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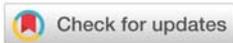
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Research Article

Study of Immunogenicity and Protective Efficacy of Live MDCK- derived Pandemic Influenza Vaccine

Abstract

Background: The threat of pandemic A/H1N1 influenza is still a matter of considerable public concern. Influenza outbreak in 2009 underlined the importance of rapid production of a sufficient vaccine reserve for pandemic and interpandemic periods. One promising way to allay this concern is development of cell culture-derived live attenuated influenza vaccines (LAIV), because this technology makes it possible to produce a considerable amount of vaccine over a short period of time.

Objectives: The goal of this work was to study the immunogenic and protective properties of the Vector-Flu vaccine in animal models.

Methods: We have developed Vector-Flu, a live attenuated vaccine against pandemic influenza, based on the cold-adapted reassortant vaccine strain for LAIV, A/17/California/2009/38(H1N1), and produced in certified MDCK cells. The immunogenicity and protective efficacy of Vector-Flu vaccine were studied in ferrets and mice.

Results: A double immunization with the live MDCK-derived Vector-Flu influenza vaccine induced a high level of neutralizing antibodies in ferret serum both to the pandemic A/Chita/3/2009(H1N1) influenza virus strain, isolated in Russia, and to the pandemic A/California/7/2009(H1N1) strain. Intranasal immunization of mice induced levels of serum antibodies sufficient to protect them when aerosol-challenged with 100 infectious doses of A/Chita/3/2009(H1N1) strain.

Conclusion: Theoretical estimates of protective antibody levels necessary for protecting humans from the disease caused by pandemic A/H1N1(2009) influenza virus, and the experimental data from animal models (ferrets and mice), suggest that the Vector-Flu vaccine is able to protect humans after a single immunization.

Introduction

The threat of pandemic A/H1N1 influenza remains a matter of considerable public concern. Recent influenza outbreaks underline the importance of rapid production of a reserve of vaccine sufficient for pandemic and interpandemic periods [1]. Traditional vaccines intended for seasonal influenza do not satisfy this demand, because they are unable to induce cross-reactive antibodies to pandemic flu [2]. Live influenza vaccines developed in the cell culture offer potential advantages because it is possible to produce a considerable amount of these vaccines over a short period, their use eliminate concerns about allergic reactions to egg proteins, and they allow the antigenic composition of the vaccine to be closer to the circulating influenza virus strains.

In collaboration with the Institute of Experimental Medicine

(St Petersburg, Russia), the State Research Centre for Virology and Biotechnology VECTOR (Novosibirsk, Russia), developed a live attenuated vaccine against pandemic influenza, Vector-Flu, derived from the cold-adapted A/17/California/2009/38(H1N1) vaccine strain and produced in the certified MDCK cells. The goal of this work was to study the immunogenic and protective properties of the Vector-Flu vaccine in ferret and mouse animal models.

Methods

Viruses

The A/California/7/2009(H1N1) pandemic influenza virus strain was obtained from CDC (Atlanta, GA). The A/17/California/2009/38 (H1N1) vaccine strain was generated at the Institute of Experimental Medicine by reassortment of the cold-adapted attenuated A/Leningrad/134/17/57(H2N2) master

donor virus with the A/California/7/2009(H1N1) pandemic strain. The A/Chita/3/2009(H1N1) influenza virus was obtained from VECTOR's Collection of Microorganisms.

Animals

ICR outbred mice 3–4 weeks of age and were obtained from VECTOR's Animal Breeding Facility. Female ferrets 4–5 month of age were obtained from the Animal Farm "Rodniki" (Pushkino, Moscow region, Russia) and delivered in a vehicle certified for animal shipment. All manipulations with laboratory animals were performed in accordance with the research protocol approved by the Bioethical Committee of VECTOR.

Vaccine

The live MDCK-derived Vector-Flu vaccine was produced at the Vector in accordance with the established production specifications. The vaccine was produced in Madin-Darby canine kidney (MDCK) cells infected with A/17/California/2009/38(H1N1) virus in a serum-free nutrient medium. The resulting vaccine bulk was freeze-dried after adding a stabilizer. One vaccine dose 0,2 ml contained 6.6–6.8 log EID₅₀ of influenza virus. Vaccine was produced in three different batches ##1, 2, 3.

Determination of Influenza virus Infectious activity

The infectious activity of influenza virus was determined by titration in 10–12-day-old chick embryos. 10-fold dilutions (0.2 ml) of virus-containing fluid were inoculated into the allantoic cavity of chick embryos (four embryos per dilution). The embryos were incubated for 48 hours at a temperature of 35°C. After the incubation, the allantoic fluid was harvested from the embryos to determine the virus infectious activity by agglutination reaction with 1% chicken red blood cells. The virus titer was calculated according to the Reed–Muench method and expressed as log₁₀ EID₅₀/0.2 ml [3].

Determination of focus-forming units

The monolayer of continuous MDCK cells grown in 96-well plates was rinsed twice with the serum-free medium (Eagle's MEM, Biolut, Russia), 100 U/ml penicillin, 100 µg/ml streptomycin, 300 µg/ml L-glutamine, 10 µg/ml DEAE-dextran (Sigma), and 5 µg/ml trypsin TPCK (Sigma). 10-fold serial dilutions of the tested virus suspension were prepared in sterile microtubes by adding 50 µl of the tested sample to 450 µl of the serum-free medium. Appropriate 10-fold dilutions of the virus suspension were inoculated into wells of the plate with MDCK cell monolayer. Each virus dilution (100 µl) was added to four wells. After incubation at 35°C in 5% CO₂ for 18 hours, the medium was removed from the wells and the plate was rinsed twice with phosphate-buffered saline solution (PBS). Subsequently, 100 µl of cooled acetone (80% in PBS) were added to each well, incubated for 10–15 min; then acetone was removed, and the plate was rinsed twice with PBS. A 1:5000 dilution of the mouse monoclonal antibodies to type A influenza virus NP protein (CDC, Atlanta, lot 03-0325L) was prepared in 1% bovine serum albumin and 0.1% Tween 20. 100 µl of this

solution were added to each well for 60 min. Then the wells were rinsed four times and covered with the affinity-purified goat antibodies to mouse IgG conjugated with horseradish peroxidase (Sigma, lot 024K4794). After 1 hr incubation the wells were rinsed four times with PBS and incubated with the substrate solution, 3-amino-9-ethylcarbazole (AEC; Sigma, lot 21K8201). After 30-min incubation the substrate solution was removed, the plate was rinsed once with PBS, and the infected cells (colored pink–brown) were counted using an Olympus CK40 microscope. The virus titer was calculated after averaging the number of focus-forming units (FFU) over four wells for the same virus dilution as $T = \text{FFU}_{\text{mean}} * 10^{N+1}$ FFU/ml, where N is the number of 10-fold dilutions of virus-containing sample.

Control of immunogenicity of the vector-flu vaccine

Group of 10 mice were anesthetized with diethyl ether before intranasal administration of the Vector-Flu vaccine (50 µl to each nostril) at a dose of 6.0 log EID₅₀ with an interval of 10 days between immunizations. 21 days after the last immunization, the animals were bled from retroorbital sinus and serum antibody titers were determined by hemagglutination and microneutralization assays.

Eighteen ferrets were anesthetized to take blood samples (5 ml) from the caudal vein; they were then immunized with 200 µl of the Vector-Flu vaccine at 6.6–6.8 log EID₅₀ dose delivered into each nostril. The control animals received MEM medium as placebo. One ferret group was immunized with a single dose and another group received two doses of vaccine with a 10-day interval between vaccinations. At day 21 after the last immunization, the sedated animals were bled from the caudal vein to obtain sera and the antibody titers were determined by hemagglutination inhibition and microneutralization assays.

Hemagglutination inhibition (HAI) test

HAI was performed by a routine technique [4] with some modifications [5]. The assayed sera were pre-treated with the receptor destroying enzyme (RDE) (Denka, Japan). The hemagglutination reaction was performed in 96-well plates with 1% chicken red blood cells (RBC). The HAI titer was determined as the reciprocal dilution of the last row which contained non-agglutinated RBC.

Microneutralization assay

The assay was performed in compliance with the WHO guidelines [4] with some modifications [2,5,6]. The studied sera were first subject to thermal inactivation at 56°C for 30 min and serial 2-fold dilutions were prepared starting from 1:20 dilution. MDCK cells supplemented with equal volumes of serum and influenza virus were mixed and incubated in 96-well plates in 5% CO₂ at 37°C. The presence of the virus was detected by enzyme immunoassay using the monoclonal antibodies to type A influenza virus NP protein (WHO Collaborating Center for Influenza, CDC, Atlanta, GA). Optical density was read at 492 nm in a micro-plate spectrophotometer Multiscan EX (Thermo Scientific, Finland). Neutralizing antibody titer (IC_{50cell}) was defined as reciprocal of the highest serum dilution that provided 50% inhibition of the virus growth in cell culture.

Replication of vector-flu vaccine in ferrets

The ferrets were anesthetized with chloroform vapors and received intranasally either 200 µl of 6.6–6.8 log EID₅₀ Vector-Flu vaccine to each nostril, or placebo for control animals. Three and 14 days after immunization, six animals from the vaccinated group and two animals from the control group were humanely euthanized. Organ specimens were excised under sterile conditions, placed into tubes, and stored at a temperature of –70°C until assayed. The examined specimens included the blood, large and small intestines, spleen, lungs, trachea, brain cortex, olfactory bulbs, and nasal septa. The samples were thawed immediately before titration, homogenized under aseptic conditions in a sterile mortar with the nutrient medium containing antibiotics, and used for virus titration in chick embryos.

Protective efficacy of vector-flu vaccine in mice

Determination of aerosol infectious dose (MAID₅₀) for mice. The experiments on aerosol infection of mice were performed in a BSL–3 laboratory facility using an Omron nebulizer (Omron, Japan), a small dynamic chamber and impinger samplers. An Omron pneumatic nebulizer with an air flow of 4L/min (at a pressure of 0.6 atm) and the virus suspension flow of 0.25 ml/min were used to produce aerosol particles with a median mass diameter of 1.2 µm. During the experiment, a negative pressure of 20 mm water was maintained relative to the pressure in the isolation chamber. The samplers were filled with 10 ml of liquid sorbent (DMEM, Vector, Russia) supplemented with L-glutamine, antibiotics, and 1 µg/ml trypsin). The time of animal exposure and sampling of bioaerosol was 2 min. The concentration of influenza virus in samples (liquid sorbent from sampler and lung homogenates) was determined according to the FFU protocol.

To determine MAID₅₀, eight mice were used for each infective dose. After 3 days post infection, the mice were euthanized by cervical dislocation to excise the lungs, which were separately homogenized. The homogenates were centrifuged at 5000 rpm, and the supernatant was frozen at –70°C until assayed. The virus concentration was calculated in FFU/ml for each sample based on the results of titration in MDCK cells. The virus dose inhaled by mice when exposed to the aerosol was calculated as C*T*W, where C is the aerosol concentration—the amount of biologically active virus particles per cm³; T is exposure time; and W is a pulmonary minute volume of animals. The W value for small laboratory animals was calculated as $W = 2.1 * P^{3/4}$ (cm³/min), where P is the animal weight (g). For a 20 g mouse, $W = 2.1 * 20^{3/4} = 19.9$ cm³/min. The MAID₅₀ is defined as the minimal aerosol infectious dose which resulted in detection of any virus in the lungs of 50% of the mice 3 days after infection. The MAID₅₀ was calculated by probit analysis.

Evaluation of protective efficacy of Vector-Flu vaccine in mice. Immunized mice which received two doses of vaccine were challenged 21 days after the last immunization, along with placebo-immunized animals, by the aerosol exposure to A/Chita/2009(H1N1) pandemic influenza virus strain at doses of 10 to 100 MAID₅₀. Three days after the challenge, animals were

euthanized, their lungs removed and influenza virus content determined according to the FFU protocol. The protection coefficient was calculated as a difference between log MAID₅₀ values for the immunized and control animals.

Results and Discussion

Immunogenicity of vector-flu vaccine

Ferrets are sensitive to a wide range of human influenza virus isolates without any preliminary adaptation and similarly to humans display the sensitivity of the upper respiratory tract to seasonal influenza virus strains. Ferrets are widely used for modeling influenza infection, because they most closely mimic the clinical signs of this disease characteristic of humans. Immunohistochemical studies confirm that unadapted human influenza virus strains are able to infect and reproduce in the cells of the ferret tracheal and bronchial epithelium [7–10].

Mice are also widely used as a model when studying influenza. They are inexpensive and a wide set of commercial immunochemical reagents specific of this model are available. However, mice are resistant to infection by the majority of primary human influenza virus isolates; therefore, the corresponding studies are performed using the mouse-adapted strains A/PR/8/1934(H1N1) or A/WSN/1933(H1N1) [7–8]. However, the pandemic A/H1N1 influenza virus strains isolated in 1918 and 2009 appeared to be infective for mice without any preliminary passages in these animals. Because it was demonstrated that the main human pathology feature caused by the pandemic strains A/H1N1(2009) is a virus infection localized to the lungs [11], and that influenza infection develops in mice in the lower respiratory tract, the mouse model is of special interest for testing vaccine candidates against the pandemic 2009 A/H1N1 influenza virus.

Ferret immune response data to administration of the live MDCK-derived Vector-Flu vaccine are listed in the table 1. First and foremost, high serum titers of the ferrets immunized with A/H1N1 pandemic influenza virus strain (up to 1:5120 by HAI after a single dose vaccination) should be noted. Second dose vaccination with the A/17/California/2009/38(H1N1) vaccine strain increased the HAI titers from 1:1097 to 1:1493, while the geometric mean titers (GMTs) determined by microneutralization assay, went from 1:1016 to 1:2032, respectively.

The responses of ferret immune sera to the A/Chita/3/2009(H1N1) pandemic influenza virus strain were the least pronounced: the HAI GMTs after the first and second immunizations were 1:296 and 1:373, respectively, versus 1:640 and 1:3225 for the A/California/7/2009 strain. However, the inhibitory activity of the ferret immune sera towards the A/Chita/3/2009 and A/California/7/2009 strains determined by microneutralization assay did not differ in a statistically significant manner, amounting to 1:871 and 1:806 after the first immunization and 1:1881 and 1:1280 after the second one (Table 1).

Thus, neutralizing antibodies to pandemic A/H1N1 influenza virus strains are induced in ferrets already after a single

Table 1: The titers of ferret blood serum to A/H1N1 influenza virus strains before and after immunization with the Vector-Flu vaccine determined by HAI and microneutralization assay.

Serological method	HAI									Microneutralization								
	A/Chita/3/ 2009			A/California/7/2009			A/17/California/2009/38			A/Chita/3/ 2009			A/California/7/2009			A/17/California/2009/38		
Influenza virus strain	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2
Number of vaccinations	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2
Serum no. / vaccine batch no.																		
01-Jan	<20	320		<20	640		<20	640		<40	640		<40	640		<40	640	
02-Jan	<20	320		<20	640		<20	640		<40	640		<40	640		<40	640	
03-Jan	<20	320		<20	320		<20	640		<40	640		<40	640		<40	640	
04-Jan	<20		640	<20		2560	<20		2560	<40		2560	<40		2560	<40		2560
05-Jan	<20		320	<20		2560	<20		1280	<40		2560	<40		1280	<40		2560
06-Jan	<20		320	<20		5120	<20		1280	<40		1280	<40		640	<40		2560
07-Feb	<20	160		<20	320		<20	1280		<40	1280		<40	640		<40	640	
08-Feb	<20	320		<20	640		<20	1280		<40	1280		<40	640		<40	1280	
09-Feb	<20	320		<20	320		<20	640		<40	640		<40	640		<40	1280	
10-Feb	<20		320	<20		2560	<20		640	<40		1280	<40		1280	<40		2560
11-Feb	<20		320	<20		5120	<20		1280	<40		2560	<40		1280	<40		2560
12-Feb	<20		320	<20		2560	<20		1280	<40		1280	<40		1280	<40		1280
13-Mar	<20	320		<20	640		<20	1280		<40	640		<40	1280		<40	1280	
14-Mar	<20	160		<20	1280		<20	2560		<40	1280		<40	640		<40	1280	
15-Mar	<20	640		<20	2560		<20	2560		<40	1280		<40	2560		<40	2560	
16-Mar	<20		640	<20		5120	<20		1280	<40		2560	<40		2560	<40		2560
17-Mar	<20		320	<20		5120	<20		2560	<40		2560	<40		1280	<40		1280
18-Mar	<20		320	<20		1280	<20		2560	<40		1280	<40		640	<40		1280
Placebo 1	<20	<20		<20	<20		<20	<20		<40	<40		<40	<40		<40	<40	
Placebo 2	<20	<20		<20	<20		<20	<20		<40	<40		<40	<40		<40	<40	
Placebo 3	<20		<20	<20		<20	<20		<20	<40		<40	<40		<40	<40		<40
Placebo 4	<20		<20	<20		<20	<20		<20	<40		<40	<40		<40	<40		<40
GMT ¹ over the group		296	373		640	3225		1097	1493		871	1881		806	1280		1016	2032
SD of the GMT		139	141		718	1538		784	723		337	675		649	699		622	640

¹Mean geometric titer.

intranasal immunization. After a second dose immunization, ferrets displayed a statistically significant seroconversion of the virus-neutralizing antibodies for pandemic influenza virus strains and the vaccine influenza virus strain. The Vector-Flu vaccine is immunogenic against both A/Chita/3/2009 strain of pandemic A/H1N1 influenza virus isolated in Russia, and the pandemic influenza virus strain A/California/7/2009.

Vector-Flu vaccine appeared to be less immunogenic in mice. The virus-neutralizing titers of serum antibodies to the A/California/7/2009 and A/Chita/3/2009 strains in the immunized mice varied in the range of 1:160–1:320, which is one order of magnitude lower compared to the immune response observed in ferrets which received two doses of the vaccine. The HAI titers against A/California/7/2009 and A/Chita/3/2009 strains were 1:640–1:1280, which is also lower than the analogous characteristics obtained in ferrets. Nonetheless, the level of specific antibodies induced by the two dose vaccination was

sufficient to protect mice against an aerosol infection with the A/Chita/3/2009 strain (Table 2).

Safety testing of vector-flu vaccine in ferrets

The performed experiments demonstrated that the vaccine strain A/17/California/2009/38(H1N1) does not reproduce in the ferret respiratory tract on day 3 after intranasal immunization of ferrets with the Vector-Flu vaccine (Table 3). No virus was isolated from the specimens of blood, lungs, trachea, nasal cavity, olfactory bulb, anterior and posterior brain regions, spleen, and large and small intestines. Signs of weight loss, lethargy, respiratory and neurologic signs were not observed in animals for up to 14 days post-infection. On day 14, the virus was also undetectable in all the studied tissues.

Thus, it was demonstrated that the vaccine strain A/17/California/2009/38(H1N1) was undetectable in the studied

organs 3 and 14 days (observation period) after the intranasal immunization of ferrets with the Vector-Flu vaccine. These findings together with the data on immunogenicity indicate the short time of virus reproduction in ferrets and complete virus elimination from the tissues during 3 days.

Vector-flu vaccine protective efficacy in mice

Resistance of the vaccinated animals to infective agent was assessed in the mice challenged with the A/Chita/3/2009 pandemic influenza virus strain, where vaccine appeared least effective according to HAI data (Table 1). Table 4 lists the MAID₅₀ values of the A/Chita/3/2009 strain for non-immunized ICR mice. Based on these data, we calculated the MAID₅₀ for the A/Chita/3/2009 strain at 8.4 FFU, with a 95% confidence interval of 4.6 to 18 FFU.

One of the important criteria for host sensitivity to influenza virus is the presence of the corresponding sialic acids on the membranes of target cells. As a rule, the human influenza viruses preferentially bind to the cells, which contain the sialic acids with α 2, 6 galactose bond on their membrane [12]. Such receptors are predominant in the cells of the human upper respiratory tract [13–14]. By contrast, the cells with α 2,3-linked

sialic acids on the membrane are predominantly present in the mouse respiratory tract [13]. This discrepancy explains why the primary human influenza virus isolates do not reproduce in mouse organs without preliminary adaptation. The human influenza virus isolates bind weakly to the cells of the mouse alveolar epithelium, whereas they bind strongly to human and ferret bronchial and tracheal tissues [7]. A distinctive and specific characteristic of the infection caused by the pandemic A/H1N1(2009) influenza virus strains is localization of the virus to the lower respiratory tract in both humans [11] and mice [15–17]. Thus, the ability of vaccine to induce high levels of serum class G immunoglobulins (IgG), which, through passive air–blood barrier diffusion are present in the alveolar serous fluid, is of special importance [18].

To assess protective effect conferred by two dose Vector-Flu immunization, groups of mice were challenged with 10, 30, and 100 MAID₅₀ of influenza virus aerosol (strain A/Chita/3/2009). The data (Table 2) demonstrate a high degree of protection of the vaccine and make it possible to estimate the coefficient of mouse protection after such immunization, which exceeds 2.0.

Comparison of virus neutralization *in vitro* in MDCK cell culture and *in vivo* in mouse lungs makes it possible to assess the level of vaccine-induced serum antibodies sufficient to protect mouse lungs from influenza infection at an infective dose of up to 1,000 FFU. Indeed, taking into account (i) the values of the dissociation constants for the binding of hemagglutinin to sialic acids on the target cell surface [19] and assuming that the dissociation constants for influenza virus in cell culture and pulmonary serous fluid are equal, and (ii) the ratio of the sensitive cells in a well of microplate and in the mouse lungs (the volume of the mouse serous fluid is approximately 20 μ l [20], we can infer that the IgG concentration in the mouse lung surfactant providing 50% neutralization of influenza virus (MIC₅₀) is 8 MIC₅₀ based on the microneutralization assay data. By analogy to the definition of 50% inhibitory concentration in cell culture previously referred to, MIC₅₀ is the IgG concentration in the mouse lung surfactant that provides for a 50% inhibition of the virus replication in the lung cells. Therefore, the microneutralization serum titer of 1:160 against the pandemic strain A/Chita/3/2009 resulting by two dose intranasal immunization corresponds with the antibody concentration in mouse lungs equal to 20 MIC₅₀ (160/8). These titers are estimated to protect almost 90% of mice from an aerosol infection with influenza virus at a dose up to 100 MAID₅₀ (Table 2). Taking into account an increase in the number of sensitive cells in the lungs and the volume of pulmonary serous fluid, extrapolation of these calculations for larger mammals suggests that the level of serum virus-neutralizing antibodies which provides protection in ferrets and humans from the virus pneumonia during respiratory infection with pandemic A/H1N1(2009) influenza virus should be approximately the same that is necessary to protect mice, i.e., the titer of serum antibodies determined by microneutralization assay should correspond to approximately 1:200–1:500. The results shown in table 1 suggest that such required antibody level in the blood of immunized ferrets is reached after a single immunization with the Vector-Flu vaccine.

Table 2: Protection of mice from aerosol challenge of the A/Chita/3/2009 (H1N1) pandemic influenza virus strain. Mice received either with two doses of Vector-Flu vaccine or placebo prior to the challenge.

Infective dose, MAID ₅₀	10	30	100
Vector-Flu vaccine immunized mice, infected/total	0/8	0/8	1/8
Placebo immunized mice, infected/total	8/8	8/8	8/8

Table 3: Influenza virus A/17/California/2009/38 (H1N1) load in ferret tissues after intranasal immunization with the Vector-Flu vaccine (log EID₅₀/ml).

Organ	Day 3 after vaccination			Day 14 after vaccination		
	Vaccine batch no.			Vaccine batch no.		
	1	2	3	1	2	3
Blood	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Lungs (one lobe)	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Trachea (3-cm fragment)	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Nose (septa)	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Olfactory bulb	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Brain (anterior + posterior)	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Spleen	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Intestines (small + large)	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5

Table 4: Determination of MAID₅₀ values for the ICR mice with A/Chita/3/2009 (H1N1) pandemic influenza virus strain.

Virus dose, FFU/mouse	Number of mice in group, infected/exposed	Mean virus titer in the mouse lungs over the group, log FFU/ml
0.4	0/8	–
1	0/8	–
4	2/8	4.3
54	8/8	4.4

1 MAID₅₀ for ICR mouse = 8.4 FFU (95% confidence level, 4.6–18 FFU)

Thus, immunization with two doses of Vector-Flu vaccine protects mice from aerosol infection with the A/Chita/3/2009(H1N1) pandemic influenza virus strain and is likely to induce a protective antibody levels both in ferrets and in humans.

Conclusions

Immunization with two doses of the live MDCK-derived Vector-Flu influenza vaccine induced a high level of neutralizing antibodies in the ferret serum both to A/Chita/3/2009(H1N1) influenza virus strain, and A/California/7/2009(H1N1) pandemic strain. Intranasal immunization of mice with the same vaccine induced serum antibodies to the highly pathogenic influenza virus strain A/Chita/3/2009, and was sufficient for protecting mice from aerosol challenge of 100 MAID₅₀ (1 MAID₅₀ = 8.4 FFU). These data make it possible to estimate that the coefficient of mouse protection after a two dose immunization exceeds 2.0.

The A/17/California/2009/38(H1N1) vaccine strain did not display any signs of virulence and did not infect brain tissues of mice and ferrets after intranasal administration. The theoretical estimates of the protective antibody level necessary for protecting humans from the virus pneumonia and the experimental data for model animals (ferrets and mice) suggest that the Vector-Flu vaccine may be able to protect humans from pandemic A/H1N1(2009) influenza virus after a single immunization.

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