Protective Role of Human Intravenous Immunoglobulin from Influenza A Virus Infection in Mice

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Abstract: Intravenous immunoglobulin (IVIG) has been manufactured from pooled plasma of 10,000 or more units from healthy donors. Recently, we reported that the IVIG manufactured even before the 2009 influenza pandemic contained antibodies reactive to seasonal H1N1 and pandemic H1N1 2009 (H1N1 pdm) viruses. In this study, we used an animal model to evaluate the efficacy of IVIG against influenza infections. A seasonal influenza H1N1 strain (New Caledonia, A/NC/20/99) and an H1N1 pdm strain (A/Osaka/168/2009) were used. The BALB/c and severe combined immunodeficiency mice (SCID; C.B-17/lcr-scid/scid) were also used. Mice inoculated with A/NC/20/99 or A/Osaka/168/2009 were administrated IVIG and monitored for 3 weeks. The administration of IVIG 48 h before and after inoculation with a mouse-adapted seasonal H1N1 virus, resulted in survival rates of 80 and 88%, respectively. The rate among control mice was 30%. In addition, infectivity in lungs from IVIG-treated mice also decreased significantly. Similar effects of IVIG on the survival rate were obtained with H1N1 pdm. Thus, IVIG was shown to be effective against both viruses in mice.

Keywords: IVIG, Influenza, 2009 Pandemic Influenza, Animal model.

INTRODUCTION

The influenza virus is currently the most important public health concern in the world, especially since the appearance of the 2009 pandemic influenza A/H1N1 (H1N1 pdm) virus. Human intravenous immunoglobulin (IVIG), a product manufactured from plasma derived from more than 10,000 units from healthy donors, most of whom have had natural infections with seasonal influenza viruses as well as vaccinations, could contain a wide variety of antibodies effective for protection against influenza infections.

In Japan, IVIG has not been yet approved for influenza virus infections, although it was recommended for influenza encephalopathy by the *Study Group for Influenza Encephalopathy* [1] and the Japanese Respiratory Society [2].

The mechanism of its effect against influenza is not yet clear. However, Yunoki *et al*. [3] and Hong *et al*. [4] reported that IVIG contains significant titers of hemagglutination-inhibition (HI) and viral neutralization (VN or MN) antibodies against not only seasonal H1N1 but also H1N1 pdm. The cross-reacting antibody against H1N1 pdm seemed to be derived from natural influenza viral infections as well as vaccinations. Here, we examined the efficacy of IVIG against these influenza infections in mice.

MATERIALS AND METHODS

Viruses

A seasonal influenza H1N1 strain (New Caledonia, A/NC/20/99) and an H1N1 pdm strain (A/Osaka/168/2009) were used. Before the experiment, the H1N1 and H1N1 pdm strains were passaged 5 times in BALB/c mice and 4 times in the severe combined immunodeficiency mice (SCID; C.B-17/lcr-scid/scid, CLEA Japan Inc., Japan). The mouse-adopted viruses were named mo-A/NC/20/99 and mo-A/Osaka/168/2009, respectively. To evaluate the adaptation, BALB/c mice (4-week-old males) were infected intranasally with 100 or 1000 focus-forming units (FFUs) of mo-A/NC/20/99 (n=10) or mo-A/Osaka/168/2009 (n=7), then observed for 3 weeks.

Human Intravenous Immunoglobulin (IVIG)

The IVIG (Venoglobulin-IH®; Benesis Corporation, Japan) used was the same lot previously show to contain

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1:160 HI and 1:640 VN titers against seasonal H1N1 (A/NC/20/99) and 1:8 HI and 1:64 VN titers against H1N1 pdm (A/Osaka/168/2009) [3].

Infection with mo-A/NC/20/99

A total of 40 BALB/c mice (4-week-old males) were divided into four groups (n=10 per group). The mice were infected intranasally with mo-A/NC/20/99 at 1000 FFUs per head for the evaluation of IVIG's effect on survival for 3 weeks. To investigate the efficacy of IVIG treatment at 48 h before inoculation (hbi) or at 48 h post inoculation (hpi), each group of mice were intraperitoneally administered with IVIG at 5 mg per head (corresponding to 250 mg/kg) at 48 hbi, and at 48 and 72 hpi with 1000 FFUs per head. As a control, the H1N1-inoculated BALB/c mice were orally administered 100 mg/kg of Oseltamivir (Tamiflu®, Chugai Pharmaceutical Co., Ltd., Japan) for 4 days, and observed for 3 weeks. The dissected lung was fixed with 10% formalin-PB and prepared for tissue sectioning and HE stained for histological examination.

Infection with mo-A/Osaka/168/2009

A total of 14 BALB/c mice were inoculated intranasally with mo-A/Osaka/168/2009 at 1000 FFUs per head. The mice were divided into two groups for the evaluation of IVIG's effect on survival for 2 weeks. To evaluate the antiviral effect of IVIG on the H1N1 pdm virus in SCID mice lacking immunoglobulin and functional T and B cells, SCID mice (8 weeks old, male) were inoculated intranasally with mo-A/Osaka/168/2009 at 1000 FFUs. The mice were intraperitoneally administered IVIG at 5 mg per head (corresponding to 250 mg/kg) at 48 hpi. The untreated control group was administered the solvent of IVIG.

Evaluation of Antiviral Effects

Survival rates were monitored for 3 weeks. The BALB/c mice inoculated with mo-A/NC/20/99 or mo-A/Osaka/168/2009 were monitored. The infected SCID mice treated with or without IVIG were dissected at 3 and 5 days post inoculation (dpi). The lung from infected mice was homogenized with PBS (20% homogenate) and infectivity ($TCID_{50}$) in the lung was determined on days 3 and 5 post administration of IVIG.

We used the commercial statistical analysis software (SPSS 15.0 J, SPSS Japan Inc., Tokyo, Japan) for all statistical analyses in this study. Independent t-test was used for comparisons of averages for two groups between IVIG treatment and untreatment control.

RESULTS

Viral Adaptation to the Mice

The BALB/c mice inoculated with the seasonal H1N1 strain at 100 and 1000 FFUs per head were observed for 3 weeks (data not shown). The survival rate was 30% among the mice infected with 1000 FFUs and 100% among those infected with 100 FFUs. In contrast, the survival rate of BALB/c mice infected with H1N1 pdm at 1000 FFUs was 33%. The histological analysis of lungs from both BALB/c and SCID mice showed hemorrhagic pneumonia. HE-stained sections of dissected lung tissue from BALB/c mice infected with 1000 FFUs of mo-A/NC/20/99 are shown in Fig. (**1**). The titer of virus hereafter was 1000 FFUs per head.

Efficacy Against H1N1 and H1N1 pdm

As shown in Fig. (**2A**), the treatment of BALB/c mice with IVIG at 48 hpi with seasonal H1N1 was highly

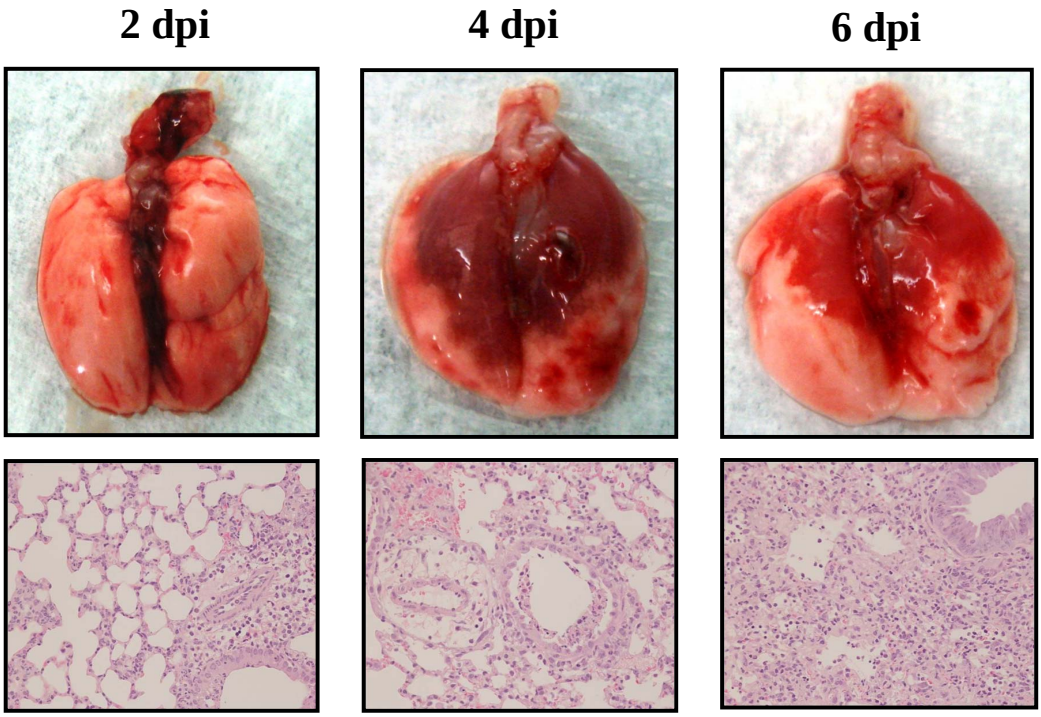

Fig. (1). HE-stained tissue sections of dissected lungs from infected BALB/c mice. Upper: Photos of dissected lungs with hemorrhagic pneumonia in BALB/c mice inoculated with 1000 FFUs of mo-A/NC/20/99 at 2, 4 and 6 dpi. Lower: HE-stained tissue sections of the dissected lungs.

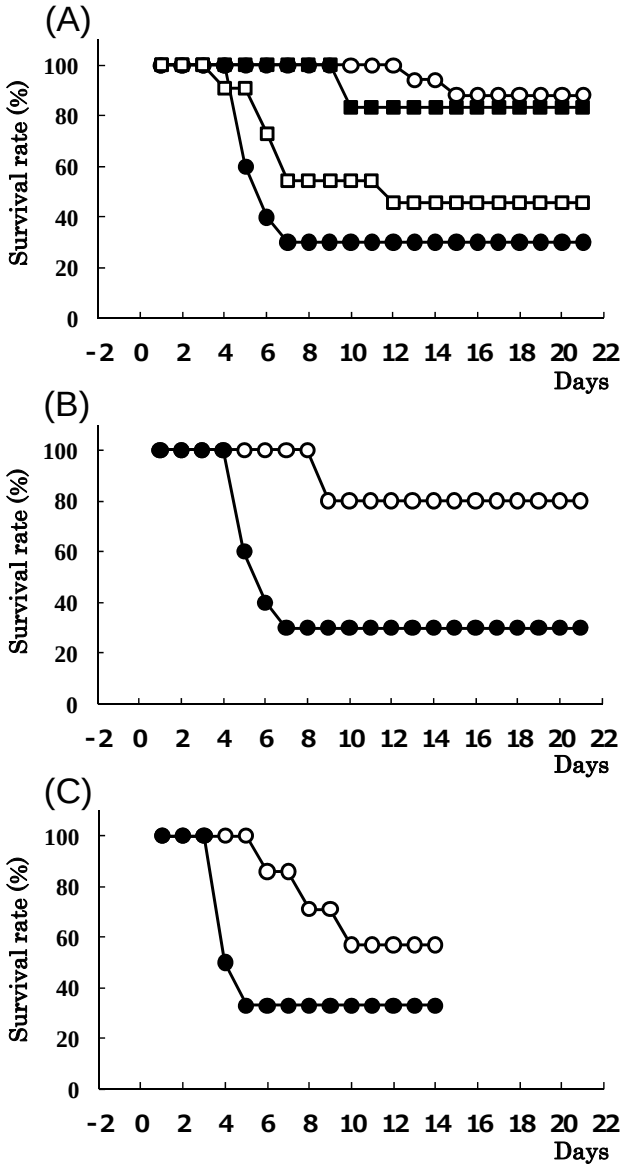

Fig. (2). Efficacy of IVIG against seasonal and pandemic H1N1 infections. **A**) To test the efficacy of IVIG after inoculation with seasonal H1N1, BALB/c mice inoculated with 1000 FFUs of mo-A/NC/20/99 per head were left untreated (closed circle) or else treated with 5 mg of IVIG (open circle) or a total of 2 mg of Oseltamivir (closed square) per head at 48 hpi. In addition, the inoculated mice were treated with 5 mg of IVIG per head at 72 hpi (open square). **B**) To test the efficacy of IVIG against seasonal H1N1 before inoculation of the virus, BALB/c mice were left untreated (closed circle) or treated with 5 mg of IVIG per head (open circle) at 48 hbi with the same dose of H1N1 as above. **C**) To test the efficacy of IVIG against pandemic H1N1 after inoculation of the virus, BALB/c mice inoculated with 1000 FFUs of mo-A/Osaka/168/2009 per head were left untreated (closed circle) or treated with 5 mg of IVIG per head (open circle) at 48 hpi.

effective, since the survival rate was significantly increased to 88%, compared with 30% in the control. Interestingly, when mice were treated with Oseltamivir, a similar survival rate (80%) was obtained. When mice were treated at 72 hpi,

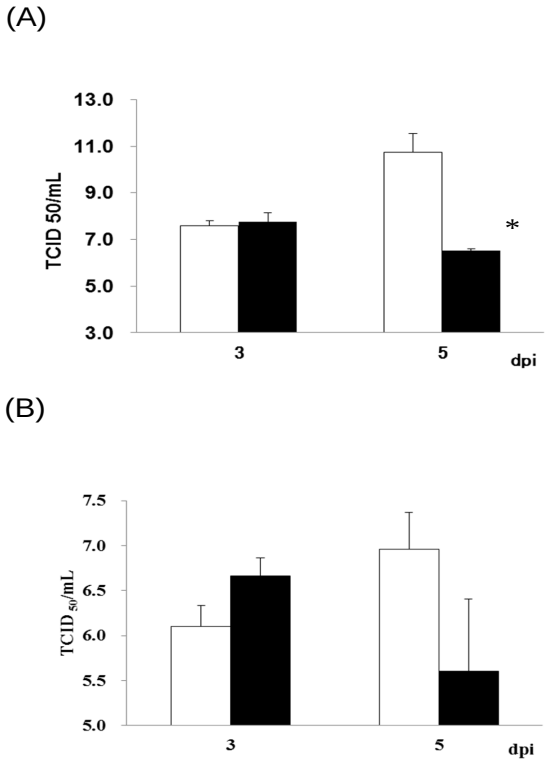

Fig. (3). Effect of IVIG treatment on viral propagation in the infected mouse lung. The amount of infectious virus in the lung from infected (closed bar) and control mice (open bar) at 3 and 5 days post inoculation (dpi) with seasonal H1N1 (**A**) and H1N1 pdm (**B**). The data showed the average and standard deviations of virus titer. Asterisk showed the statistical significance $P<0.05$.

the survival rate greatly decreased to 45.5%, suggesting the delayed treatment reduced by half the survival rate.

We also evaluated the preventive effect of IVIG against seasonal H1N1 infections in BALB/c mice. As shown in Fig. (**2B**), a similar survival rate, 80%, was achieved with the intramuscular administration of IVIG (5 mg/head) at 48 hbi. Interestingly, severe clinical manifestations, such as respiratory symptoms and cyanosis, were not observed in all mice in the pretreated groups, suggesting that pre-treatment with IVIG may prevent the life-threatening influenza virus infection at least in mice. This suggestion was supported by a significant reduction of viral titers in the lungs from BALB/c mice treated with IVIG at 48 hpi: $10^{10.7}$ versus $10^{6.5}$ $TCID_{50}$/ml in control and IVIG-treated mice, respectively (Fig. **3A**).

The treatment of BALB/c mice with IVIG increased the survival rate to 58% at 48 hpi with H1N1 pdm, the survival rate being 33% for control mice (Fig. **2C**). In fact, the experiments using SCID mice for H1N1 pdm revealed that the propagation of this virus also tended to decrease after IVIG treatment (Fig. **3B**).

DISCUSSION

IVIG proved highly effective against influenza viral infections in mice, raising the survival rate from 30% to 88% for seasonal H1N1 infections and from 33% to 58% for H1N1 pdm infections. The treatment's efficacy depended on infection status, and was at least comparable with that

of Oseltamivir treatment at 48 hpi. Interestingly, the administration of IVIG within 48 hpi significantly increased the survival rate of H1N1-infected individuals. This efficacy was also observed in the mice infected with H1N1 pdm. The effect of IVIG differed between the seasonal and pandemic H1N1. The difference in survival rates could be due to different HI and VN titers of the IVIG against the viruses, i.e., 1:160 HI and 1:640 VN titers against seasonal H1N1 (A/NC/20/99) and 1:8 HI and 1:64 VN titers against H1N1 pdm (A/Osaka/168/2009) [3].

Immunoglobulin is believed to be a multi-functional plasma product [5]. There are several options against influenza-related diseases. Generally, IVIG has been used against complications such as influenza encephalopathy and the development of severe pneumonitis whereas Oseltamivir has been used against viral replication. Interestingly, the use of SCID mice revealed that IVIG reduced the viral titer, though only the 1:8 HI and 1:64 VN titers against H1N1 pdm. Thus, IVIG could be an option for treating serious influenza and the complications associated with it. In fact, in several cases, IVIG treatments have proven effective against influenza encephalopathy, severe pneumonitis and flu-related respiratory tract complications [6, 7]. IVIG could be a second-line option for influenza with seasonal H1N1 and H1N1 pdm in patients with hematologic malignancies [8]. In addition, immunoglobulin G_2 deficiency and bacterial co-infections seem to exacerbate H1N1 pdm infections [9-11].

Our study showed a significant effect of IVIG against the influenza virus in an animal model. The effectiveness of IVIG seems to be derived from Japanese blood donors who had received the seasonal influenza vaccines or had a history of natural infections with seasonal influenza viruses. Interestingly, the IVIG lot reactive with H1N1 pdm was detected even before the outbreak of H1N1 pdm [3]. The rate of donors who have antibodies cross-reactive with H1N1 pdm has increased in the population since the H1N1 pdm outbreak and the administration of trivalent inactivated influenza vaccine [12]. This finding suggests that vaccinations against the influenza virus generate cross-reacting antibodies to reassortant H1N1 pdm and may prevent lethal illness. Thus, the circulation of vaccinations may play an important role in producing cross-reactive immunoglobulin against new subtypes of the influenza virus. This study revealed the efficacy of IVIG for the prevention and treatment of both seasonal and pandemic H1N1 infections.

CONFLICT OF INTEREST AND ACKNOWLEDGEMENT

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