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Efficacy of A/H1N1/2009 split inactivated influenza A vaccine (GC1115) in mice and ferrets

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To evaluate the efficacy of a non-adjuvant A/H1N1/2009 influenza A vaccine (GC1115), we demonstrated the immunogenicity and protective efficacy of GC1115 in mouse and ferret models. The immunogenicity of GC1115 was confirmed after intramuscular administration of 1.875, 3.75, 7.5, and 15 μg hemagglutinin antigen (HA) in mice and 7.5, 15, and 30 μg HA in ferrets at 3-week intervals. A single immunization with GC1115 at HA doses > 7.5 μg induced detectable seroconversion in most mice, and all mice given a second dose exhibited high antibody responses in a dose-dependent manner. The mice in the mock (PBS) and 1.875 μg HA immunized groups succumbed by 13 days following A/California/04/09 infection, while all mice in groups given more than 3.75 μg HA were protected from lethal challenge with the A/California/04/09 virus. In ferrets, although immunization with even a single dose of 15 or 30 μg of HA induced detectable HI antibodies, all ferrets given two doses of vaccine seroconverted and exhibited HI titers greater than 80 units. Following challenge with A/California/04/09, the mock (PBS) immunized ferrets showed influenza-like clinical symptoms, such as increased numbers of coughs, elevated body temperature, and body weight loss, for 7 days, while GC1115-immunized ferrets showed attenuated clinical symptoms only for short time period (3–4 days). Further, GC1115-immunized ferrets displayed significantly lower viral titers in the upper respiratory tract (nasal cavity) than the mock vaccinated group in a dose-dependent manner. Taken together, this study demonstrates the immunogenicity and protective efficacy of GC1115 as a non-adjuvanted vaccine.

Keywords: H1N1 pandemic, influenza, vaccination, immunogenicity, protective efficacy

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Introduction

Influenza A virus is a member of the family *Orthomyxoviridae* and causes severe respiratory disease in animals including humans (Louten, 2016). In 2009, a novel swine-derived influenza A (H1N1) virus was responsible for numerous cases of febrile respiratory illness globally. Epidemiological data from the 2009 H1N1 influenza pandemic indicate that the outbreak of influenza-like respiratory illness originated in the Mexican town of La Gloria, Veracruz in mid-February 2009 (Fraser *et al.*, 2009). By the end of April 2009, the international spread and clusters of human-to-human transmission prompted World Health Organization (WHO) to elevate the pandemic alert from phase 3 to phase 4 and shortly thereafter to phase 5, indicating the existence of human-to-human spread in at least two countries and signs of an imminent pandemic (Neumann *et al.*, 2009). Therefore, the first influenza A virus pandemic in the 21st century was declared on June 11, 2009 by the WHO owing to the emergence and rapid global spread of a novel influenza A (H1N1) virus, hereafter referred to as the pandemic (H1N1) 2009 virus (Munster *et al.*, 2009; Dawood *et al.*, 2012).

In September 2009, the FDA approved injectable egg-based H1N1 vaccines manufactured by CSL, Novartis, and Sanofi Pasteur and a nasal H1N1 vaccine (live attenuated) developed by MedImmune. Although Novartis and GSK market H1N1 vaccines both with and without adjuvants (MF59.C1 and AS03, respectively), only non-adjuvanted vaccines have been authorized in the USA. The adjuvanted subunit (use only some parts of the virus) Focetria (Novartis, MF59.C1 adjuvant) and split (the virus has been disrupted by a detergent) Pandemrix (GSK, AS03 adjuvant) vaccines were approved by the European Medicines Agency (EMA) in 2009. Because of global pandemic prevalence, during the H1N1 influenza pandemic of 2009 there was no guarantee of availability of vaccine stocks for import to all countries, which resulted in a vaccine shortage. Although the addition of adjuvant to vaccines might help induce a stronger immune response in hosts, compared with non-adjuvanted vaccines, vaccines containing adjuvants are relatively expensive and require additional safety testing in clinical trials before they are licensed for use. Further, there are only limited numbers of adjuvants for human use, most of which are patented by large pharmaceutical companies. Therefore, there is some limitation for use of proper adjuvants in emergency situations such as during a pandemic situation.

In this study we developed a non-adjuvanted, monovalent split p2009 H1N1 vaccine (GC1115) and evaluated its protective efficacy in animals. To demonstrate the vaccine efficacy, we adapted two well-known influenza infection mo-

dels, mice and ferrets (Itoh *et al.*, 2009; Maines *et al.*, 2009; Munster *et al.*, 2009), and inoculated them with different hemagglutinin antigen (HA) doses. Our results demonstrate that immunization with GC1115 induces hemagglutination inhibition (HI) titers in a dose-dependent manner in both mice and ferrets and inhibits viral shedding through the nasal route and viral replication in the respiratory tract. Thus, there is a positive correlation between the HI titers induced by non-adjuvanted GC1115 vaccine and its efficacy against lethal challenge of pdm2009 H1N1 virus.

Materials and Methods

Viruses

The GC1115 vaccine was produced using the 2009 pandemic vaccine seed strain, (A/California/07/09, H1N1; NYMC X179A) and A/California/04/09(H1N1; CA/04) was used for virus challenge experiments. Challenge virus stocks were generated in 11-day-old embryonated chicken eggs, and the titers were calculated as $\log_{10}$ EID_{50}/ml (Reed and Muench, 1938). Stock viruses were stored at -80°C and thawed immediately before use. In addition, the 50% lethal dose for mice was defined as the EID_{50} that resulted in 50% mortality and was calculated using the method proposed by Reed and Muench. The A/CA/ 04/09 virus was handled in an approved biosafety level 3 facility for use in both *in vitro* and *in vivo* experiments.

Vaccine generation

The 2009 H1N1 pandemic influenza vaccine seed virus was propagated in 11-day-old-embryonated eggs at 37°C for 72 h. The allantoic fluid containing viruses was harvested and concentrated to 1/10th of the original volume using a molecular cut-off concentrator apparatus (300K, Sarto Flow 40, Sartorius Biotech). The concentrates were purified using sucrose density gradient centrifugation and were subsequently inactivated using formalin. The inactivation of the vaccine was confirmed by the absence of virus growth (HA titration with turkey RBC) in two consecutive passages in 11-day-old-embryonated eggs. The HA protein content in the purified bulk vaccine was determined using the standard single-radial-immunodiffusion technique using standard sera and antigens obtained from the NIBSC (Schild *et al.*, 1975).

Vaccination and virus challenge

Four-week-old BALB/c mice were purchased from Samtako, and 15–16-week-old ferrets (*Mustela putorius furo)* were purchased from Marshall Co. Ltd. All animals were confirmed to be seronegative for influenza A viruses using serologic assay. Groups of mice (10 per group) were intramuscularly vaccinated with two doses of inactivated GC1115 containing 1.875, 3.75, 7.5, or 15 μg HA or whole inactivated 15 μg HA vaccine in 0.2 ml of sterile phosphate-buffered saline (PBS) at a 3-week interval. Two weeks after the last vaccination, mice were intranasally challenged with 6.0 $\log_{10}$ EID_{50}/ml of CA/04 virus. The control group received 30 μl of sterile PBS. The kinetics of survival was analyzed by Kaplan-Meier curves using GraphPad Prism version 5.00 for Windows (GraphPad Software).

Groups of ferrets (eight per group) were intramuscularly vaccinated with two doses of the inactivated GC1115 containing 7.5, 15, or 30 μg HA in 0.5 ml of sterile PBS at a 3-week interval. Two weeks after the last immunization, they were challenged with 10^6 EID_{50} of CA/04 virus in the nasal cavity. The control group received 0.5 ml of PBS.

Serologic assays

HI assays were performed as described previously (Kendal, 1982; Lu *et al.*, 1999). Briefly, serum samples were treated with receptor-destroying enzyme (RDE; Denka Seiken) to inactivate nonspecific inhibitors using a final serum dilution of 1:10. RDE-treated sera were serially diluted 2-fold, and an equal volume of virus (8 HA units/50 μl) was added to each well of a microplate. The microplates were incubated at room temperature for 30 min followed by the addition of 0.5% (v/v) turkey red blood cells (RBCs). The plates were gently mixed and incubated at 37°C for 30 min. HI titer was determined using the reciprocal of the last dilution that did not exhibit agglutination of turkey RBCs. The detection limit for HI assay was set as < 20 HI units.

Sera, nasal wash samples, and tissue collection

Sera from mice and ferrets were collected 2 weeks after each vaccination and were stored at -80°C until use. Lung tissues of mice (3, 5, and 7 dpi) and ferrets (3 and 7 dpi, 4 ferrets/time point) were harvested and homogenized with an equal volume (1 ml/g tissue) of PBS containing antibiotics [penicillin G (2×10^6 U/L), polymyxin B (2×10^6 U/L), gentamicin (250 mg/L) nystatin (0.5×10^6 U/L), ofloxacin HCl (60 mg/L), and sulfamethoxazole (0.2 g/L)].

Tissue homogenates were clarified by centrifugation at 12,000 × *g* for 10 min at 4°C, following which the supernatants were transferred to new tubes. To evaluate the viral replication in the upper respiratory tracts of ferrets, nasal wash samples were collected at 2, 3, 5, and 7 dpi. The collected lung and nasal wash samples were serially diluted 10-fold and then inoculated in 11-day-old embryonated chicken eggs for viral titration according to the Reed-Muench method; the results were expressed as $\log_{10}$ EID_{50}/ml or $\log_{10}$ EID_{50} per gram of tissue ($\log_{10}$ EID_{50}/g) collected (Reed and Muench, 1938).

The limit of virus detection was set at < 0.1 $\log_{10}$ EID_{50}/ml or $\log_{10}$ EID_{50}/g, and the viral titers were compared using the standard Student's *t*-test.

Histopathology

Mice immunized with each vaccine were infected with 6.0 $\log_{10}$ EID_{50}/ml of CA/04 virus. At 5 dpi, mice lungs were harvested in each group. The samples were fixed in 10% neutral-buffered formalin and embedded in paraffin. Histological assessment was conducted using standard hematoxylin and eosin staining. The slides were viewed using an Olympus h 71 (Olympus) microscope and DP controller software to capture images (magnification × 200).

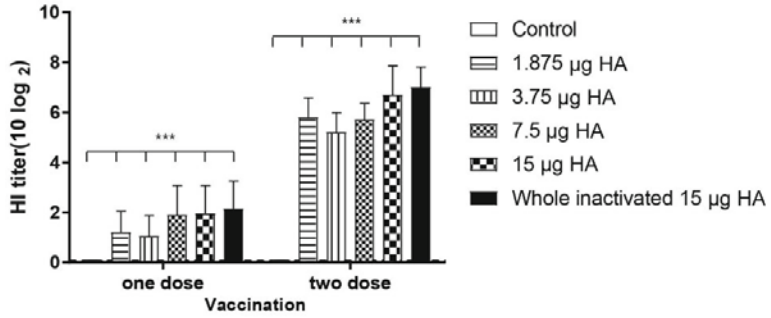

Fig. 1. Hemagglutination inhibition (HI) titers following immunization of mice with the split/inactivated GC1115 or whole inactivated vaccine. Four-week-old BALB/c mice (n = 10/group) were immunized twice at a 3-week interval. PBS was administered by intramuscular injection to the control group and 1.875, 3.75, 7.5 or 15 μg HA antigen was given to each of the other groups. Asterisks indicate statistical significance between control and immunized group as determined by Mann-Whitney U test (*** indicates $P < 0.0001$).

Statistical analysis

To assess significant differences, viral titers were compared between the control and GC1115-immunized groups. Asterisks indicate statistical significance between the control and GC1115-immunized groups as determined using Mann-Whitney U test (*$P < 0.05$, **$P < 0.001$, ***$P < 0.0001$). All statistical analyses were performed using GraphPad Prism version 5.00.

Ethics Statement

All experiments involving animals were conducted in accordance with relevant national and international guidelines for animal handling at Bioleaders Corp (BLS-ABLS-12-009) and Animal Use and Care by Laboratory Animal Research Center in Chungbuk National University, a member of the International Animal Care and Usage Committee (CBNUA-767-14-11).

Results

Efficacy of GC1115 in mice

Immunogenicity of GC1115 : Although single doses of 1.875 and 3.75 μg HA of GC1115 could not induce an antibody response sufficient for the HI assay, most mice that received > 7.5 μg HA of the vaccine exhibited relatively high geometric mean titers (GMTs) of 33.11–45.37 HI units (Fig. 1). In the two-dose immunization groups, all immunized mice exhibited antibody responses regardless of the dose with GMTs of 557.15 (1.875 μg HA), 367.58 (3.75 μg HA), 497.41 (7.5 μg HA), and 1059.52 (15 μg HA) HI units (Fig. 1). Further, the 15 μg HA dose group induced comparable HI titers to the mice receiving 15 μg of whole inactivated vaccine. This demonstrates that the GC1115 vaccine can induce strong immune responses after two doses in mice.

Clinical symptoms followed by wild type CA/04 virus infection : While the PBS control group exhibited 100% mortality and a gradual loss in body weight until death follow-

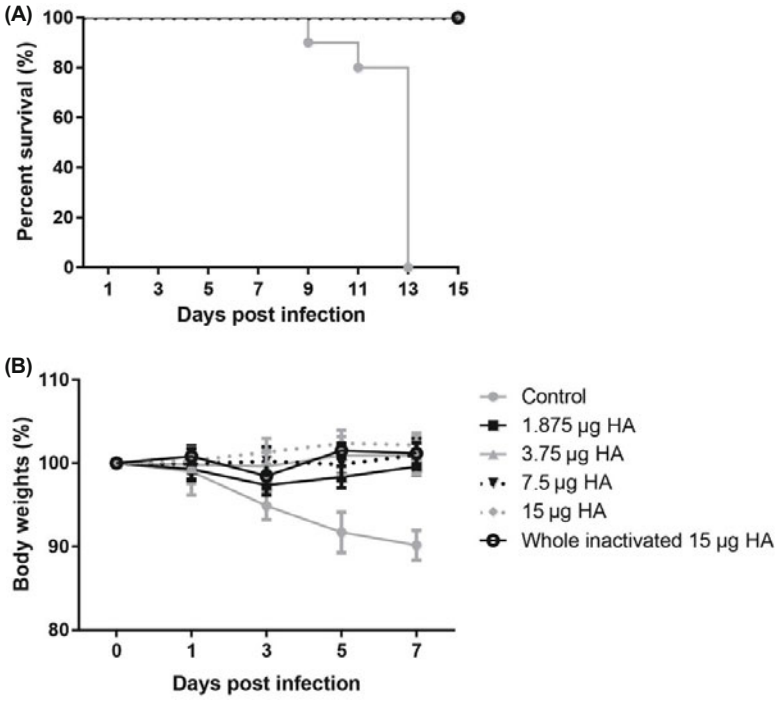

Fig. 2. Survival rate and percent body weight loss in mice after challenge with the A/California/04/09 (H1N1) influenza virus. Groups of mice were immunized twice at a 3 week-interval, and then intranasally infected with the virus. The survival rate (A) and body weight loss (B) were monitored.

Table 1. Clinical symptoms in GC1115-immunized mice following A/California/04/09 (H1N1) virus infection

Groups	HA dose (μg)	Clinical symptoms									
		Day 0		Day 1		Day 3		Day 5		Day 7	
		Activity[a]	Ruffled fur[b]	Activity	Ruffled fur	Activity	Ruffled fur	Activity	Ruffled fur	Activity	Ruffled fur
Control	PBS	–	–	–	–	+	++	++	++	++	++
GC1115	1.875	–	–	–	–	+	+	+	+	–	–
	3.75	–	–	–	–	+	+	–	–	–	–
	7.5	–	–	–	–	–	+	–	–	–	–
	15	–	–	–	–	–	+	–	–	–	–
Whole inactivated vaccine	15	–	–	–	–	–	+	–	–	–	–

[a] Activity was scored using three grades (-, normal; +, decreased; ++, significantly decreased)
[b] Appearance was categorized based on fur condition (-, normal; +, coarse fur; ++, extremely coarse fur)

ing challenge with the CA/04 virus, all GC1115- and whole inactivated vaccine immunized mice exhibited only mild body weight loss (less than 7%) and were protected from lethal challenge with CA/04 virus (Fig. 2). Further, the control mice exhibited fur ruffling from a lack of grooming and a steady decline in activity by 3 dpi, whereas mice immunized with ≥ 3.75 μg HA of GC1115 regained normal activity and recovered to their pre-challenge condition (Table 1). At 5 dpi, the control group displayed significantly decreased activity, which continued until 7 dpi. While the 1.875 μg HA GC1115-immunized group exhibited moderate clinical symptoms such as activity loss and coarse fur until 5 dpi, they recovered to the pre-challenge condition by 7 dpi (Table 1). The mice receiving ≥ 7.5 μg HA dose of GC1115 showed comparable protective efficacy with mice vaccinated with 15 μg of whole inactivated H1N1 in regard to survival, body weight loss, and clinical symptoms (Table 1 and Fig. 2).

Lung virus titers followed by wild type CA/04 virus infection : In the control group, the highest lung viral titer was observed at 3 dpi (4.5 $\log_{10}$ EID_{50}/ml) and persisted until 7 dpi (3.5 $\log_{10}$ EID_{50}/g) (Fig. 3). However, viral titers peaked at 3 dpi in all GC1115-immunized groups (1.0–2.25 $\log_{10}$ EID_{50}/g) and decreased at 5 dpi before becoming undetectable at 7 dpi. The decreased lung viral titers of each vaccine group tended to be proportional to antigen amount in the vaccine (Fig. 3). These results confirmed that two doses of the non-adjuvanted, split GC1115 vaccine provides protection to mice against CA/04 challenge.

Histopathology in lungs of mice followed by wild type CA/04 virus infection : Lung tissue from PBS-treated control mice showed severe and extensive inflammation following CA/04 virus infection, including large amounts of mononuclear cell

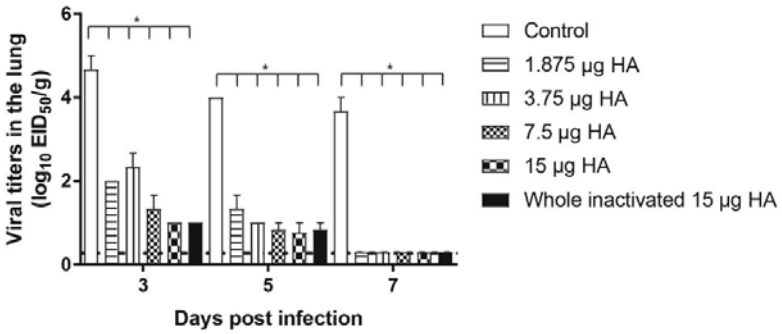

Fig. 3. Lung virus titers of GC1115 or whole inactivated vaccine immunized mice infected with A/California/04/09 (H1N1). Lungs were collected at 3, 5, and 7 dpi with A/California/04/09 (H1N1), and the viral titers were calculated using embryonated chicken eggs ($\log_{10}$ EID_{50}/ml). Asterisks indicate statistical significance between control and immunized group (* indicates $P < 0.05$) as determined by Mann-Whitney U test.

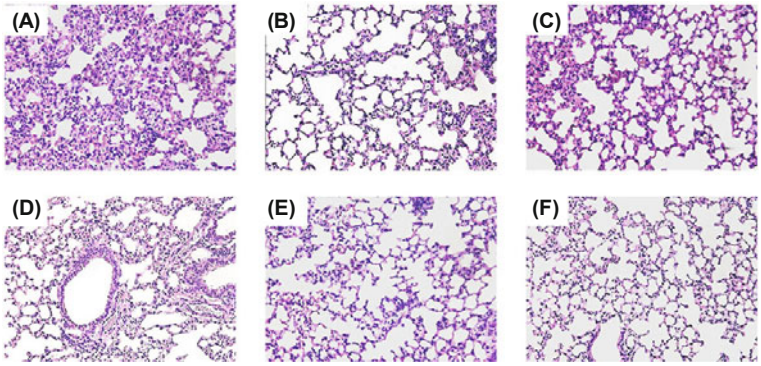

Fig. 4. Histopathology of lungs from each group of mice followed by challenge with A/California/04/09 (H1N1) virus. Each lung was collected at 5 dpi and processed for H&E staining for histopathology evaluation. (A) Control (B) 1.875 μg HA (C) 3.75 μg HA, (D) 7.5 μg HA, (E) 15 μg HA, or (E) whole inactivated 15 μg HA vaccine. The lung from the control (PBS-treated) showed more inflamed tissue than that seen in GC1115 immunized mice. Magnification × 200.

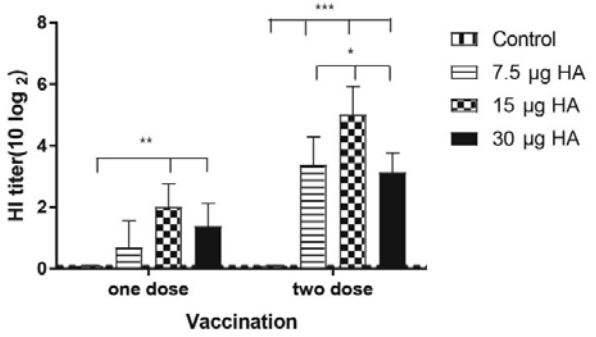

Fig. 5. Hemagglutination inhibition (HI) titers following split/inactivated GC1115 vaccine immunization of ferrets. GC1115 was administered at 0, 7.5, 15 or 30 μg HA per dose, and HI titers were measured 3 weeks and 5 weeks after immunization. Asterisks indicate statistical significance between the control and immunized group (** indicates $P < 0.001$, and *** indicates $P < 0.0001$) or between 15 μg HA and other immunized groups (* indicates $P < 0.05$) determined by Mann-Whitney U test

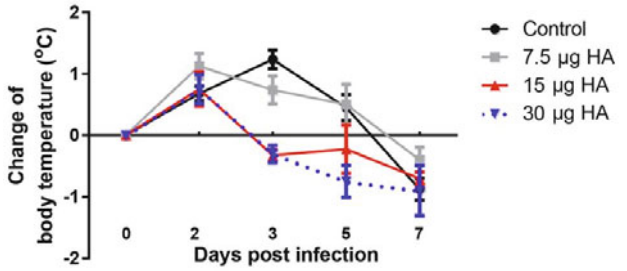

Fig. 6. Change in mean body temperature of A/California/04/09 (H1N1)-infected vaccinated ferrets. Body temperatures were monitored for 7 dpi. The 0 on y axis stands for the mean initial body temperature of each group prior to A/California/04/09 (H1N1) virus infection.

infiltrates in the alveoli, interstitial septa, and perivascular spaces (Fig. 4A). In comparison, lungs from GC1115-immunized mice exhibited decreased lesions, in which inflammation was localized to limited areas of the lungs (Fig. 4B to F). Moreover, lungs from 15 μg HA immunized mice demonstrated only small areas of inflammatory infiltrates and a minimal increase in the cellularity of the interstitial septa comparable to that seen following immunization with 15 μg of the whole inactivated vaccine (Fig. 4E and F).

Efficacy of GC1115 in ferrets

Immunogenicity of the GC1115 vaccine in ferrets : To evaluate the immunogenicity of the GC1115 vaccine in ferrets, groups of animals were immunized twice at a 3-week interval. Three weeks after the first vaccination, blood samples were collected and the antibody response was assessed. The 7.5 μg HA group exhibited an antibody response of 20–40 HI, whereas the 15 and 30 μg HA groups exhibited titers of 40–80 HI units (Fig. 5). Following the second vaccination, antibody levels significantly increased to 103.75 (7.5 μg HA), 320 (15 μg HA), and 87.24 (30 μg HA) HI units (Fig. 5). It is noteworthy that the highest antibody response was observed in the 15 μg HA group rather than the 30 μg HA group (Fig. 5). No antibodies were detected in the control group.

Clinical symptoms followed by wild type CA/04 virus infection : Following infection with wild-type A/California/04/09 (H1N1) virus, the PBS-treated ferrets (control group) exhibited increased body temperature as early as 2 dpi (mean temperature increase: 0.7°C), reaching a peak by 3 dpi (mean temperature increase: 1.4°C), then gradually decreasing until 7 dpi (Fig. 6), suggesting an active CA/04 infection. In the 7.5 μg HA immunized group, the body temperature of ferrets was also elevated at 2 dpi and gradually decreased to the normal range by 7 dpi (Fig. 6). However, ferrets immunized with 15 or 30 μg HA showed only slight increases of body temperature at 2 dpi, which rapidly returned to the normal temperature range as early as 3 dpi. Meanwhile, decreased food intake, increased body temperature, and decreased activity were observed in all ferret groups until 2 dpi. Although both the control and 7.5 μg HA groups exhibited higher cough numbers, greater activity loss, and reduced food intake, especially in the control group until 3 dpi, ferrets in the 7.5 μg HA group rapidly regained their activity and food intake by 4 dpi (data not shown). In contrast, the 15 and 30 μg HA groups exhibited only moderate clinical symptoms at 2 dpi, with

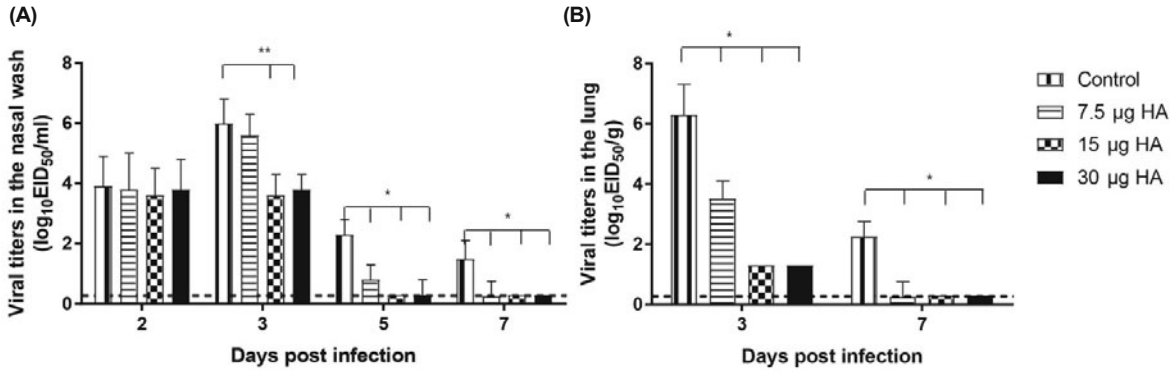

Fig. 7. Virus titers in nasal washes (A) and lungs (B) of each group of vaccinated ferrets following infection with A/California/04/09 (H1N1) virus. (A) Nasal washes were collected from each group of ferrets at 2, 3, 5, and 7 dpi and the viral titers were calculated using embryonated chicken eggs (log_{10} EID_{50}/ml). The data shown are mean values plus standard deviations for 4–8 ferrets per group. (B) Lung tissue (n = 4) was collected from each group at 3 and 7 dpi, and the viral titers were calculated using embryonated chicken eggs (log_{10} EID_{50}/ml). Asterisks indicate statistical significance between control and immunized groups (* indicates $P < 0.05$, and ** indicates $P < 0.001$) as determined by Mann-Whitney U test.

increased food intake and recovery of their physical movements by 3 dpi. By 5 dpi, only the control ferrets displayed complete nose obstruction due to excessive mucous production and an unstable respiratory rate (data not shown).

Viral titers followed by wild type CA/04 virus infection : To evaluate the vaccine efficacy of GC1115 for inhibition of virus replication, nasal washes were collected from each group from 2 to 7 dpi and the viral titers were measured (Fig. 6). The control group displayed the highest viral titers at 3 dpi (6.0 $\log_{10}$ EID_{50}/ml), with detectable virus until 7 dpi (1.5 $\log_{10}$ EID_{50}/ml). The 7.5 μg HA group also exhibited relatively high viral titers at 3 dpi compared with the other vaccine groups ($P < 0.001$), which rapidly decreased at 5 and 7 dpi compared with the titers in the control group ($P < 0.05$). The 15 and 30 μg HA groups displayed significantly attenuated viral titers at 3 dpi compared with control and 7.5 μg HA group ($P < 0.05$) and the virus was cleared by 5 dpi (Fig. 7A). Similar to the findings for nasal swabs, the control ferrets exhibited the highest lung viral titers with 6.3 and 2.25 $\log_{10}$ EID_{50}/g at 3 and 7 dpi, respectively. All GC1115-immunized ferrets displayed significantly attenuated viral titers compared with control ferrets ($P < 0.05$), but more attenuation was observed in the 15 and 30 μg HA groups compared with the 7.5 μg HA groups ($P < 0.05$) at 3 dpi, and the virus was not detectable at 7 dpi (Fig. 7B). Taken together, two doses of GC1115 can induce sufficient immunogenicity in ferrets and significantly attenuate both clinical symptoms and CA/04 replication in the upper and lower respiratory tracts.

Discussion

The causative pandemic 2009 influenza virus, H1N1 is considered a novel strain and the pre-existing vaccines against human influenza viruses did not elicit cross-reactive antibodies to this pandemic virus (Katz *et al.*, 2009). Therefore, we conducted a preclinical study to determine the efficacy of a split inactivated 2009 pandemic influenza A (H1N1) monovalent vaccine (GC1115) using mouse and ferret models.

In mice, although single immunizations of GC1115 at 1.875 and 3.75 μg HA did not induce detectable HI antibody responses, all GC1115-immunized mice displayed 100% antibody responses following booster immunization. Upon challenging with the CA/04 virus, most of the mice in the GC1115-immunized groups exhibited decreased activity, although mice vaccinated with more than 3.75 μg HA/dose regained normal activity and displayed attenuated lung viral titers (Table 1 and Fig. 3).

Among animal models, the ferret model is considered to be the most suitable small animal model for conducting influenza research related to humans because this species is extremely susceptible to infection by human influenza viruses without prior host adaptation (Belser *et al.*, 2011). Hence, to further evaluate the vaccine efficacy of GC1115, two doses of vaccine at 7.5, 15, or 30 μg HA were administered at a 3-week interval. While single immunization using GC1115 only induced moderate HI titers irrespective of the dose, booster immunization significantly increased the titers compared with the control group ($P < 0.001$) (Fig. 5). Interestingly, ferret treated with 15 μg HA displayed the highest HI titers in sera compared with other immunized groups ($P < 0.05$). This result suggests that too high a dose of the antigen might interfere with antibody production. Similar results were reported in several clinical vaccine trials that showed there were no significant differences in immunogenicity between the 15 and 30 μg HA group with respect to HI titers (Greenberg *et al.*, 2009; Zhu *et al.*, 2009; Cheong *et al.*, 2011). Further, according to metadata analysis, the immunized antibody titers were similar between ferrets and humans (Cheong *et al.*, 2011). These results suggest that similar to the seasonal vaccine doses, the 15 μg HA dose of GC1115 could be an accurate antigen dose for pandemic 2009 H1N1 vaccines.

Following infection with the CA/04 virus all GC1115 immunized ferrets displayed similar viral titers in the upper respiratory tract (3.75–4.0 $\log_{10}$ EID_{50}/ml) at 2 dpi, with the mock (PBS) control group showing the highest virus titers (as 6.0 $\log_{10}$ EID_{50}/ml) which persisted until 7 dpi (1.75 $\log_{10}$ EID_{50}/ml). However, all GC1115-immunized groups displayed significantly decreased viral titers from 5 dpi, and no virus was detected in nasal swabs from the 15 and 30 μg HA groups at 7 dpi (Fig. 7A), suggesting inhibition of viral shedding and early viral clearance in response to GC1115 vaccination.

In conclusion, our results demonstrate that GC1115 immunization induces high HI titers in a dose-dependent manner in both mice and ferrets. Further, vaccination of ferrets with GC1115 reduces shedding of the CA/04 virus through the nasal route and inhibits viral replication in the lungs suggesting a positive correlation between the induced HI titers and immunization method adequacy. Hence, this study demonstrates that the non-adjuvanted GC1115 vaccine induces proper immunogenicity and protective efficacy against H1N1 infection of hosts.

Acknowledgements

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Conflicts of Interest

No potential conflicts of interest were disclosed.

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