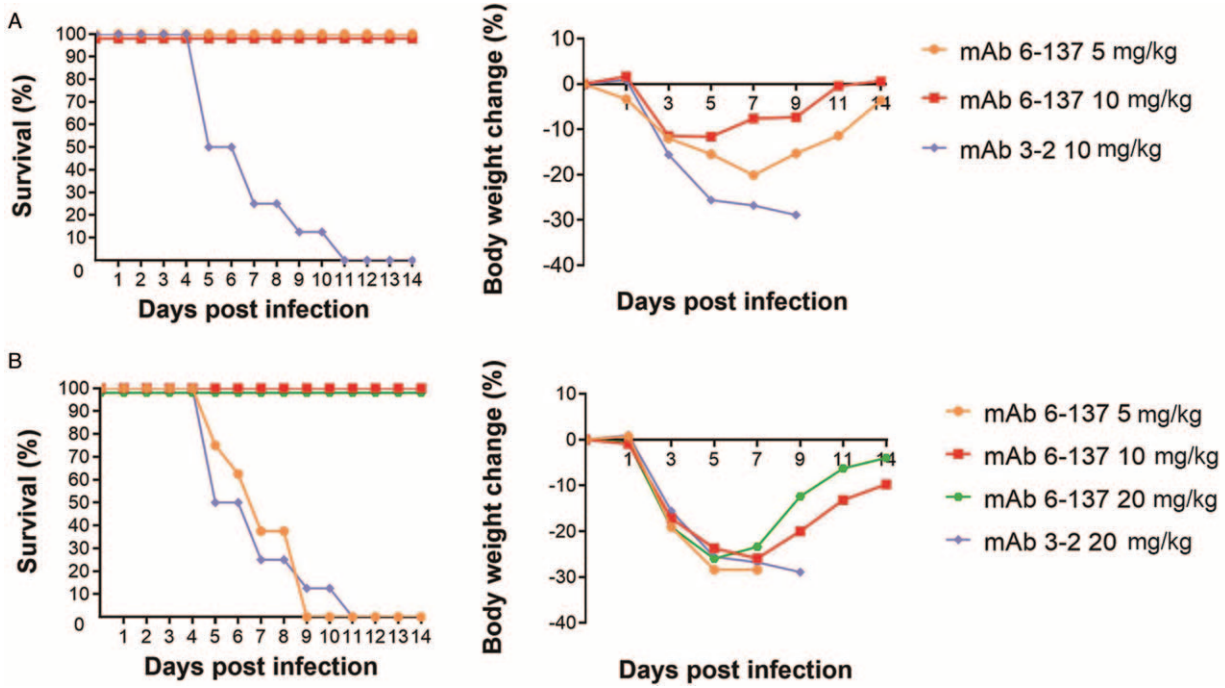

Figure 2: Prophylactic and therapeutic efficacy of human mAb 6-137 protects mice against the challenge of H7N9. (A) BALB/c mice were dosed with two different concentrations of the human neutralizing mAbs and subsequently challenged for five MLD_{50} of H7N9 influenza A/Anhui/1-YK_RG25/2013; (B) BALB/c mice were infected intranasally with 5 MLD_{50} of H7N9 influenza A/Anhui/1-YK_RG25/2013 2 h before passively immunizing with the human neutralizing mAbs. Mice were followed for survival (left) and body weight change (right). mAb: Monoclonal antibody; MLD: Median lethal doses.

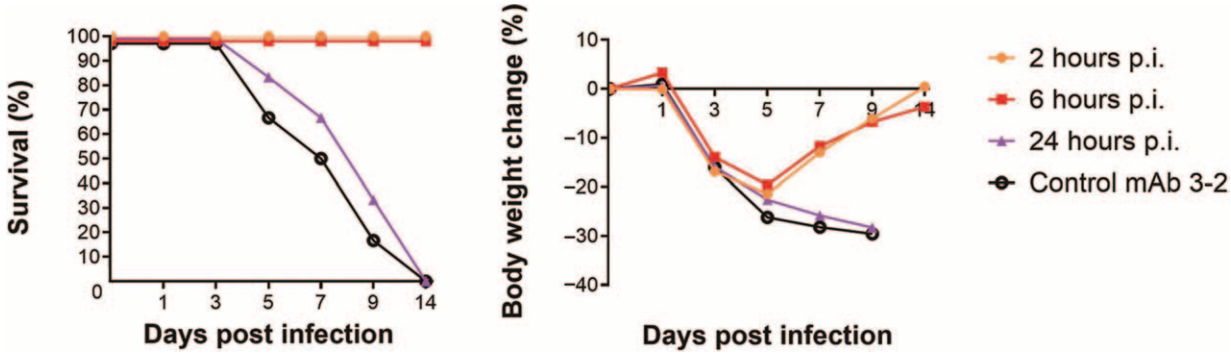

Figure 3: Therapeutic efficacy of human mAb 6-137 in mice after the challenge of H7N9 at 2, 6, and 24 h post-infection. Mice received mAb 6-137 at 20 mg/kg at 2, 6, and 24 h postinfection with Ave MLD_{50} of H7N9 influenza A/Anhui/1/2013. Mice were followed for survival curves (left) and body weight loss (right). Control antibody (mAb 3-2) was administered at the highest concentration at 2 h post-challenge. mAb: Monoclonal antibody; MLD: Median lethal doses; p.i.: post infection.

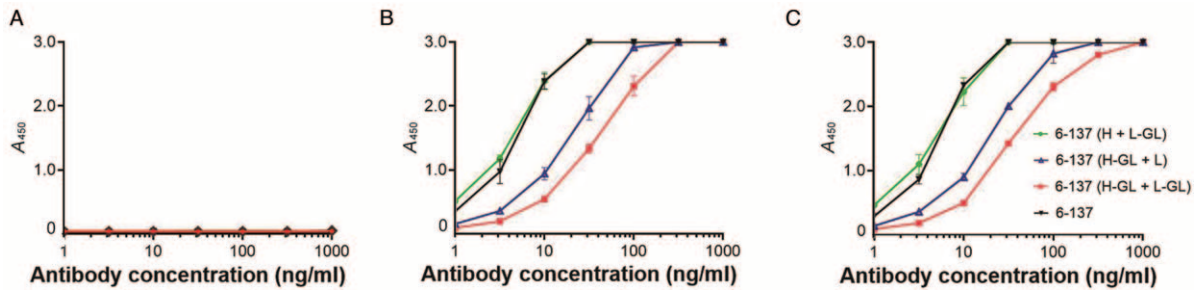

Figure 4: The binding affinity of human neutralizing mAb 6-137 germline version. The binding affinity of the germline antibodies 6-137 (H-GL or L-GLor H-GL+ L-GL) with (A) H1, (B) H7 HA trimers, and (C) H7 HA1 were compared with the matured mAb 6-137 and determined by ELISA. ELISA: Enzyme-linked immunosorbent assay; HA: Hemagglutinin; H-GL: High-chain germline; L-GL: Light-chain germline; mAb: Monoclonal antibody.

subtypes of H7 viruses.[19] Although the human infection with H7N9 has emerged sporadically, no evidence of sustained human-to-human transmission has come to light. Besides, the H7N9 viruses have the highest risk score. They are generally characterized for possessing moderate to high potential pandemic risk.[20] Similarly, the H7N9 virus is a potentially deadly strain, which can cause numerous cases with a high mortality rate.[21] Moreover, the prolonged hospital stays with severe disease progress of H7N9 infected patients were also associated with the late recruitment of the neutralizing antibodies, as well as $CD8^+/CD4^+$ T cells.[22] Unfortunately, an efficient H7N9 vaccine is still unavailable and limited to the general public. Therefore, identifying human neutralizing antibodies that can respond naturally to the H7N9 viruses by determining the molecular basis for the neutralization mechanism is essential for developing the prophylactic and therapeutic biological treatment for H7N9.

The early outbreak of virus isolates H7N9 A/Shanghai/1/2013 was predominantly connected to avian receptors, although this strain had limited human receptor binding. However, the later dominant prevalent strain of H7N9 A/Anhui/1-YK_RG25/2013 demonstrated preferential binding to the avian receptors, increasing binding to human receptors.[23] Interestingly, the adaptation of viruses to human transmission might involve multiple genetic factors, but the receptor-binding properties of the HA glycoprotein remain very crucial.[24,25] In our study, we used blood samples collected from the previously infected patients of H7N9 A/Nanjing/1/2013 strain (has a similarity of 99.8% with H7N9 A/Anhui/1-YK_RG25/2013 strain), during the first wave of an H7N9 outbreak in Jiangsu Province [Supplementary Table 2, http://links.lww.com/CM9/A948]. However, none of the sequence differences caused the change in the amino acid at the virus's receptor-binding domain (RBD) region. We sorted the peripheral blood plasma cells from the donors and obtained human mAb 6-137. This mAb demonstrated a strong binding affinity to a branch of the H7 virus of various sources, with a significant neutralization potency to the H7N9 A/Nanjing/1/2013. More importantly, both prophylactic and therapeutic efficacy of the human mAb 6-137 were observed in the H7N9 A/Anhui/1-YK_RG25/2013 lethally challenged mice. These results indicate that this antibody might target a relatively well-conserved epitope among circulating H7N9 viruses. Besides, as a naturally generated antibody following the immune response to the H7N9 infection, the human mAb 6-137 is specific to H7, but it could not recognize either H1 or H3. Moreover, the relatively high specificity to H7 of the human mAb 6-137 is distinct from some other previously reported broadly neutralizing mAbs of H7N9.[26]

The epitopes on the H7 HA, which are recognized by the human mAb 6-137, were not defined yet. But the human mAb 6-137 located around the RBD region on HA1 of the H7N9 HA considers its evident inhibition ability in HAI. Further study is essential for determining the epitope of clone 6-137 by cryo-EM or acquisition of the mutant escape virus. Post-challenge, the significant protection of the mAb 6-137 was observed in mice at 2 h but not at the 6 or 24 h later. These results further indicate the capability of mAbs for blocking the entry of the H7N9 virus into cells at an initial step in the infection process. An effective intervention at the early phase of the infection of H7N9 could be of high importance for slowing the progress of the disease and improving the prognosis. This would be further important for the condition where the development of resistance to oseltamivir and the adamantanes has already been reported for H7N9.[27] Additionally, as a human-origin mAb, the mAb 6-137 is more likely to have a superior safety profile than the mouse neutralizing mAbs against the H7N9 virus.[28] The human-origin mAbs do not have the antigenicity for mouse xenogeneic proteins and thus are less likely to attack the human immune system. Several other human origins with various neutralizing potencies, neutralizing antibodies against H7N9, have been recognized previously.[29] These neutralizing antibodies and the mAb 6-137 were generated initially in response to these natural infectious H7N9 viruses. Moreover, they had protected the infected individual, which could represent the potential interventions in the event of an H7N9 pandemic. This study identifies a highly efficient neutralizing human mAb 6-137 against H7N9 from previously H7N9 infected individuals. Further, the human mAb 6-137 is a promising prophylactic or therapeutic biological treatment for H7N9 exposure or infection. However, additional tests using other animal models are required to determine further the potential efficacy and utility of the passive immunity of the mAb 6-137 in humans.

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Conflicts of interest

None.

References

1. Gao R, Cao B, Hu Y, Feng Z, Wang D, Hu W, *et al*. Human infection with a novel avian-origin influenza A (H7N9) virus. New Engl J Med 2013; 368:1888–1897. doi: 10.1056/NEJMoa1304459.
2. Lam TTY, Wang J, Shen Y, Zhou B, Duan L, Cheung CL, *et al*. The genesis and source of the H7N9 influenza viruses causing human infections in China. Nature 2013; 502:241–244. doi: 10.1038/nature12515.
3. Iuliano AD, Jang Y, Jones J, Todd-Davis C, Wentworth DE, Uyeki TM, *et al*. Increase in human infections with avian influenza A (H7N9) virus during the fifth epidemic - China, October 2016-February 2017. MMWR Morb Mortal Wkly Rep 2017; 66:254. doi: 10.15585/mmwr.mm6609e2.
4. Wang X, *et al*. Epidemiology of avian influenza A H7N9 virus in human beings across five epidemics in mainland China, 2013-17: an epidemiological study of laboratory-confirmed case series. Lancet Infect Dis 2017; 17:822–832. doi: 10.1016/S1473-3099(17)30323-7.
5. Bart SA, Hohenboken M, Cioppa GD, Narasimhan V, Dormitzer PR, Kanesa-Thasan N. A cell culture-derived MF59-adjuvanted

pandemic A/H7N9 vaccine is immunogenic in adults. Sci Transl Med 2014; 6:234ra255. doi: 10.1126/scitranslmed.3008761.
6. Tharakaraman K, Subramanian V, Cain D, Sasisekharan V, Sasisekharan R. Broadly neutralizing influenza hemagglutinin stem-specific antibody CR8020 targets residues that are prone to escape due to host selection pressure. Cell Host Microbe 2014; 15:644–651. doi: 10.1016/j.chom.2014.04.009.
7. Ekiert DC, Friesen RHE, Bhabha G, Kwaks T, Jongeneelen M, Yu W, *et al*. A highly conserved neutralizing epitope on group 2 influenza A viruses. Science 2011; 333:843–850. doi: 10.1126/science.1204839.
8. Corti D, Voss J, Gamblin SJ, Codoni G, Macagno A, Jarrossay D, *et al*. A neutralizing antibody selected from plasma cells binds to group 1 and group 2 influenza A hemagglutinins. Science 2011; 333:850–856. doi: 10.1126/science.1205669.
9. Tan GS, Lee PS, Hoffman RMB, Sanchez BM, Krammer F, Leon PE, *et al*. Characterization of a broadly neutralizing monoclonal antibody that targets the fusion domain of group 2 influenza A virus hemagglutinin. J Virol 2014; 88:13580–13592. doi: 10.1128/JVI.02289-14.
10. Coudeville L, Bailleux F, Riche B, Megas F, Andre P, Ecohard R. Relationship between haemagglutination-inhibiting antibody titres and clinical protection against influenza: development and application of a bayesian random-effects model. BMC Med Rese Methodol 2010; 10:18. doi: 10.1186/1471-2288-10-18.
11. Krause JC, Tsibane T, Tumpey TM, Huffman CJ, Basler CF, Crowe JEJr, *et al*. A broadly neutralizing human monoclonal antibody that recognizes a conserved, novel epitope on the globular head of the influenza H1N1 virus hemagglutinin. J Virol 2011; 85:10905–10908. doi: 10.1128/JVI.00700-11.
12. Benjamin E, Wang W, McAuliffe JM, Hill FJP, Kallewaard NL, Chen Z, *et al*. A broadly neutralizing human monoclonal antibody directed against a novel conserved epitope on the influenza virus H3 hemagglutinin globular head. J Virol 2014; 88:6743–6750. doi: 10.1128/JVI.03562.
13. Ouisse LH, Rolland LG, Devilder MC, Osborn M, Moyon M, Visentin J, *et al*. Antigen-specific single B cell sorting and expression-cloning from immunoglobulin humanized rats: a rapid and versatile method for the generation of high affinity and discriminative human monoclonal antibodies. BMC Biotechnol 2017; 17:3. doi: 10.1186/s12896-016-0322-5.
14. Stettler K, Beltramello M, Espinosa DA, Graham V, Cassotta A, Bianchi S, *et al*. Specificity, cross-reactivity and function of antibodies elicited by Zika virus infection. Science 2016; 353:823–826. doi: 10.1126/science.aaf8505.
15. Yang ZY, Wei CJ, Kong WP, Wu L, Xu L, Smith DF, *et al*. Immunization by avian H5 influenza hemagglutinin mutants with altered receptor binding specificity. Science 2007; 317:825–828. doi: 10.1126/science.1135165.
16. Serological Detection of Avian Influenza A (H7N9) Virus Infections by Turkey Haemagglutination-Inhibition Assay 2015. Geneva: World Health Organization; 2013.
17. Serological Detection of Avian Influenza A (H7N9) Infections by Microneutralization Assay 2015. Geneva: World Health Organization; 2013.
18. Schmeisser F, Vasudevan A, Verma S, Wang W, Alvarado E, Weiss C, *et al*. Antibodies to antigenic site A of influenza H7 hemagglutinin provide protection against H7N9 challenge. PloS One 2015; l10:e0117108. doi: 10.1371/journal.pone.0117108.
19. Stevens J, Blixt O, Tumpey TM, Taubenberger JK, Paulson JC, Wilson IA. Structure and receptor specificity of the hemagglutinin from an H5N1 influenza virus. Science 2006; 312:404–410. doi: 10.1126/science.1124513.
20. CDC. Influenza Risk Assessment Tool (IRAT). Atlanta, GA: US Department of Health and Human Services, CDC, 2017. Available from: https://www.cdc.gov/flu/pandemic-resources/national-strategy/risk-assessment.htm. [Accessed November 7, 2021]
21. Uyeki TM, Cox NJ. Global concerns regarding novel influenza A (H7N9) virus infections. New Engl J Med 2013; 368:1862–1864. doi: 10.1056/NEJMp1304661.
22. Wang Z, Wan Y, Qiu C, Quiñones-Parra S, Zhu Z, Loh L, *et al*. Recovery from severe H7N9 disease is associated with diverse response mechanisms dominated by CD8+T cells. Nat Commun 2015; 6:6833. doi: 10.1038/ncomms7833.
23. Shi Y, Zhang W, Wang F, Qi J, Wu Y, Song H, *et al*. Structures and receptor binding of hemagglutinins from human-infecting H7N9 influenza viruses. Science 2013; 342:243–247. doi: 10.1126/science.1242917.
24. Chen Z, Wang J, Bao L, Guo L, Zhang W, Xue Y, *et al*. Human monoclonal antibodies targeting the haemagglutinin glycoprotein can neutralize H7N9 influenza virus. Nat Commun 2015; 6:6714. doi: 10.1038/ncomms7714.
25. Ha Y, Stevens DJ, Skehel JJ, Wiley DC. X-ray structures of H5 avian and H9 swine influenza virus hemagglutinins bound to avian and human receptor analogs. Proc Natl Acad Sci USA 2001; 98:11181–11186. doi: 10.1073/pnas.201401198.
26. Tharakaraman K, Subramanian V, Viswanathan K, Sloan S, Yen HL, Barnard DL, *et al*. A broadly neutralizing human monoclonal antibody is effective against H7N9. Proc Natl Acad Sci USA 2015; 112:10890–10895. doi: 10.1073/pnas.1502374112.
27. Hai R, Schmolke M, Leyva-Grado VH, Thangavel RR, Margine I, Jaffe EL, *et al*. Influenza A(H7N9) virus gains neuraminidase inhibitor resistance without loss of in vivo virulence or transmissibil-ity. Nat Commun 2013; 4:2854. doi: 10.1038/ncomms3854.
28. Tan GS, Leon PE, Albrecht RA, Margine I, Hirsh A, Bahl J, *et al*. Broadly-reactive neutralizing and non-neutralizing antibodies directed against the H7 influenza virus hemagglutinin reveal divergent mechanisms of protection. PLoS Pathog 2016; 12: e1005578. doi:10.1371/journal.ppat.1005578.
29. Wang J, Chen Z, Bao L, Zhang W, Jin Q. Characterization of two human monoclonal antibodies neutralizing influenza A H7N9 viruses. J Virol 2015; 89:9115–9118.

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