



Preparation and characterization of egg yolk immunoglobulin Y specific to influenza B virus

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ARTICLE INFO

Article history:

Received 12 June 2011

Revised 13 November 2011

Accepted 15 November 2011

Available online 22 November 2011

Keywords:

Antiviral

Influenza B virus

Egg yolk immunoglobulin (IgY)

Positive immunization

Plaque reduction assay

ABSTRACT

The aim of this study was to prepare egg yolk immunoglobulin (IgY) for use in the prevention and treatment of influenza B viral infections. Laying hens were immunized with inactivated influenza B virus (IBV), and IgY was isolated from the egg yolk by multiple polyethylene glycol (PEG) 6000 extraction and ammonium sulfate purification steps. The titers and specificity of the purified antibodies were assessed. The specific IgY titer increased beginning the second week after the first immunization, with the titer peaking at the fifth week. The yield of IgY was 76.5 mg per yolk, and the purity was 98.2%. The use of western blotting and the hemagglutination inhibition (HI) test demonstrated that IBV-specific IgY binds specifically to influenza B virus proteins, and a plaque reduction assay revealed the neutralization efficacy of IBV-specific IgY at reducing influenza infection in MDCK cells. Furthermore, when mice were treated intranasally prior to or after influenza B virus infection, IBV-specific IgY protected the mice from influenza infection or reduced viral replication in their lungs, respectively. These findings indicate that IgY is an easily prepared and rich source of antibodies that offers a potential alternative strategy for preventing and treating influenza B infections.

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1. Introduction

Influenza is one of the most common acute viral diseases, and it causes systemic symptoms of variable degrees, such as mild fatigue, respiratory failure, and even death. Approximately 10–20% of the world's population suffers from seasonal influenza, resulting in 250,000–500,000 deaths every year (World Health Organization, 2003). Influenza B virus is one of the pathogens involved in localized outbreaks of seasonal influenza (Saat et al., 2010). Children recovering from influenza B who receive aspirin treatment may suffer a rare illness, Reye syndrome, which can affect the brain and liver (Ashe, 1975; Corey et al., 1976; Reynolds et al., 1972). Much effort has been made to prevent and cure influenza B viral infections. Antiviral drugs, such as neuraminidase inhibitors, are widely used to treat influenza. However, influenza B virus is increasingly resistant to these agents (Sheu et al., 2008; Sugaya et al., 2007). Although vaccination can provide protection, virtually none of the current vaccines is effective enough for use as a therapy.

Passive immunization based on specific antibodies provides an alternative strategy for the prevention and treatment of infectious diseases (Keller and Stiehm, 2000). The availability of specific antibodies is the key for immunotherapy. Several approaches have

been utilized to develop safe, effective, and inexpensive antibodies, including humanized monoclonal antibodies (Mabs) (Goncalvez et al., 2008) and human recombinant antibodies (Sun et al., 2008). Mabs are specific for single epitopes and may not offer full protection clinically, although they can be produced at a high titer.

Immunoglobulin Y (IgY) antibodies, the predominant serum immunoglobulin in birds, reptiles, and amphibians, are transferred from the serum of females to the egg yolk, where they offer passive immunity to embryos and neonates (Patterson et al., 1962). IgY can be extracted at high yield from the yolks of immunized hens by several simple processes. IgY is a safe and inexpensive antibody that has been successfully used to prevent and treat bacterial and viral infections in animals (Nguyen et al., 2010; Wallach et al., 2011). However, the antiviral activity of IgY against influenza B virus has not been extensively investigated.

This study aimed to produce IgY specific to influenza B virus and assess its *in vitro* antiviral activity against influenza B virus. Our results provide a foundation for future alternative prophylactic and therapeutic medicines for influenza.

2. Materials and methods

2.1. Virus and cell lines

The virus strain, B/Guangdong/867/2009, was generously provided by Guangdong CDC (Guangzhou, Guangdong, China) and prepared as described previously (Qiu et al., 1992). Virus stock was diluted 1:500 in 0.9% sodium chloride solution

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and injected into the allantoic cavity of 9-day-old, specific pathogen-free, embryonated eggs (0.2 mL/egg). After incubating for 72 h at 35 °C, the eggs were stored overnight at 4 °C, and the allantoic fluid was harvested and centrifuged at 2000 g for 30 min at 4 °C. Solid polyethylene glycol (PEG) 6000 (Amresco, USA) was added to a final concentration of 8% (w/v) and stirred for 1 h at 4 °C. The precipitate was collected by centrifugation at 30,000g for 1 h and resuspended in 0.5 mL of phosphate-buffered saline (PBS). The preparation was centrifuged through 30% sucrose onto a 60% sucrose cushion at 20,000g for 1 h at 4 °C. Virus removed from the interface was diluted 1:3 in NTE buffer (10 mmol/L Tris-HCl, 100 mmol/L NaCl, 1 mmol/L ethylenediamine tetra-acetic acid). The diluted virus was layered over a 10 mL 30–60% sucrose gradient and centrifuged at 20,000g for 1 h at 4 °C. The virus was collected and diluted 1:5 in NTE buffer followed by sedimenting at 150,000g for 1 h at 4 °C. The pellet was dissolved in a small volume of NTE buffer and stored at –80 °C.

Madin-Darby canine kidney (MDCK) cells (Catalogue No. GN023) were obtained from the Cell Bank of the Committee of the Type Culture Collection of the Chinese Academy of Sciences and maintained in complete Dulbecco's modified Eagle's medium (DMEM) containing 5% fetal bovine serum.

2.2. Immunization of hens

Five high-health-status, 20-week-old Roman laying hens housed at an experimental animal center were immunized intramuscularly with influenza B virus (HA titer of 64) that had been inactivated using beta-propiolactone. For the first immunization, 1 mg of antigen (viral protein) was emulsified with an equal volume of complete Freund's adjuvant (FA) (Sigma, USA), and incomplete FA was similarly used for the subsequent boosting immunizations. The hens were immunized a total of five times at intervals of two weeks. Eggs were collected after the initial immunization. Eggs laid by non-immunized hens were collected for use as the negative control.

2.3. Isolation and purification of IgY

A rapid and simple method adapted from previous studies (Ko and Ahn, 2007; Polson et al., 1980) was used to extract IgY from yolk. Briefly, the yolk was separated from the white by egg separators, and a volume of buffer containing 14% PEG6000 (w/v) equivalent to three volumes of yolk was added. The mixture was stirred at room temperature (RT) for 30 min and was centrifuged at 5000g for 20 min at 10 °C. The supernatant was collected and filtered through four layers of sterile gauze. The volume of the filtrate was measured, and PEG6000 was added by gentle stirring to adjust the final polymer concentration to 12% (w/v). The material was centrifuged at 5000g for 20 min at 10 °C. The pellet was dissolved to the original volume of yolk in phosphate buffer, solid ammonium sulfate was added to reach 50% saturation, and the mixture was stirred overnight at 4 °C. The precipitate was collected by centrifugation and washed with 33% saturated ammonium sulfate. The precipitate was dialyzed against PBS and freeze-dried, and the powder obtained was stored at –20 °C.

2.4. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed to determine the purity of IgY. A 15% polyacrylamide gel was used with a Mini-PROTEAN® 3 cell (Bio-Rad Laboratories, USA). The analysis was conducted under reducing conditions; the sample was mixed with sample buffer and held for 5–8 min at 100 °C. Ten microliters of the sample was loaded into each well. Pre-stained protein standard (Fermentas, Lithuania) was used as a molecular weight marker. The protein bands were visualized with Coomassie Brilliant Blue R250 (Fluka USA). The gel was analyzed using Bio-Rad image analysis software.

2.5. Enzyme-linked immunosorbent assay

The titer of IgY against influenza B virus was measured by an indirect non-competitive enzyme-linked immunosorbent assay (ELISA) according to previously reported methods with modifications (Zhao et al., 2003; Zhen et al., 2008). A 96-well micro-titer plate was coated with inactivated influenza B virus containing 0.58 mg/mL protein in carbonate–bicarbonate buffer (0.05 M, pH 9.6) at 100 µL/well. Serial dilutions of specific IgY were incubated in the pre-coated and blocked plate for 1 h before the bound IgY was detected with 100 µL/well HRP-conjugated rabbit anti-chicken IgY (1:5000) (Promega, USA). After incubation for 1 h at 37 °C, the plate was washed four times with PBS containing 0.05% Tween 20 (PBST). Next, 100 µL/well 3,3',5,5'-tetramethylbenzidine (Amresco, USA) substrate was added and incubated for 15 min at 37 °C. The color development was stopped with 2 M sulfuric acid (50 µL/well), and the optical density (OD) was measured on a micro-titer plate reader (Tecan f200, Switzerland) at 450 nm. The reproducibility of the experiment was ascertained by including a blank control (PBS) and a negative control (IgY derived from non-immunized hens) in each plate. IBV-specific IgY titer was defined as the maximum dilution multiple of the sample with an OD value that was 2.1 times that of the negative control.

2.6. Western blotting assay

To confirm the specificity of anti-IBV IgY to influenza B virus proteins, western blotting was conducted using a previously published method with some modifications (Qiu et al., 1992). Briefly, 100 µL of purified influenza B virus containing 1–2 mg/mL protein, as determined by the Bradford method, was mixed with 100 µL of electrophoresis sample buffer. The disrupted virus preparation was subjected to SDS–PAGE in a 14% slab polyacrylamide gel separated by a 4% stacking gel at 100 V for 3.5 h at RT. After electrophoresis, the gel was equilibrated in transfer buffer for 15–30 min, after which the viral proteins were electrically transferred onto a nitrocellulose (NC) membrane (Osmonics, USA) for 1 h at 350 mA at 4 °C. The NC membrane was cut into 0.5-cm strips, which were blocked with Tris-buffered saline containing 0.05% Tween 20 (TBS-T) and 5% non-fat dry milk for 1 h at RT and was incubated overnight at 4 °C with a 1:100 dilution of specific or nonspecific IgY. After incubation, the strips were washed four times with PBS-T and incubated with HRP-conjugated rabbit anti-chicken IgY (Promega, USA) diluted 1:1000. Following 2 h of incubation at RT, the strips were washed three more times. After washing, the strips were incubated in developing buffer containing 4 mg 3,3'-diaminobenzidine tetrahydrochloride (Aladdin, China) in 5 mL Tris-HCl and 15 µL hydrogen peroxide for 3–5 min. This reaction was stopped by rinsing with distilled water. The strips were photographed after development.

2.7. Hemagglutination inhibition (HI) test

HI test was carried out following the standard protocol (Meijer et al., 2006). Twofold serial dilutions of RDE-treated IgY, starting at 1:2, were incubated with equal volumes of inactivated influenza B virus with a titer of 4 HA units in U-bottom 96-well plates for 60 min at RT. At the end of the incubation, 25 µL of a freshly prepared 1% guinea pig (*Cavia porcellus*) erythrocyte suspension was added, and the mixture was homogenized. A virus control (containing virus and erythrocytes alone) and a blank control (containing erythrocytes and buffer alone) were included on the plate. The plates were allowed to stand at RT until a positive hemagglutination reaction developed in the virus control wells and the erythrocyte control showed a button at the bottom of the well, and the reading was subsequently taken. The HI titer was defined as the reciprocal of the highest dilution of IgY that completely prevented viral hemagglutination.

2.8. Plaque reduction assay

To determine the effect of IgY on influenza B virus replication, a plaque reduction assay was conducted as described (Landry et al., 2000). The original stock of influenza B virus was subjected to two additional passages in chick embryo allantoic cavities. The 50% tissue culture infection dose (TCID₅₀), and the multiplicity of infection (MOI) of the virus were determined. MDCK cells were seeded in 12-well plates, 2.5×10^5 per well, and cultured for 24 h to ensure the cells were 95–100% confluent. IgY, starting at 2 mg/mL with 2-fold serial dilutions, was incubated with an equal volume of 0.01 MOI influenza B virus at 37 °C for 30 min. Next, 200 µL of the incubated mixture was added to MDCK cell monolayers in the 12-well plates; a cell control (PBS and cells alone) and a virus control (virus and cells alone) were also included. After absorption for 2 h at 37 °C, the wells were overlaid with 1.5 mL of agarose-containing overlay medium. The plates were incubated for 72 h at 37 °C in an incubator with 5% CO₂, and then the overlay was carefully aspirated. The monolayers were fixed in 10% formalin in phosphate-buffered saline and were stained overnight with 1% crystal violet in 50% ethanol. The plaques were counted microscopically at low power. The 50% inhibitory concentration (IC₅₀) of IBV-specific IgY against influenza B virus was calculated by the Reed–Muench method.

2.9. Mouse infection and treatment trials

Mouse experiments were carried out to test the protective effect of the specific IgY in influenza B virus-infected mice. Female BALB/c mice that were 8 weeks old were obtained from the Experimental Animal Center of Guangdong Province (Foshan, Guangdong, China) and were maintained at the animal facilities of Sun Yat-sen University (Guangzhou, Guangdong, China). The mice were randomly divided into four groups of 10. The stock virus (B/Guangdong/867/2009) (25 µL/mouse, $10^{6.5}$ TCID₅₀/mL) was used to challenge the mice. Challenge trials in the mice were carried out as follows. The first group was inoculated intranasally with virus (25 µL/mouse, $10^{6.5}$ TCID₅₀/mL) and received PBS (25 µL/mouse) treatment 2 h before infection. The second group was infected with virus ($10^{6.5}$ TCID₅₀/mL) and were treated with IgY (10 mg/mL, 25 µL/mouse) 2, 26, 50, and 74 h post-infection. The third group received IgY (10 mg/mL, 25 µL/mouse) treatment 2 h prior to infection. The last group was not infected with virus and did not receive IgY treatment. The mice were monitored daily for weight changes for 7 days post-infection. At days 3 and 6 following infection, three mice from each group were sacrificed by inhalation of CO₂, and their lungs were collected. The right lung lobes were fixed in 10% neutral-buffered formalin overnight, embedded in paraffin and stained with hematoxylin and eosin (H&E). The remaining lungs were homogenized in 1 mL of cold PBS and clarified by centrifugation at 4 °C. The clarified homogenates were inoculated into 9-day-old, specific pathogen-free embryonated chicken eggs. After

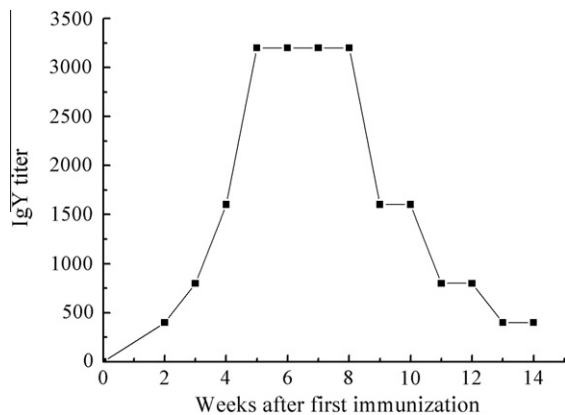


Fig. 1. The titer of anti-influenza B virus IgY antibody in egg yolks increases over time. Egg yolks were collected from immunized hens over a course of 100 days after the first immunization.

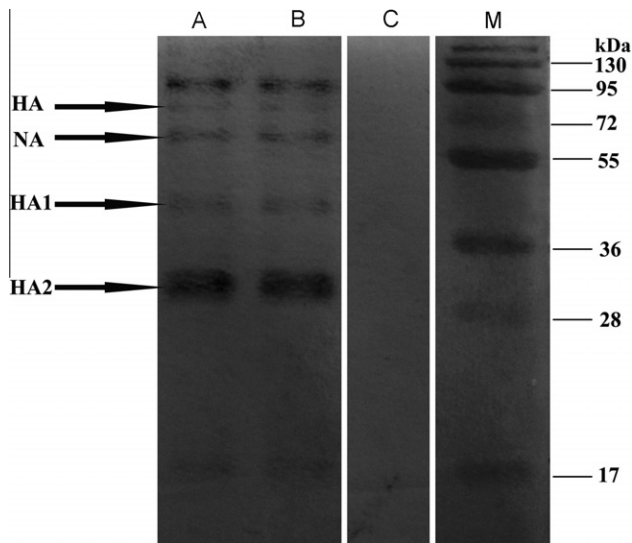


Fig. 2. Western blot analysis of IBV-specific IgY response to influenza B virus proteins. The virus was subjected to SDS-PAGE under reducing conditions, electrically transferred onto nitrocellulose membranes and probed with IgY from immunized and non-immunized hens (lane M: molecular maker; lanes A–B: immunized IgY diluted 1:500; lane C: non-immunized IgY diluted 1:500).

being passaged once in eggs, virus collected from the allantoic fluid was titrated by hemagglutination test. Viral nucleic acid in the lung homogenate was quantitatively detected by real-time reverse-transcription PCR (RT-PCR) using an Ag Path-ID™ One-step RT-PCR kit (Applied Biosystems). The primers and probe were as follows: Flu B Forward, 5'-TCC TCA ACT CAC TCT TCG AGC G-3'; Flu B Reverse, 5'-CGG TGC

TCT TGA CCA AAT TGG-3'; Flu B probe, 5'-CCA ATT CGA GCA GCT GAA ACT GCG GTG-3'. Positive virus and diethylpyrocarbonate (DEPC)-treated water were included as positive and negative controls, respectively.

2.10. Statistics

The data are expressed as the mean \pm standard error of the mean. Statistical differences were analyzed using Student's t-test.

3. Results

3.1. Isolation and purification of IgY

Isolation of IgY from yolk yielded 8.6 mg IgY/mL yolk. After two ammonium sulfate purification steps, the purity of IgY was significantly increased to 98.2% from 72.1%, and the yield was 5.1 mg IgY/mL egg yolk.

SDS-PAGE revealed the IgY preparation to be pure and dissociated into two protein bands with molecular weights of 68 kDa and 27 kDa. This electrophoretic pattern is consistent with that in a previous report (Devi et al., 2002).

3.2. Titer of anti-influenza B virus IgY in egg yolks

The anti-influenza B virus IgY produced by the immunized hens increased over time, as revealed by ELISA (Fig. 1). The specific IgY titer increased starting the second week after the first immunization, with the titer peaking at the fifth week. After 4 weeks, the antibody titer slowly declined.

3.3. Immunoreactivity of IBV-specific IgY to influenza B virus

By immunoblotting, IBV-specific IgY was found to specifically bind to the membrane proteins of influenza B virus (Fig. 2). The molecular weights of the viral proteins detected by IgY correspond to those of HA, HA1, HA2, and neuraminidase (NA), which is in accordance with previous results using HA and NA-specific monoclonal or polyclonal antibodies for detection (Qiu et al., 1992).

3.4. HI activity of IgY

In the HI assay, the attachment of influenza B virus to red blood cells was prevented by specific IgY, and the maximum dilution at which efficacy was observed was 1:128. In contrast, there was no HI effect observed with IgY derived from the egg yolks of non-immunized control hens (Fig. 3).

3.5. Inhibition of influenza B virus replication by IgY

To confirm further the antiviral activity observed with the HI test, a plaque reduction assay was performed on MDCK cell mono-

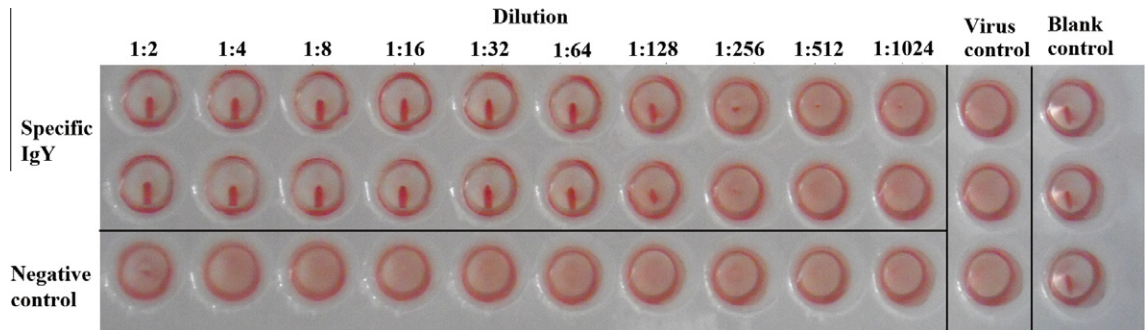


Fig. 3. HI test using IgY against influenza B virus. Twofold serial dilutions of IgY were used in the presence of 4 hemagglutination units of influenza B virus to observe the inhibition of guinea pig erythrocyte hemagglutination by IgY. Wells containing non-specific IgY served as negative controls; wells without IgY served as virus controls; wells without virus served as blank controls.

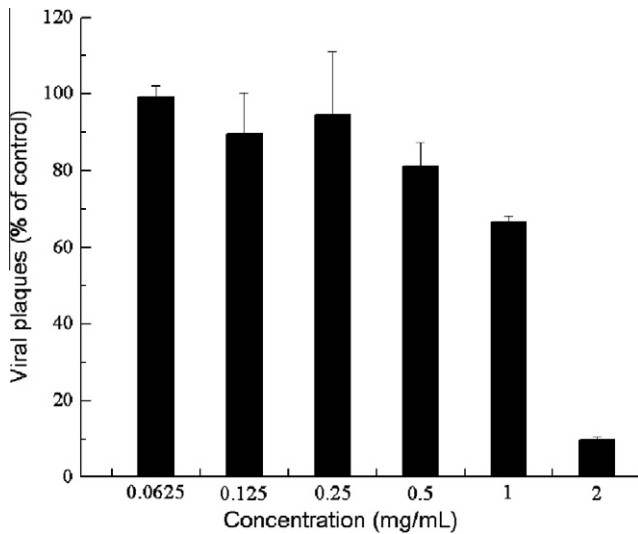


Fig. 4. IgY inhibition of influenza B virus replication. Influenza B virus (MOI 0.01) was pre-incubated with different concentrations of IgY before being added to MDCK cells. After the viral adsorption stage, 1.5 mL of agar overlay medium was added to the cells. The plaques produced on the plate were stained with crystal violet. Quantification of plaques treated with IgY. The results are from three independent experiments. The virus control was set as 100%.

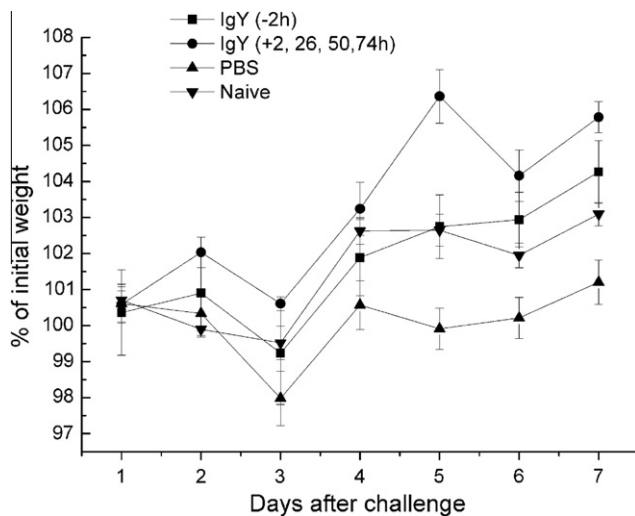


Fig. 5. Protection provided to influenza B virus-challenged mice by IgY based on weight gain. BALB/c mice were treated with 25 μ L of specific IgY 2 h before infection or 2, 26, 50, and 74 h after infection. Twenty-five microliters of influenza B virus ($10^{6.5}$ TCID₅₀/mL) was used for challenge. The values are the mean of 4–10 mice from each group. The data shown for IBV-specific IgY-treated group between day 4 and day 6 indicate a significant increase of weight ($P < 0.005$) compared to the PBS-treated group.

layers treated with a mixture of IgY pre-incubated with influenza B virus. As shown in Fig. 4, the specific IgY significantly ($P < 0.005$, IgY concentration at 2 mg/mL compared to virus control) inhibited influenza B virus replication in MDCK cells with an IC₅₀ of 1.68 mg/mL.

3.6. IgY provides *in vivo* protection to virus-challenged mice

Mouse experiments were carried out to determine the prophylactic and/or therapeutic efficacy of IBV-specific IgY in infected mice. Challenged mice receiving daily IgY treatment for 3 days beginning 2 h after virus challenge started to lose weight on the

Table 1
Protective effect of IgY in challenged mice.

Experimental group	RT-PCR analysis	
	3 days post-infection	6 days post-infection
PBS-treated mice ^a	3/3 Positive	3/3 Positive
IgY-treated mice ^b	2/3 Positive	1/3 Positive
IgY-pre-treated mice ^c	0/3 Positive	1/3 Positive
Naive mice ^d	0/3 Positive	0/3 Positive

^a Infected mice treated with PBS.

^b Infected mice treated with IgY at 2, 26, 50, and 74 h post-infection.

^c Infected mice that received IgY treatment 2 h prior to infection.

^d Mice that did not receive virus infection or treatment. Three mice from each group were euthanatized at the indicated day, and the lungs were taken for real-time one-step RT-PCR analysis.

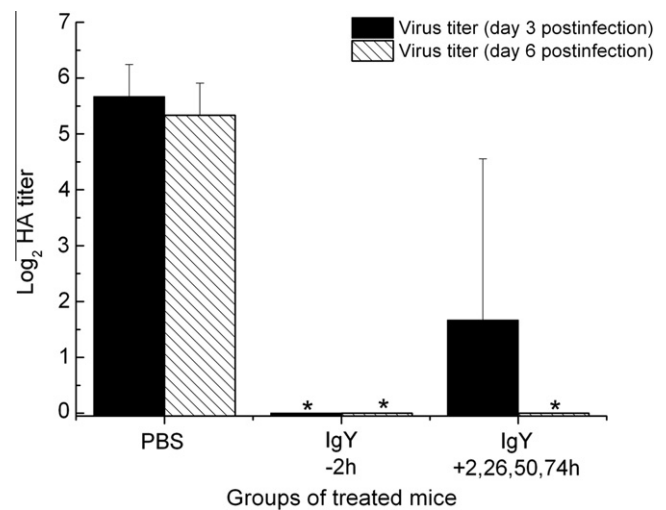


Fig. 6. Viral titers in the lungs of challenged mice receiving IgY treatment. BALB/c mice received 25 μ L of specific IgY 2 h before (–2 h) or 2, 26, 50, and 74 h after infection (+2, 26, 50, 74 h). Infected mice treated with PBS prior to infection or naive mice were included as controls. Twenty-five microliters of influenza B virus ($10^{6.5}$ TCID₅₀/mL) was used for challenge. Viral titers (HA) in the lungs were determined on day 3 and day 6 after infection. Lung homogenates from the indicated days were inoculated into and passaged in embryonated chicken eggs; the virus was titrated by hemagglutination test. * $P < 0.005$: significant difference compared to the PBS-treated group.

second day, but they recovered quickly with increasing weight gain. In contrast, infected mice that did not receive IgY treatment gained almost no weight during the trial period (Fig. 5).

Quantitative RT-PCR (Table 1) showed that challenged mice treated intranasally with specific IgY were protected from viral replication. The mice that received a single IgY treatment before infection were completely protected, whereas the mice that received multiple post-infection treatments were partially protected from viral reproduction. The partial protection may have been the result of intranasal lesions caused by the multiple intranasal IgY treatments during the trial.

To confirm further the RT-PCR results, embryonated chicken eggs were inoculated with virus. After one allantoic cavity passage, the fluids were harvested for HA testing. As anticipated, viral titers were significantly lower ($P < 0.005$) in mice that received IgY treatment than in PBS-treated mice (Fig. 6). There was no detectable viral titer in the mice pre-treated with IgY.

Lung histopathology analysis showed marked bronchiolar mucosa lesions and leukocyte infiltration in the challenged mice treated with PBS (Fig. 7D and d), whereas neither inflammatory cells nor lesions were observed in the lungs of infected mice that received IgY treatment, (Fig. 7B–C and b–c). These results indicate

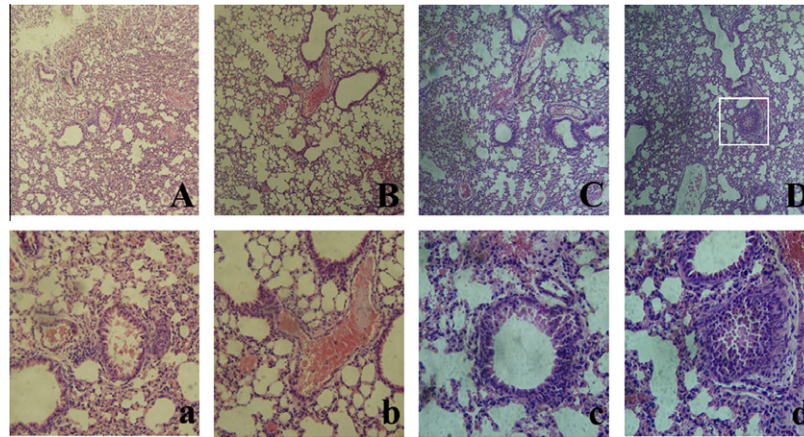


Fig. 7. Histopathological changes in mouse lungs after influenza B virus infection with or without IgY treatment. Lungs were fixed in neutral-buffered 10% formalin, and sections were stained with H&E. (A–D) Magnification is $\times 100$; (a–d) Magnification is $\times 400$. (A, a) Lung from a naive mouse. (B, b) Lung from mouse received IgY treatment 2 h prior to infection. (C, c) Lung from mouse treated with IgY 2, 26, 50, and 74 h post-infection. (b and c) Neither inflammatory cells nor lesions were observed in the lungs of either naive control or IgY-treated mice. (D, d) Lung from a mouse treated with PBS alone before infection; pulmonary inflammation, marked bronchiolar mucosa lesions and leukocyte infiltration were apparent.

that the virus neutralization activity of IgY promoted viral clearance in the infected mice.

4. Discussion

The prospect of passive immunotherapy for infectious diseases using specific IgY has received considerable attention in recent years. Several studies on pathogen-specific IgY have demonstrated the therapeutic efficacy of IgY, including in the treatment of *S. mutans* – caused dental caries (Fan et al., 2003), *H. pylori* – caused gastric ulcers (Attallah et al., 2009) and rotavirus-associated diarrhea (Sarker et al., 2001). However, IgY remains an underused resource for the treatment of influenza. Although influenza virus infections can be cured by the administration of anti-influenza drugs, such as adamantane compounds and neuraminidase inhibitors, large-scale administration of antiviral chemical compounds may lead to the emergence of resistant strains. IgY derived from the egg yolks of immunized hens is safe and inexpensive and has no known resistance issues. Importantly, IgY possesses remarkable pathogen-neutralizing activity in the respiratory tract and lungs. Therefore, intranasal or oral administration (in an unabsorbed manner) of IgY antibody for immunotherapy may be a promising strategy for the treatment of influenza.

In this study, anti-influenza virus IgY was easily produced on a large scale within a few months. Using this type of strategy, it would be possible to respond rapidly in the early stages of a new influenza pandemic, with only the antigen needing to be changed for immunization. To isolate IgY from egg yolks, we adopted a PEG extraction method followed by several steps of ammonium sulfate precipitation. The method is simple, efficient, and safe compared with water dilution, chloroform extraction, and gel-filtration chromatography, and, therefore, it is suited to large-scale isolation of IgY from egg yolk. The PEG used in this process, which is of low toxicity, is widely used in pharmaceutical production.

Western blotting results suggest that IBV-specific IgY may be able to neutralize the activity of both neuraminidase and hemagglutinin, which mediate viral attachment by interacting with a fusion protein in a specific manner to induce membrane fusion. The antigenic specificity of anti-IBV IgY was determined in an HI test using inactivated influenza B virus, and erythrocyte hemagglutination was inhibited by IBV-specific IgY at a dilution

of 1:128. The ability of IBV-specific IgY to inhibit influenza B virus replication was further confirmed in a plaque reduction assay.

More importantly, in mouse experiments, we found that IgY treatment provided prophylactic and therapeutic efficacy in challenged mice. When specific IgY was delivered intranasally, the viral titer in the lungs of infected mice decreased significantly. This viral clearance was probably due to the intrinsic neutralization ability of IgY. A recent study by Nguyen et al. (2010) reported that IgY also protects H1N1 and H5N1 influenza virus-infected mice. In another recent report, Wallach et al. demonstrated a cross-protection effect of IgY in H1N1 and H5N1-treated mice. Furthermore, Wallach et al. (2011) showed the feasibility of increasing the production of IgY antibodies using inactivated H1N1 and H3N2 viruses together. For this reason, influenza A virus may also be potentially combined with influenza B to increase further the production of universal IgY antibodies.

IgY possesses remarkable resistance to extreme pH values, temperature, and pepsin. IgY is stable at pH 4–9 and up to 65 °C in aqueous conditions (Shimizu et al., 1992), and it retains antigen-binding activity in the presence of pepsin at pH 4–6 (Shin et al., 2002). These properties make it possible to make functional foods containing IgY and pathogen-specific antibody medicines for infectious disease control.

In conclusion, the results presented in this study indicate the possibility of 'universal' passive immunotherapy using specific IgY for influenza virus infection. Our results also suggest that egg yolk from immunized laying hens may offer a new large-scale source of low-cost antibodies. Efforts are underway in our laboratory to increase the potency of IgY against influenza virus infections. In a future study, we hope to demonstrate the antiviral potency of IgY in a drug-resistant influenza virus infection animal model.

Acknowledgments

This work was financially supported by the Guangdong Province 211 project and the Tianhe district project (No. 104G193). The authors would like to thank Professor David J. Hampson of Murdoch University, Australia, for assisting with language editing. The authors acknowledge Dr. Huaqian Wang and Dr. Zheng Jie for many helpful discussions during the preparation of the manuscript.

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