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A cross-reactive neutralizing monoclonal antibody protects mice from H5N1 and pandemic (H1N1) 2009 virus infection

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ABSTRACT

A novel influenza (H1N1) virus caused an influenza pandemic in 2009, while highly pathogenic H5N1 avian influenza viruses have continued to infect humans since 1997. Influenza, therefore, remains a serious health threat. Currently, neuraminidase (NA) inhibitors are the mainstay for influenza therapy; however, drug-resistant mutants of seasonal H1N1 and H5N1 viruses have emerged highlighting the need for alternative therapeutic approaches. One such approach is antibody immunotherapy. Here, we show that the monoclonal antibody C179, which recognizes a neutralizing epitope common among H1, H2, H5, and H6 hemagglutinins (HAs), protected mice from a lethal challenge with various H5N1 and pandemic (H1N1) 2009 viruses when administered either intraperitoneally or intranasally. The protective efficacy of intranasally inoculated C179 was comparable to that of intraperitoneal administration. Our results suggest that direct administration of this anti-influenza antibody to viral replication sites is an effective strategy for prophylaxis and therapy.

1. Introduction

The first fatal case of highly pathogenic avian H5N1 influenza virus infection of a human was reported in Hong Kong in 1997 (Claas et al., 1998; Subbarao et al., 1998). Since then, over 470 people have been infected with H5N1 viruses, with a mortality rate of about 60% (http://www.who.int/csr/disease/avian_influenza/en/). As outbreaks of H5N1 virus infection of poultry have spread worldwide, the virus' glycoprotein, hemagglutinin (HA), has evolved into multiple phylogenetically distinct clades and subclades (Chen et al., 2006; Webster and Govorkova, 2006; WHO, 2008). Meanwhile, in 2009, a novel influenza virus, pandemic (H1N1) 2009 virus, caused a pandemic with serious public health issues (Khan et al., 2009).

Vaccines offer effective prophylaxis for influenza, whereas, for therapy, two classes of drugs are available: M2 ion channel blockers (amantadine and rimantadine) (Dolin et al., 1982; Hay et al., 1985) and neuraminidase (NA) inhibitors (oseltamivir and zanamivir) (Zambon and Hayden, 2001). However, viruses resistant to M2 ion channel blockers have emerged (Belshe et al., 1989; Bright et al., 2006; He et al., 2008; Shiraishi et al., 2003) and spread worldwide. In fact, currently circulating seasonal H1N1 and H3N2 viruses, as well as some H5N1 viruses, are resistant to M2 ion channel blockers (Bright et al., 2006; He et al., 2008). M2 ion channel blockers, therefore, are no longer the first choice for influenza therapy. The NA inhibitors are effective against seasonal influenza viruses, including those resistant to M2 ion channel blockers; however, oseltamivir-resistant viruses have appeared sporadically (Kiso et al., 2004). Indeed, during the 2007–2008 season, oseltamivir-resistant seasonal H1N1 viruses emerged and spread worldwide (Cheng et al., 2010; Meijer et al., 2009). Moreover, oseltamivir-resistant pan-

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demic (H1N1) 2009 and H5N1 viruses have also been reported (Le et al., 2005; Wang et al., 2010), raising concerns regarding the management of influenza virus infections. Taken together, these resistance patterns underscore the need for novel antiviral therapeutic options.

Antibody immunotherapy is an effective therapeutic strategy with high potency. In fact, human monoclonal antibody (mAb)-based immunotherapy has been used to treat numerous human diseases, including respiratory tract infections (Desjardin and Snydman, 1998; Frogel et al., 2010; Groothuis and Simoes, 1993). Monoclonal antibody-based immunotherapy may, therefore, be of value in the management of influenza outbreaks, including those caused by highly pathogenic avian H5N1 and pandemic (H1N1) 2009 viruses. Influenza A viruses are sub-classified according to the antigenicity of their two major surface proteins: HA (H1–H16), which mediates cell entry and membrane fusion, and NA (N1–N9), which facilitates virion release. However, antigenic drift occurs in human influenza viruses, necessitating modification and re-administration of the vaccine to ensure protection. Therefore, the ideal antibodies for influenza immunotherapy would be cross-reactive among the HAs of different subtypes and their drift variants. Recently, several monoclonal antibodies cross-reactive with influenza A viruses of different HA subtypes, including H5, have been shown to be highly protective in mouse and ferret models (Chen et al., 2009, 2010; Friesen et al., 2010; Khurana et al., 2009; Sui et al., 2009; Throsby et al., 2008; Yoshida et al., 2009). C179, which was raised against H2 HA more than 15 years ago, was the first anti-HA monoclonal antibody shown to be subtype cross-reactive, neutralizing viruses of the H1, H2, and H5 subtypes (Okuno et al., 1993). C179 recognizes a common epitope among H1, H2, H5, and H6 (Smirnov Iu et al., 1999), and neutralizes virus by inhibiting the fusion process (Okuno et al., 1993). It has been shown to protect mice from a mouse-adapted seasonal H1N1 virus strain (Okuno et al., 1994), but its efficacy against highly pathogenic H5N1 avian and pandemic (H1N1) 2009 influenza viruses has not been demonstrated in animal models.

Although the studies to date with anti-HA monoclonal antibodies have shown these antibodies to be highly protective in animal models, these studies all used intraperitoneal or intravenous inoculation. Other inoculation routes (e.g., intranasal inoculation) have not been evaluated (Friesen et al., 2010; Throsby et al., 2008; Yoshida et al., 2009). Here, to bridge this gap, we evaluated the prophylactic and therapeutic efficacy of C179 against various H5N1 viruses and a pandemic (H1N1) 2009 virus, comparing its efficacy via intraperitoneal and intranasal administration in a mouse model.

2. Materials and methods

2.1. Antibodies

The monoclonal antibody C179 was produced from BALB/c mouse immunized with A/Okuda/57 (H2N2) (Okuno et al., 1993). It recognizes a common epitope on H1, H2, H5, and H6 HAs (Smirnov Iu et al., 1999).

The mouse monoclonal antibody (mAb) H3-32.1 recognizes only H3 HA and was used as a control.

The mAbs C179 and H3-32.1 were purified from ascites by affinity chromatography using an IgG purification kit (Pierce) or HiTrap Protein G Columns (GE Healthcare) before use.

2.2. Viruses and cells

The following virus strains were used in this study: A/Vietnam/UT3040/04 (H5N1, clade 1; VN3040), A/Vietnam/UT31312III/07 (H5N1, clade 2.3.4; VN31312III), A/swine/Indonesia/UT2071/05 (H5N1, clade 2.1.1; SwIDN2071), A/Indonesia/UT3006/05 (H5N1, clade 2.1.3; IDN3006), A/chicken/Miyazaki/K11/07 (H5N1, clade 2.2; CkMiyazaki), A/California/04/09 (pandemic (H1N1) 2009; CA04), mouse-adapted A/California/04/09 (pandemic (H1N1) 2009; MA-CA04), and A/WSN/33 (H1N1; WSN). These viruses were propagated in Madin–Darby canine kidney (MDCK) cells, which were maintained in Eagle's minimal essential medium (MEM) supplemented with 5% newborn calf serum (SIGMA) and cultured at 37 °C in 5% CO_2.

2.3. Neutralizing activity of C179 against H5N1 and pandemic (H1N1) 2009 viruses

Viruses were serially diluted by ten-fold in MEM and mixed with C179 (100 μg) or phosphate-buffered saline (PBS). The virus–C179 or –PBS mixture was then added to MDCK cells in 12-well tissue culture plates. After a 72 h inoculation, cytopathic effects (CPEs) were assessed. The ratio of the 50% tissue culture infectious dose ($TCID_{50}$) of the virus–PBS mixture and virus–C179 mixture was determined and used as the neutralizing activity. $TCID_{50}$ was calculated by the methods of Reed and Muench (1938).

2.4. Prophylactic and therapeutic efficacy studies in mice

We used 4- to 5-week-old female BALB/c mice (Japan SLC) for all animal experiments. For the prophylactic efficacy study, C179 or the control antibody H3-32.1 was inoculated into four mice per group via the intraperitoneal (15 mg/kg) or intranasal (2.5 mg/kg) route in 0.5 ml or 50 μl, respectively. One or 24 h after administration, mice were infected with H5N1 viruses, WSN, CA04, or MA-CA04 via the intranasal route in 50 μl volumes per mouse with 50 MLD_{50} (dose required to kill 50% of mice), 50 MLD_{50}, 10^5 plaque-forming units (PFU), or 10 MLD_{50}, respectively. Mice were monitored daily for mortality (for H5N1 viruses, WSN, and MA-CA04) or body weight changes (for CA04) for 14 days after virus challenge.

For the therapeutic efficacy study, the experimental design duplicated that of the prophylaxis study, with the following exceptions. We first inoculated mice intranasally with 50 MLD_{50}, 50 MLD_{50}, 10^5 PFU, or 10 MLD_{50} of H5N1 viruses, WSN, CA04, or MA-CA04, respectively. At 24, 48, or 72 h post-challenge, the virus-infected mice received C179 or the control mAb H3-32.1 via the intraperitoneal or intranasal route.

Three mice per group challenged with CA04 were euthanized on day 3 or 6 post-challenge. Their lungs were collected in a 9-fold volume of MEM supplemented with 0.3% bovine serum albumin (BSA: SIGMA). The amount of virus in the lung homogenates was then determined by plaque assays in MDCK cells.

2.5. Statistical analysis

Statistically significant differences in survival between mouse groups treated with C179 and the untreated group were determined by the log-rank and generalized Wilcoxon (Gehan) tests by use of R software ver. 2.11.1.

Dunnett's multiple comparison method was used to evaluate statistically significant differences between virus titers in the lungs of mice treated with C179 and the untreated groups.

3. Results

3.1. Amino acid conservation of the proposed epitope recognized by C179 in H5N1 and pandemic (H1N1) 2009 viruses

The epitope of C179 is believed to consist of two regions, the A region (amino acids 318–322 in HA1) and the B region (amino

Table 1
Amino acid sequences of the C179 epitope.

Virus strains	A region (HA1 318–322[a])	B region (HA2 47–58)
Okuda57 (H2N2)	TGLRN	GITNKVNSVIEK
WSN (H1N1)	TGLRN	GITNKVNSIIEK
H5N1 viruses		
VN3040 (clade 1)	TGLRN	G**V**TNKVNSII**D**K
SwIDN20712071/05 (clade 2.1.1)	TGLRN	G**V**TNKVNSII**D**K
IDN3006 (clade 2.1.3)	TGLRN	G**V**TNKVNSII**D**K
CkMiyazaki (clade 2.2)	TGLRN	G**V**TNKVNSII**D**K
VN31312III (clade 2.3.4)	TGLRN	G**V**TNKVNSII**D**K
Pandemic (H1N1) 2009 virus		
CA04	TGLRN	**E**ITNKVNSVIEK

The epitope recognized by C179 is proposed to consist of an A region (HA1 318–322aa) and a B region (HA2 47–58aa) (Okuno et al., 1993). The amino acid differences from Okuda57 or WSN are shown in bold. The rectangle shows the amino acid difference between Okuda57 and WSN.

[a] H2 numbering.

acids 47–58 in HA2) (H2 numbering) (Okuno et al., 1993). An amino acid change at position 318 in HA1 or position 52 in HA2 abolishes C179 recognition (Okuno et al., 1993). Comparison of the amino acid sequence of the A region showed complete conservation of the sequence among A/Okuda/57 (H2N2; Okuda57), A/WSN/33 (H1N1; WSN), all H5N1 viruses examined, and A/California/04/09 (H1N1; CA04). For the B region, the H5N1 and CA04 virus sequences differed from those of Okuda57 or WSN at two or one positions, respectively (Table 1). In addition, the amino acid at position 55 in HA2 differed among Okuda57, WSN, H5N1, and CA04 viruses. However, Val or Ile at this position did not appear to affect C179 binding to the viruses since the antibody efficiently neutralized WSN (see below).

3.2. C179 neutralizes various H5N1 and pandemic (H1N1) 2009 viruses in vitro

To examine the efficacy of C179 against H5N1 and pandemic (H1N1) 2009 viruses in vitro, the neutralizing activity of the antibody was measured. Neutralization assays, in which the mAb concentration that neutralizes a given infectious dose of virus is determined, are commonly used to evaluate the neutralizing activity of antibodies against influenza viruses. However, complete neutralization of H5N1 viruses was observed only at the highest concentration of the C179 antibody tested (i.e., 100 μg/μl) and virus growth was observed at the next dilution (i.e., 50 μg/μl). We, therefore, evaluated the neutralizing activity of C179 by comparing virus titers in the absence (PBS served as a control) or presence of this antibody (Table 2). C179 neutralized WSN most efficiently, followed by pandemic (H1N1) 2009 virus, and to a much lesser extent, H5N1 viruses.

3.3. Intraperitoneal treatment of C179 protects mice from lethal challenge with H5N1 or pandemic (H1N1) 2009 viruses

To examine the prophylactic and therapeutic efficacy of C179 against H5N1 viruses in vivo, mice were inoculated intraperitoneally with a single dose of C179 (15 mg/kg) at the following time points (prophylaxis, 24 h or 1 h pre-virus challenge; and therapy, 24 h, 48 h, or 72 h post-virus challenge). Mice were then challenged by intranasal inoculation of 50 MLD_{50} (dose required to kill 50% of mice) of various H5N1 viruses (A/Vietnam/UT3040/04 (clade 1; VN3040), A/Vietnam/UT31312III/07 (clade 2.3.4; VN31312III), A/swine/Indonesia/UT2071/05 (clade 2.1.1; SwIDN2071), A/Indonesia/UT3006/05 (clade 2.1.3; IDN3006), A/chicken/Miyazaki/K11/07 (clade 2.2; CkMiyazaki)) or WSN virus. Survival of infected mice was monitored daily for 14 days (Fig. 1). While all of the control mice, who received a control mAb H3-32.1 instead of C179 (see Supplementary Fig. S1), and the untreated control mice died by day 9 post-challenge, most (74%) of the C179-inoculated mice survived during the monitoring period. Furthermore, the groups with 100% survival did not show any clinical signs. The prophylactic and therapeutic efficacies of C179 were high for VN3040 and CkMiyazaki, as well as for WSN, and relatively high for SwIDN2071; they were, however, low for IDN3006 and VN31312III (Fig. 1). These results indicate that C179 can protect mice from lethal challenge of various clades and subclades of H5N1 viruses, albeit to differing degrees.

Next, we examined the efficacy of C179 against CA04, a representative pandemic (H1N1) 2009 virus. Because a high dose of CA04 is required for lethality in mice (MLD_{50}: 6.3×10^5 PFU) (Itoh et al., 2009; Maines et al., 2009), we evaluated changes in body weight of mice intranasally challenged with 10^5 PFU of CA04 (Fig. 2A). Mice inoculated with C179 before virus challenge exhibited little to no weight loss, whereas all of the control mice that received H3-32.1 instead of C179 lost a substantial amount of body weight. For therapy, recovery of body weight loss was improved by C179 when administrated for 24 h.

To further evaluate the efficacy of C179 against CA04, three mice per group were euthanized on days 3 and 6 post-challenge and virus titers in the lungs were determined by plaque assays in MDCK cells (Fig. 2B). On day 3 post-challenge, the virus titers in the lungs of the C179-inoculated mice were significantly lower than those of the untreated control mice when C179 was administered 24 h, but not 1 h, before challenge or after challenge. However, on day 6 post-challenge, a significant reduction in lung virus titer as a result of C179 inoculation was more prominent in most treatment regimens, with the exception of the animals treated with the antibody 1 h after challenge.

We also examined the efficacy of C179 against a mouse-adapted CA04, MA-CA04 (MLD_{50}: 3.7×10^4 PFU) (Sakabe et al., unpublished data) in a murine lethal infection model (Fig. 3). When mice were inoculated with C179 prior to challenge with this virus, C179 effectively protected them (≥75%) from a lethal dose (10 MLD_{50}) of MA-CA04, which killed all of H3-32.1-treated control mice (see Supplementary Fig. S2) and the untreated mice by 7 days post-challenge. The prophylactic efficacy of C179 against MA-CA04 (Fig. 3) was significant. By contrast, therapeutic treatment with C179 afforded limited protection against lethal challenge with MA-CA04. These results indicate that C179 protects mice from pandemic (H1N1) 2009 virus infection prophylactically, but not therapeutically when administered intraperitoneally.

3.4. Intranasal C179 treatment provided protective efficacy against H5N1 and pandemic (H1N1) 2009 viruses

For intraperitoneal treatment, we used 15 mg/kg of C179. To examine whether we could reduce the amount of antibody for

Table 2
Neutralizing activity index of C179.[a]

Virus strains	Virus titer (log_{10} $TCID_{50}$/ml) in the presence of		Neutralizing activity[b]
	PBS	C179	
WSN (H1N1)	7.8	2.8	5.0
H5N1 viruses			
VN3040 (clade 1)	8.3	7.3	1.0
SwIDN2071 (clade 2.1.1)	8.3	7.3	1.0
IDN3006 (clade 2.1.3)	8.5	7.3	1.2
CkMiyazaki (clade 2.2)	7.3	6.5	0.8
VN31312III (clade 2.3.4)	8.5	7.5	1.0
Pandemic (H1N1) 2009 virus			
CA04	7.5	3.5	4.0
MA-CA04	6.8	3.8	3.0

[a] Virus was mixed with C179 or PBS and incubated for 1 h, then the $TCID_{50}$ of the mixture was determined in MDCK cells.
[b] The neutralizing activity index was determined by dividing the virus titers in the presence of PBS by those in the presence of C179.

treatment, we next examined the protective efficacy of intranasal treatment with C179 against H5N1 and pandemic (H1N1) 2009 viruses. Mice were inoculated with C179 (2.5 mg/kg) intranasally 24 or 1 h before or 24, 48 or 72 h after virus challenge. Mice were challenged with WSN, SwIDN2071, IDN3006 (50 MLD_{50}, respectively), or MA-CA04 (10 MLD_{50}; we could not use a higher dose because of the lower titer of the virus stock compared with that of the other test viruses) and monitored daily for survival for 14 days (Fig. 4). While all of H3-32.1-treated control mice (see Supplementary Fig. S3) and untreated control mice died by day 12

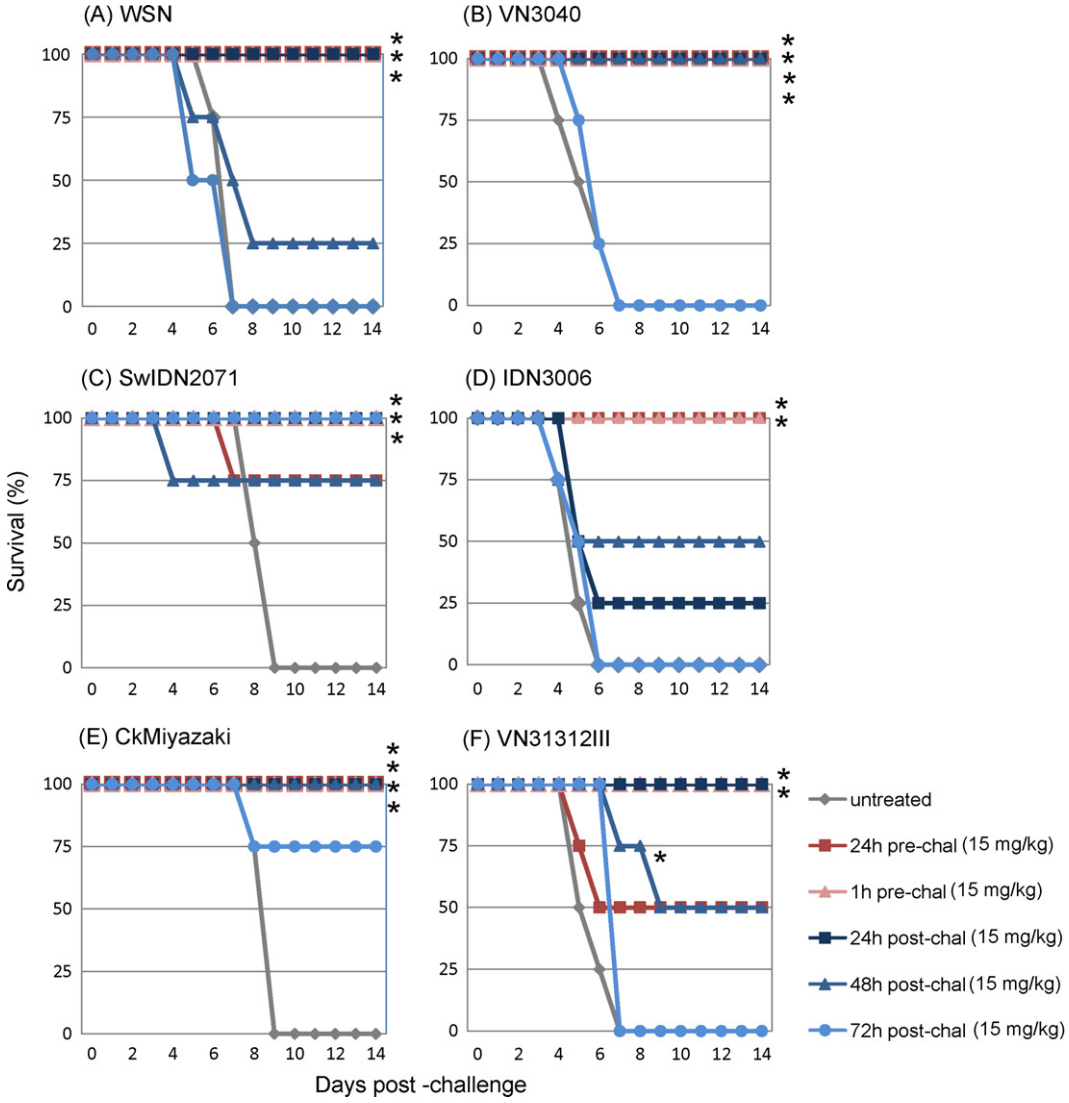

Fig. 1. Prophylactic and therapeutic efficacy of C179 against WSN (H1N1) and H5N1 viruses. Four mice per group were intraperitoneally inoculated with C179, 24 or 1 h pre- (prophylactic) or 24, 48, or 72 h post- (therapeutic) challenge (chal) with a lethal dose of WSN (A), VN3040 (B), SwIDN2071 (C), IDN3006 (D), CkMiyazaki (E), or VN31312III (F). Survival was monitored daily for 14 days. $^{*}p < 0.05$: significant difference compared to the untreated control (log-rank and generalized Wilcoxon (Gehan) tests).

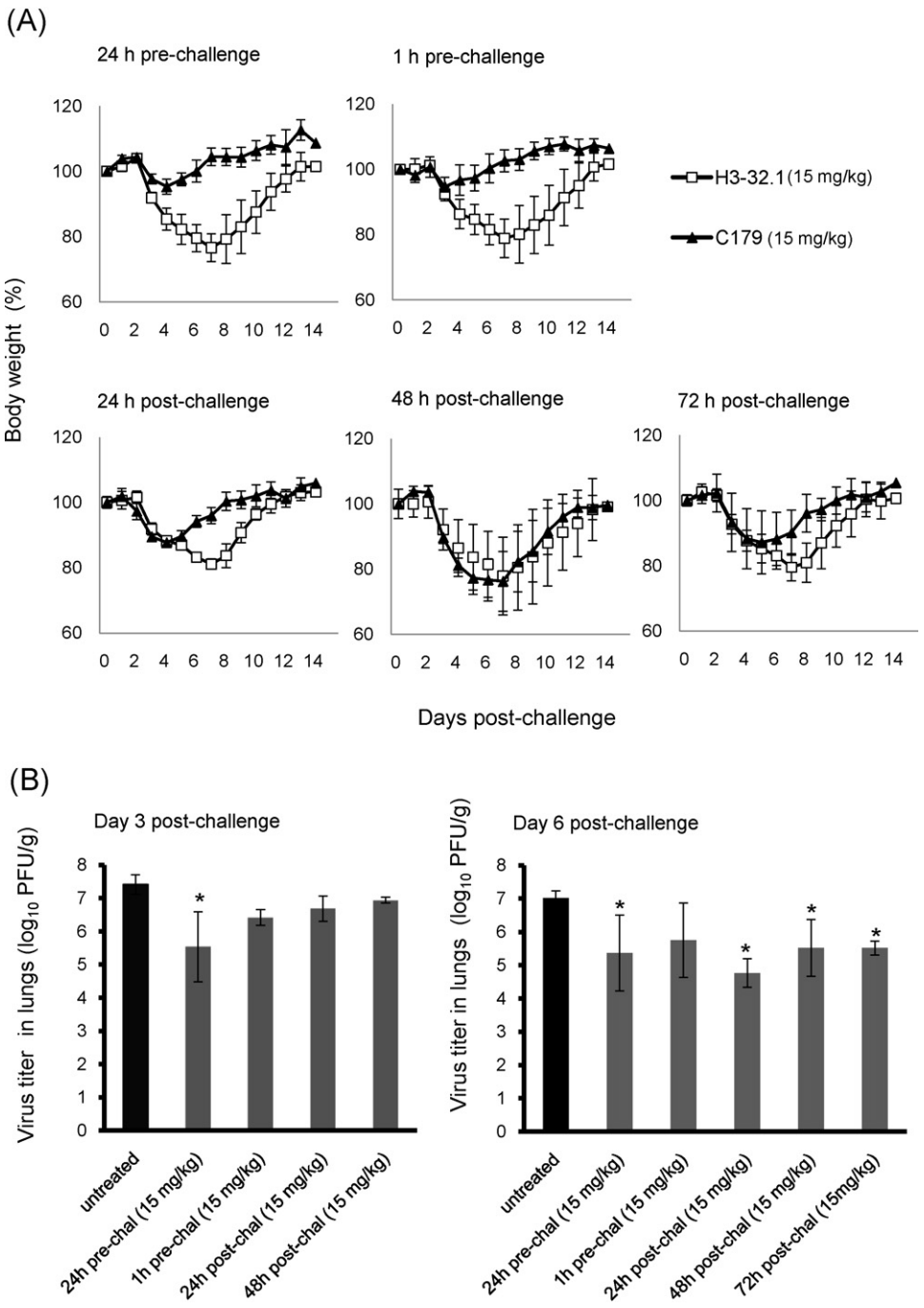

Fig. 2. Body weight changes in mice infected with CA04 (A). Four mice per group were intraperitoneally inoculated with C179 (green) or H3-32.1 (red) at the indicated time points, and then challenged with CA04. Body weights were monitored daily. The values are means ± SD. All of the control mice that received H3-32.1 at the 24 h or 1 h pre-challenge and the 24 h post-challenge time points lost a significant amount of body weight compared to those treated with C179 (*t* test, $p < 0.05$). Virus titers in the lungs of mice inoculated with C179 (B). Three mice per group were intraperitoneally inoculated with C179 at the indicated time points and then challenged with CA04. On days 3 and 6 post-challenge (chal), mice were euthanized and virus titers in the lungs were measured by plaque assays in MDCK cells. The results are expressed as the mean ± SD. *$p < 0.05$: significant difference compared to the untreated control (Dunnett's multiple comparison method).

post-challenge. Some (68%) of C179-inoculated mice survived during the monitoring period. Mice in groups with 100% survival did not show any clinical signs. The prophylactic and therapeutic efficacies of intranasally inoculated C179 were significantly high for WSN, relatively high for SwIDN2071, but low for IDN3006 and MA-CA04 (Fig. 4). Interestingly, the protective efficacy of intranasally inoculated C179 at a dose of 2.5 mg/kg was comparable to that achieved via the intraperitoneal route with a 15 mg/kg dose for all of the viruses tested, suggesting that direct administration of this anti-influenza antibody to the viral replication site may reduce the amount of antibody needed for prophylaxis and therapy.

4. Discussion

Here, we show that the subtype cross-reactive monoclonal antibody C179 protects mice from H5N1 and pandemic (H1N1) 2009 viruses, despite its low neutralizing activity in vitro. Furthermore, we found that C179 provides protective efficacy via two different routes of administration. In particular, a substantially lower dose

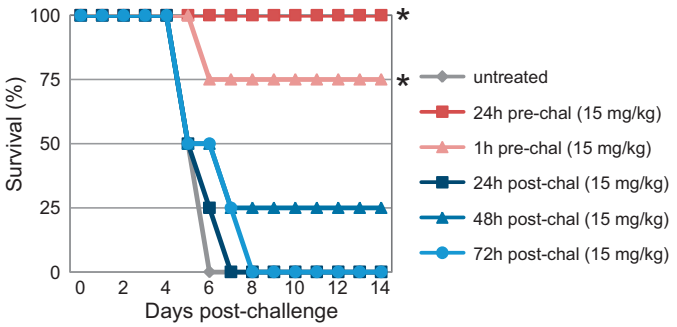

Fig. 3. Prophylactic and therapeutic efficacy of C179 against MA-CA04. Four mice per group were intraperitoneally inoculated with C179 at the indicated time points and then challenged with a lethal dose of MA-CA04. Survival was monitored daily for 14 days. *$p < 0.05$: significant difference compared to the untreated control (log-rank and generalized Wilcoxon (Gehan) tests).

of intranasally administered antibody was as effective as intraperitoneal administration. Recently, multiple cross-reactive anti-HA antibodies with high potency have been reported (Chen et al., 2009, 2010; Friesen et al., 2010; Kashyap et al., 2008; Sui et al., 2009; Yoshida et al., 2009). Our results suggest that intranasal administration of anti-influenza virus antibodies should be considered as a route of inoculation for these antibodies.

The epitope recognized by C179 is highly conserved among H1N1, H5N1, and H2N2 viruses (Table 2). However, the in vitro neutralizing activity of C179 varied among these viruses, suggesting that regions other than the proposed C179 epitope (Okuno et al., 1993) affect C179 recognition.

C179 protected mice from H5N1 and pandemic (H1N1) 2009 viruses effectively, although it showed low neutralizing activity in vitro. It may be that this antibody exhibits its protective efficacy by not only inhibiting HA fusion (Okuno et al., 1993), but also by inducing other mechanisms, such as opsonization. Further studies are needed to gain more insights into the protective mechanism of this antibody.

Prophylactic administration of C179 to mice was effective for all of the virus strains examined in this study. In addition, C179 therapeutic use was also effective for some H5N1 viruses. Although the therapeutic efficacy for pandemic (H1N1) 2009 virus was limited, C179 did extend the lives of the test mice (Figs. 3 and 4). These results suggest that daily administration of C179 after challenge may be more effective for pandemic (H1N1) 2009 virus.

In this study, we found that intranasal administration required a lower dose of antibody for protection against influenza virus infection compared to intraperitoneal administration. Thus, the intranasal administration of antibody for influenza treatment may offer cost-effective benefits.

It is difficult to predict both when an influenza pandemic will occur and what kind of virus will cause a pandemic, as exemplified by the pandemic of 2009. Due to the limitations with existing vaccines and currently licensed anti-influenza drugs, it is prudent to develop new prophylactic and therapeutic measures. Intranasal administration of an antibody as immunotherapy may be a promising option for controlling future pandemics and epidemics.

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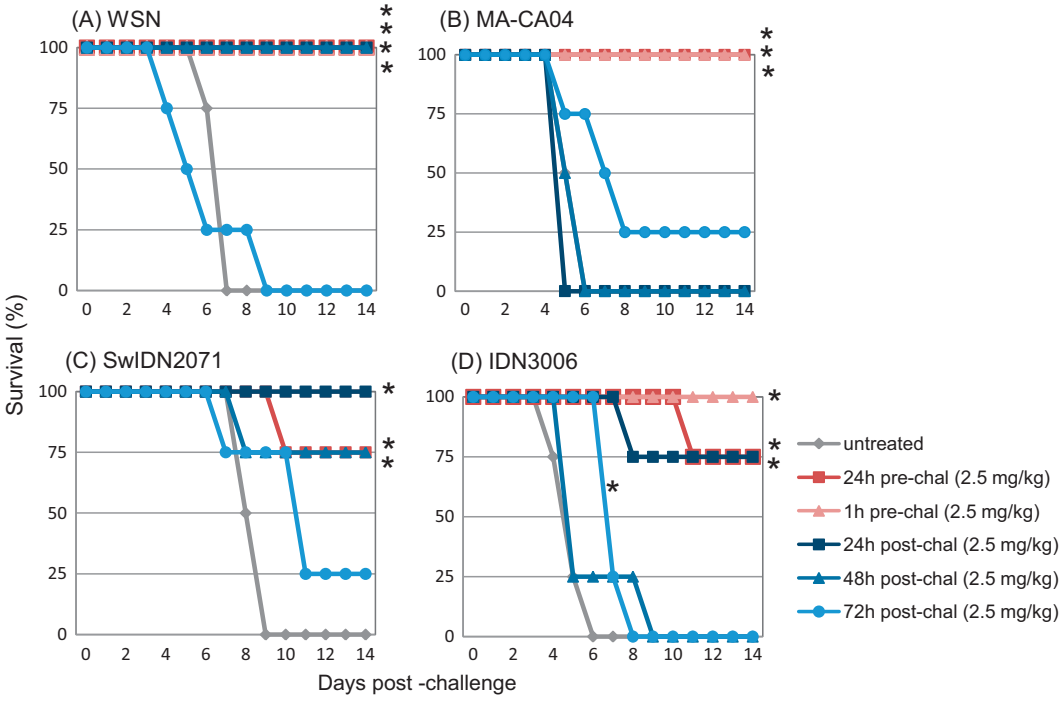

Fig. 4. Prophylactic and therapeutic efficacy of intranasally inoculated C179 against WSN (H1N1), H5N1 and pandemic (H1N1) 2009 viruses. Four mice per group were intranasally inoculated with C179 at the indicated time points and then challenged with a lethal dose of WSN (A), MA-CA04 (B), SwIDN2071 (C), or IDN3006 (D). Survival was monitored daily for 14 days. *$p < 0.05$: significant difference compared to the untreated control (log-rank and generalized Wilcoxon (Gehan) tests).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.antiviral.2010.09.007.

References

Belshe, R.B., Burk, B., Newman, F., Cerruti, R.L., Sim, I.S., 1989. Resistance of influenza A virus to amantadine and rimantadine: results of one decade of surveillance. J. Infect. Dis. 159, 430–435.

Bright, R.A., Shay, D.K., Shu, B., Cox, N.J., Klimov, A.I., 2006. Adamantane resistance among influenza A viruses isolated early during the 2005–2006 influenza season in the United States. JAMA 295, 891–894.

Chen, H., Smith, G.J., Li, K.S., Wang, J., Fan, X.H., Rayner, J.M., Vijaykrishna, D., Zhang, J.X., Zhang, L.J., Guo, C.T., Cheung, C.L., Xu, K.M., Duan, L., Huang, K., Qin, K., Leung, Y.H., Wu, W.L., Lu, H.R., Chen, Y., Xia, N.S., Naipospos, T.S., Yuen, K.Y., Hassan, S.S., Bahri, S., Nguyen, T.D., Webster, R.G., Peiris, J.S., Guan, Y., 2006. Establishment of multiple sublineages of H5N1 influenza virus in Asia: implications for pandemic control. Proc. Natl. Acad. Sci. U.S.A. 103, 2845–2850.

Chen, Y., Luo, W., Wu, W.L., Fang, Z., Xia, L., Gui, X., Chen, H., Shih, J.W., Xia, N., 2010. Humanized antibodies with broad-spectrum neutralization to avian influenza virus H5N1. Antiviral Res. 87, 81–84.

Chen, Y., Qin, K., Wu, W.L., Li, G., Zhang, J., Du, H., Ng, M.H., Shih, J.W., Peiris, J.S., Guan, Y., Chen, H., Xia, N., 2009. Broad cross-protection against H5N1 avian influenza virus infection by means of monoclonal antibodies that map to conserved viral epitopes. J. Infect. Dis. 199, 49–58.

Cheng, P.K., To, A.P., Leung, T.W., Leung, P.C., Lee, C.W., Lim, W.W., 2010. Oseltamivir- and amantadine-resistant influenza virus A (H1N1). Emerg. Infect. Dis. 16, 155–156.

Claas, E.C., Osterhaus, A.D., van Beek, R., De Jong, J.C., Rimmelzwaan, G.F., Senne, D.A., Krauss, S., Shortridge, K.F., Webster, R.G., 1998. Human influenza A H5N1 virus related to a highly pathogenic avian influenza virus. Lancet 351, 472–477.

Desjardin, J.A., Snydman, D.R., 1998. Antiviral immunotherapy: a review of current status. BioDrugs 9, 487–507.

Dolin, R., Reichman, R.C., Madore, H.P., Maynard, R., Linton, P.N., Webber-Jones, J., 1982. A controlled trial of amantadine and rimantadine in the prophylaxis of influenza A infection. N. Engl. J. Med. 307, 580–584.

Friesen, R.H., Koudstaal, W., Koldijk, M.H., Weverling, G.J., Brakenhoff, J.P., Lenting, P.J., Stittelaar, K.J., Osterhaus, A.D., Kompier, R., Goudsmit, J., 2010. New class of monoclonal antibodies against severe influenza: prophylactic and therapeutic efficacy in ferrets. PLoS One 5, e9106.

Frogel, M.P., Stewart, D.L., Hoopes, M., Fernandes, A.W., Mahadevia, P.J., 2010. A systematic review of compliance with palivizumab administration for RSV immunoprophylaxis. J. Manag. Care Pharm. 16, 46–58.

Groothuis, J.R., Simoes, E.A., 1993. Immunoprophylaxis and immunotherapy: role in the prevention and treatment of respiratory syncytial virus. Int. J. Antimicrob. Agents 2, 97–103.

Hay, A.J., Wolstenholme, A.J., Skehel, J.J., Smith, M.H., 1985. The molecular basis of the specific anti-influenza action of amantadine. EMBO J. 4, 3021–3024.

He, G., Qiao, J., Dong, C., He, C., Zhao, L., Tian, Y., 2008. Amantadine-resistance among H5N1 avian influenza viruses isolated in Northern China. Antiviral Res. 77, 72–76.

Itoh, Y., Shinya, K., Kiso, M., Watanabe, T., Sakoda, Y., Hatta, M., Muramoto, Y., Tamura, D., Sakai-Tagawa, Y., Noda, T., Sakabe, S., Imai, M., Hatta, Y., Watanabe, S., Li, C., Yamada, S., Fujii, K., Murakami, S., Imai, H., Kakugawa, S., Ito, M., Takano, R., Iwatsuki-Horimoto, K., Shimojima, M., Horimoto, T., Goto, H., Takahashi, K., Makino, A., Ishigaki, H., Nakayama, M., Okamatsu, M., Warshauer, D., Shult, P.A., Saito, R., Suzuki, H., Furuta, Y., Yamashita, M., Mitamura, K., Nakano, K., Nakamura, M., Brockman-Schneider, R., Mitamura, H., Yamazaki, M., Sugaya, N., Suresh, M., Ozawa, M., Neumann, G., Gern, J., Kida, H., Ogasawara, K., Kawaoka, Y., 2009. In vitro and in vivo characterization of new swine-origin H1N1 influenza viruses. Nature 460, 1021–1025.

Kashyap, A.K., Steel, J., Oner, A.F., Dillon, M.A., Swale, R.E., Wall, K.M., Perry, K.J., Faynboym, A., Ilhan, M., Horowitz, M., Horowitz, L., Palese, P., Bhatt, R.R., Lerner, R.A., 2008. Combinatorial antibody libraries from survivors of the Turkish H5N1 avian influenza outbreak reveal virus neutralization strategies. Proc. Natl. Acad. Sci. U.S.A. 105, 5986–5991.

Khan, K., Arino, J., Hu, W., Raposo, P., Sears, J., Calderon, F., Heidebrecht, C., Macdonald, M., Liauw, J., Chan, A., Gardam, M., 2009. Spread of a novel influenza A (H1N1) virus via global airline transportation. N. Engl. J. Med. 361, 212–214.

Khurana, S., Suguitan Jr., A.L., Rivera, Y., Simmons, C.P., Lanzavecchia, A., Sallusto, F., Manischewitz, J., King, L.R., Subbarao, K., Golding, H., 2009. Antigenic fingerprinting of H5N1 avian influenza using convalescent sera and monoclonal antibodies reveals potential vaccine and diagnostic targets. PLoS Med. 6, e1000049.

Kiso, M., Mitamura, K., Sakai-Tagawa, Y., Shiraishi, K., Kawakami, C., Kimura, K., Hayden, F.G., Sugaya, N., Kawaoka, Y., 2004. Resistant influenza A viruses in children treated with oseltamivir: descriptive study. Lancet 364, 759–765.

Le, Q.M., Kiso, M., Someya, K., Sakai, Y.T., Nguyen, T.H., Nguyen, K.H., Pham, N.D., Ngyen, H.H., Yamada, S., Muramoto, Y., Horimoto, T., Takada, A., Goto, H., Suzuki, T., Suzuki, Y., Kawaoka, Y., 2005. Avian flu: isolation of drug-resistant H5N1 virus. Nature 437, 1108.

Maines, T.R., Jayaraman, A., Belser, J.A., Wadford, D.A., Pappas, C., Zeng, H., Gustin, K.M., Pearce, M.B., Viswanathan, K., Shriver, Z.H., Raman, R., Cox, N.J., Sasisekharan, R., Katz, J.M., Tumpey, T.M., 2009. Transmission and pathogenesis of swine-origin 2009 A(H1N1) influenza viruses in ferrets and mice. Science 325, 484–487.

Meijer, A., Lackenby, A., Hungnes, O., Lina, B., van-der-Werf, S., Schweiger, B., Opp, M., Paget, J., van-de-Kassteele, J., Hay, A., Zambon, M., 2009. Oseltamivir-resistant influenza virus A (H1N1), Europe, 2007–08 season. Emerg. Infect. Dis. 15, 552–560.

Reed, L.J., Muench, H., 1938. A simple method of estimating fifty percent end points. Am. J. Hyg. 27, 493–497.

Okuno, Y., Isegawa, Y., Sasao, F., Ueda, S., 1993. A common neutralizing epitope conserved between the hemagglutinins of influenza A virus H1 and H2 strains. J. Virol. 67, 2552–2558.

Okuno, Y., Matsumoto, K., Isegawa, Y., Ueda, S., 1994. Protection against the mouse-adapted A/FM/1/47 strain of influenza A virus in mice by a monoclonal antibody with cross-neutralizing activity among H1 and H2 strains. J. Virol. 68, 517–520.

Shiraishi, K., Mitamura, K., Sakai-Tagawa, Y., Goto, H., Sugaya, N., Kawaoka, Y., 2003. High frequency of resistant viruses harboring different mutations in amantadine-treated children with influenza. J. Infect. Dis. 188, 57–61.

Smirnov Iu, A., Lipatov, A.S., Okuno, I., Gitel'man, A.K., 1999. A common antigenic epitope in influenza A virus (H1, H2, H5, H6) hemagglutinin. Vopr. Virusol. 44, 111–115.

Subbarao, K., Klimov, A., Katz, J., Regnery, H., Lim, W., Hall, H., Perdue, M., Swayne, D., Bender, C., Huang, J., Hemphill, M., Rowe, T., Shaw, M., Xu, X., Fukuda, K., Cox, N., 1998. Characterization of an avian influenza A (H5N1) virus isolated from a child with a fatal respiratory illness. Science 279, 393–396.

Sui, J., Hwang, W.C., Perez, S., Wei, G., Aird, D., Chen, L.M., Santelli, E., Stec, B., Cadwell, G., Ali, M., Wan, H., Murakami, A., Yammanuru, A., Han, T., Cox, N.J., Bankston, L.A., Donis, R.O., Liddington, R.C., Marasco, W.A., 2009. Structural and functional bases for broad-spectrum neutralization of avian and human influenza A viruses. Nat. Struct. Mol. Biol. 16, 265–273.

Throsby, M., van den Brink, E., Jongeneelen, M., Poon, L.L., Alard, P., Cornelissen, L., Bakker, A., Cox, F., van Deventer, E., Guan, Y., Cinatl, J., ter Meulen, J., Lasters, I., Carsetti, R., Peiris, M., de Kruif, J., Goudsmit, J., 2008. Heterosubtypic neutralizing monoclonal antibodies cross-protective against H5N1 and H1N1 recovered from human IgM+ memory B cells. PLoS One 3, e3942.

Wang, B., Dwyer, D.E., Blyth, C.C., Soedjono, M., Shi, H., Kesson, A., Ratnamohan, M., McPhie, K., Cunningham, A.L., Saksena, N.K., 2010. Detection of the rapid emergence of the H275Y mutation associated with oseltamivir resistance in severe pandemic influenza virus A/H1N1 09 infections. Antiviral Res..

Webster, R.G., Govorkova, E.A., 2006. H5N1 influenza—continuing evolution and spread. N. Engl. J. Med. 355, 2174–2177.

WHO, 2008. Toward a unified nomenclature system for highly pathogenic avian influenza virus (H5N1). Emerg. Infect. Dis. 14, e1.

Yoshida, R., Igarashi, M., Ozaki, H., Kishida, N., Tomabechi, D., Kida, H., Ito, K., Takada, A., 2009. Cross-protective potential of a novel monoclonal antibody directed against antigenic site B of the hemagglutinin of influenza A viruses. PLoS Pathog. 5, e1000350.

Zambon, M., Hayden, F.G., 2001. Position statement: global neuraminidase inhibitor susceptibility network. Antiviral Res. 49, 147–156.