Safety and immunogenicity of live attenuated influenza reassortant H5 vaccine (phase I–II clinical trials)

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Abstract

Objective Our studies aimed to evaluate in clinical trials the safety and immunogenicity of an H5 live influenza vaccine candidate obtained using classical reassortment techniques from a low pathogenicity avian influenza (LPAI) A/Duck/Potsdam/1402-6/86(H5N2) virus and the cold-adapted (ca) donor strain A/Leningrad/134/17/57(H2N2).

Methods During Phase I–II clinical trials, volunteers received intranasally two doses of reassortant influenza vaccine strain A/17/Duck/Potsdam/86/92 (H5N2) 21 days apart. Clinical examination of all vaccinees was conducted 7 days post-vaccination. Serum antibody responses were measured by hemagglutination-inhibition and microneutralization and local antibodies were estimated using an enzyme-linked immunosorbent assay test.

Results The vaccine was safe and of low reactogenicity with no febrile reactions. After revaccination 47.1–54.8% of subjects showed ≥fourfold seroconversions of Hamagglutination inhibition (HAI) antibodies to the hemagglutinin (HA) antigen of the A/17/Duck/Potsdam/86/92 (H5N2) virus and 29.4–30.8% were seroconverted to the HA antigen of the reverse genetics reassortant A/Indonesia/05/2005 × PR8 IBCDC-RG (H5N1). Virus-neutralizing antibody levels in sera of volunteers were similar to those shown in HAI test. The virus-specific nasal IgA antibody response after two vaccine doses demonstrated significant increases of ≥fourfold rise SIgA antibodies (65%) geometrical mean titers (160) and a rise in SIgA antibodies (2.8) compared with one dose.

Conclusion The live attenuated influenza vaccine candidate prepared using the LPAI A(H5N2) strain was well tolerated and elicited serum and local immune responses. There was evident cross-reactivity to the A(H5N1) strain in the HAI test.

Keywords Live attenuated influenza vaccine, pandemic.

Introduction

The live cold-adapted influenza vaccine (LAIV) has been used extensively in Russia and shown to be safe, immunogenic and highly protective among children, adults and the elderly and to provide a significant level of community (herd) immunity. 1,2 The nature of a future pandemic strain is not known. We used an A(H5N2) cold-adapted (ca) reassortant strain as a model vaccine strain, generated by classical genetic reassortment similar to that for seasonal vaccine strains. The avian A(H5N2) reassortant vaccine candidate, based on the A/Leningrad/134/17/57 (H2N2) master donor strain (MDS), demonstrated an attenuated phenotype in mice and did not infect chickens. Mice administered A(H5N2) intranasal vaccine were substantially protected from lethal challenge with highly pathogenic A/Hong Kong/483/97 (H5N1) virus and protected from pulmonary infection with an antigenically distinct virus A/Hong Kong/213/2003 (H5N1) virus. The cross-protective effect correlated with the levels of virus-specific mucosal IgA and/or serum IgG antibodies. 3 Additionally, the induction of cytokine-producing virus-specific T cells may also contribute to the broader cross-protective effect. 4 At present a number of preclinical and clinical studies of potential pandemic strains prepared using a reverse genetics technique are being carried out. 5–7 Comparisons of vaccine strains prepared by reverse genetics and classic genetic reassortment will be useful to estimate safety and efficacy of vaccines generated by both approaches.

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The aim of this study is evaluation of safety and immunogenicity of A(H5N2) vaccine in clinical trials among volunteers. The Russian pharmaceutical company ‘Microgen’ organized Phase I–II clinical trials.

Materials and methods

Vaccine strain A/17/Duck/Potsdam/86/92 (H5N2, Len17/H5)
The Len17/H5 reassortant was obtained by classical genetic reassortment on the backbone of A/Leningrad/134/17/57 (H2N2) MDS as previously described. Reverse transcript-polymerase chain reaction–restriction fragment length polymorphism (RT-PCR–RFLP) analysis and/or nucleotide sequencing of PCR-amplified DNA copies of all genes demonstrated a 7:1 genomic composition of the Len 17/H5 reassortant possessing the hemagglutinin (HA) gene from a non-pathogenic avian virus, A/Duck/Potsdam/1402-6/86 (H5N2) and the Neuraminidase (NA) gene and six genes coding for internal and non-structural proteins from the MDS. A non-pathogenic avian virus, A/Duck/Potsdam/1402-6/86 (H5N2) was antigenically similar to human and avian A/Hong Kong/97 (H5N1) isolates.

The Len17/H5 reassortant was compared to the parent virus A/Duck/Potsdam/1402-6/86 (H5N2) by receptor binding preference. We used several binding assays: (1) binding affinity to peroxidase-labelled fetuin conjugate; (2) binding affinity to sialic acid (SA) – Neu5Acα (SA) and 3’ sialilgalactose (3’SL) Neu5Acα2-3Galβ1-4Glc (3’SL); (3) binding affinity to polymeric inhibitorn – polyacrylic acid bound to 3SL groups (3’SL-PA); (4) binding affinity to other receptor determinants on a polymeric carrier.

Virus attachment to receptor analogs was determined as previously described by the method of intercurrent inhibition expressed as 50% inhibition of binding to SA. Monovalent vaccine was prepared by ‘Microgen’ manufacturing using standard technology. In the Phase I study the vaccine used had an infectivity titer of 6.9 log EID_{50}/0.5 ml and in the Phase II – 8.3 log EID_{50}/0.5 ml.

Clinical study design

This study protocol was reviewed and approved by Medical Ethics Committee of the Ministry of Health of the Russian Federation. Written inform consent was obtained from each subject. Subjects were excluded if they had allergy to eggs, bronchial asthma, chronic lungs illnesses, chronic rhinitis, acute infectious or non-infectious illnesses, and exacerbation of chronic diseases or if they are immunocompromized or were positive for human immunodeficiency virus (HIV). Subjects also were excluded if they were pregnant or participated in any other ongoing trial.

The clinical study was conducted in two parts during 2006–2007 in clinics licensed according to World Health Organization (WHO) guidance on biosecurity for pandemic live flu vaccine. All participants remained in the isolation unit for a minimum of 14 days after vaccination. During Phase I the protocol included only one group of volunteers vaccinated by live ca reassortant influenza vaccine candidate Len17/H5 and excluded a placebo group at the recommendation of the Medical Ethics Committee. A group of 20 healthy volunteers, males and females, between 21 and 49 years of age, seronegative to A(H5N2) influenza virus, were recruited for this study in August 2006. All volunteers received two doses of live attenuated reassortant influenza vaccine by 0.5 ml/dose (infectious activity 6.9 log EID_{50}/0.5 ml) intranasally by single-dose applicators with 21 days interval between doses.

The Phase II study was conducted as a double blind control study. In October 2007 a total of 100 participants aged 18–49 years were assigned in 2:1 ratio of vaccine or placebo. Volunteers in each group were given two doses of vaccine (8·3 log EID_{50}/0.5 ml) 21 days apart or two doses of placebo. Sterile phosphate buffered saline (PBS) was used as a placebo. We tested three samples of sera (pre-vaccination, after first vaccination and revaccination) from 42 vaccine group volunteers and from placebo group eight volunteers.

Safety study

All volunteers were examined by physicians each day for 7 days which included the measurement of body temperature and examination of skin, eyes, and nasopharynx. In order to determine whether the vaccine was safe, hematological, biochemical, and urine analyses were carried out among a group of 20 volunteers (Phase 1) before vaccination, 3 days and 21 days after the first dose and 3 days and 21 days after the second dose.

Immunogenicity

Peripheral blood specimens and nasal swabs were collected from volunteers before vaccination, 21 days after the first vaccination and 21 days after the second dose of vaccine. Sera samples were treated with receptor-destroying enzyme from Vibrio cholera (Denka–Seiken, Tokyo, Japan) and then were tested in duplicates for hemagglutination-inhibition (HI) H5 specific antibodies by standard procedures using horse or goose erythrocytes starting from initial dilution 1:10 (Phase I), or 1:5 (Phase II). Test antigens were A/17/Duck/Potsdam/86/92 (H5N2) and A/Indonesia/05/2005 × PR8 IBCDC-RG (H5N1).

Virus neutralizing antibodies to H5N2 virus were determined by microneutralization (MN) assay as previously described. Neutralizing antibody titers were expressed as the reciprocal of the highest dilution of serum that gave 50% neutralization of 100 TCID_{50} of virus in Madin-Darby canine kidney cells.
Influenza virus-specific IgA antibodies in nasal swabs were tested by enzyme-linked immunosorbent assay (ELISA)\textsuperscript{12} using whole purified A/17/Duck/Potsdam/86/92 (H5N2) virus at 16 HAU per 0.05 ml for absorption. The end-point ELISA titers were expressed as the highest dilution that gave an optical density (OD) greater than twice the mean OD plus three standard deviation (SD) of six negative controls.

**Statistical analysis**

Data were analyzed with statistica software (version 6.0). Geometric mean titers (GMT) with 95% confidence intervals (CIs) were calculated and used to represent the antibody response. The comparisons were made within treatment groups between pre-and postvaccinated titers (expressed as log\textsubscript{10}) after first and second vaccination using Wilcoxon matched pairs test or between vaccine and placebo group using Mann–Whitney u-test. Antibody titers were also analyzed for four-fold titer rise and by achievement of post-vaccination titers of ≥1:20 or ≥1:40 using McNemar chi-square test or Fisher’s exact test.

**Results**

**Binding Affinity phenotype**

The binding affinity phenotype of A/Duck/Potsdam/1402-6/86 (H5N2) virus takes an intermediate position between duck viruses and chicken H5 viruses. Like chicken viruses the A/Duck/Potsdam/1402-6/86 (H5N2) virus bound to 6-sulfo 3’-linked sialyoligosaccharide Su-3’SL-N[Neu5Ac2-3Galβ1-4(6-HSO\textsubscript{3})GlcNAcβ] and it is fucosylated derivate Su-SLe\textsubscript{a} [Neu5Acα2-3Galβ1-4(Fukα1-3)-(6-HSO\textsubscript{3})GlcNAcβ] (data not shown). According to its receptor specificity the A/Duck/Potsdam/1402-6/86 (H5N2) virus was similar to the strain A/Duck/Altai/1285/91 (H5N3) which has a homologous HA nucleotide sequence. According to their binding affinity the A/Duck/Potsdam/1402-6/86 (H5N2) virus and Len 17/H5 reassortant were similar to each other. Nevertheless in all experiments there were low quantitative differences in binding affinity to fetuin conjugate and 3’S-L-PA – the features of other high-yielded viruses (Table 1).

**Clinical safety evaluation**

Clinical examination of 20 volunteers who received two doses of vaccine during Phase I clinical trial indicated that the vaccine was well tolerated. No febrile reactions were observed after either the first or the second vaccination. Most reactogenicity events (40%) after the first vaccination consisted of catarrhal symptoms as pharyngeal irritation (Table 2). All symptoms registered on day 4 or 5 after vaccination were mild and had only a 1 day duration. Safety laboratory tests didn’t reveal any hematologic, biochemical or urine test abnormalities among vaccinees. Safety data from Phase II clinical trial was similar to those obtained on the phase I study.

**Serum HI antibody response to vaccination**

Of 20 participants on the phase I study who received two doses of vaccine (6·9 log EID\textsubscript{50}/0·5 ml), serum HI antibodies were measured in 17 subjects using horse erythrocytes. Pre-vaccination titers were negative by all tests (≤1:10). The number of fourfold rises and GMTs of serum HI antibody in post-vaccination antibody titers from these 17 volunteers are given in Table 3. Post-vaccination GMT’s to A(H5N2) antigen among volunteers who received two doses of vaccine were significantly higher than pre-vaccination titers. The frequency of fourfold antibody rises was significantly higher (47% ) after revaccination than after one dose (59%). The percentage of volunteers with post-vaccination serum HI titers to A(H5N2) virus ≥1:20 was 47·1% and for titers ≥1:40 it was 29·4%. There was clear evidence of cross-reactivity to the A(H5N1) antigen. The twofold rises in HI antibodies to A(H5N1) antigen among volunteers, who received two doses of A(H5N2) LAIV was 29·4%. Thus there was a 70% coincidence of antibody response in volunteers to both the A(H5N2) vaccine strain and the A/Indonesia/05/2005 × PR8 IBCDC-RG (H5N1) virus.

Sera from 42 volunteers (Phase II clinical trial) who received two doses of vaccine (8·3 log EID\textsubscript{50}/0·5 ml) were

| Virus | Subtype | Fetuin peroxidase-labelled (m \textmu{}SA) | p3SL (m \textmu{}SA) | SAx* (m \textmu{}SA) | 3’S-L (m \textmu{}SA) |
|-------|---------|------------------------------------------|---------------------|---------------------|---------------------|
| A/Duck/Potsdam/1402-6/86 (wild type) | H5N2   | 0.8                                      | 0.2                 | 3000                | 600                 |
| Len 17/H5 (reassortant) | H5N2   | 1.2                                      | 0.4                 | 3000                | 600                 |

SA, sialic acid; 3’S-L, 3’ sialylgalactose.

*concentration of SA α-form is given, which are 5% of total SA content.
tested in HI test using goose erythrocytes (Table 4). The percentage of ≥fourfold antibody rises to A(H5N2) antigen was higher after revaccination (54.8%) compared to 31% after one dose. The number of post-vaccination GMT’s rises was 2.0 after first vaccination and 2.9 after revaccination. Fifty percent of volunteers had post-vaccination serum HI titers to A(H5N2) virus ≥1:20. There were 30.8% seroconversions to A(H5N1) antigen among 39 subjects from vaccine group after revaccination. There were no detectable seroconversions in the placebo group.

**Virus microneutralization assay**

The pre- and post-vaccination serum neutralizing antibody GMT, the number of ≥fourfold rise in post-vaccination antibody titers and the number of subjects with antibody titers 1:20–1:40 are shown in Table 5. Virus-neutralizing antibodies levels in sera of 20 volunteers who received 6.9 log EID$_{50}$/0.5 ml of Len17/H5 LAIV were similar to those given in Table 2 and 3 for HI. Fifty percent of volunteers exhibited ≥fourfold rise in antibody to H5N2 vaccine strain after two doses of vaccine. In 42 subjects who received two doses of 8.3 log EID$_{50}$/0.5 LAIV, 33.3% of ≥fourfold seroconversions were observed. Antibody titers ≥1:20 after revaccination were detected in 55% vaccinees in Phase I and in 42.8% in Phase II trials. In both cases there were no statistically significant differences in post-vaccination antibody levels between one and two vaccine doses ($P > 0.05$).

In summary according to both HI and MN tests two doses of H5N2 LAIV raised 24–50% of ≥fourfold seroconversions after one dose and 71–74% after two doses (Figure 1).

**Nasal IgA antibody response to vaccination**

The virus-specific nasal IgA antibody response to vaccination in 20 volunteers who received two doses of LAIV is

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**Table 2. Reactogenicity of vaccine strain Len17/H5 in volunteers (Phase I)**

| Vaccination | Temperature | Fever reactions | Nausea | Catarrhal symptoms* |
|-------------|-------------|-----------------|--------|---------------------|
|             | Mild 37–37.5°C | Moderate 37–38.5°C | Severe ≥38.6°C |                 |
| First (n = 20) | n | % | n | % | n | % | n | % |
| Second (n = 20) | n | % | n | % | n | % | n | % |

*Hyperemia and irritation of pharynx: all symptoms registered on day 4 or 5 after vaccination were poorly expressed and had 1 day duration.

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**Table 3. Immunogenicity of vaccine strain Len17/H5 at dose of 6.9 log EID$_{50}$/0.5 ml in volunteers (HI-test)**

| HI test antigen | Vaccination | No. with ≥fourfold rise | GMT | GMT-rise | No. with HI titer ≥1:20 | No. with HAI titer ≥1:40 |
|-----------------|-------------|-------------------------|------|----------|-------------------------|-------------------------|
|                 |             | n | % | n | % | n | % | n | % |
| A(H5N2)         | 1 dose      | 17/20 | 1 | 5.9  | 7.5 | 1.3  | 3 | 17.7 | 2 | 11.8 |
|                 | 2 doses     | 17/20 | 8 | 47.1** | 15.7*** | 2.8 | 8 | 47.1* | 5 | 29.4* |
| A(H5N1)         | 1 dose      | 17/20 | 2 | 11.8 | 6.4 | 1.2  | 2 | 11.8 | 0 | 0   |
|                 | 2 doses     | 1720 | 5 | 29.4 | 8.2** | 16 | 5 | 29.4* | 1 | 5.9  |

GMT, geometric mean titers; HAI, hemagglutination-inhibition.

*Horse erythrocytes.

**The post-vaccination GMTs after revaccination were higher than respective pre-vaccination titers ($P = 0.003$) and were higher than titers after first vaccination ($P = 0.005$).**

†The post-vaccination levels with titers ≥1:20 were higher compared to pre-vaccination levels ($P = 0.02$).

††The post-vaccination levels with titers ≥1:40 were higher compared to pre-vaccination levels ($P < 0.05$).

†††The post-vaccination GMTs after revaccination were higher than respective pre-vaccination titers ($P < 0.05$).

The post-vaccination levels with titers ≥1:20 after revaccination were higher compared with pre-vaccination levels ($P < 0.05$).
The post-vaccination GMTs after 1 dose were higher than those in placebo group ($P < 0.0001$) and higher than after 2 doses of placebo ($P < 0.001$).

| Preparation | Vaccination | Tested/Total | No. with ≥fourfold rise | GMT | GMT-rise | No. with HAI titer ≥1:20 |
|-------------|-------------|--------------|-------------------------|-----|----------|--------------------------|
| Vaccine A(H5N2) | Pre-vaccination | 42/42 | – | – | 40** | – | 0 | 0 |
| | one dose | 42/42 | 13 | 31.0 | 78*** | 2.0 | 8 | 190 |
| | two doses | 42/42 | 23 | 54.8† | 11.4†† | 2.9 | 21 | 50.0††† |
| Vaccine A(H5N1) | Pre-vaccination | 39/42 | – | – | 3.6 | 0 | 0 | 0 |
| | 1 dose | 39/42 | 9 | 23.1 | 5.5 | 1.5 | 0 | 0 |
| | 2 doses | 39/42 | 12 | 30.8 | 7.1 | 2.0 | 5 | 12.8 |
| Placebo A(H5N2) | Pre-vaccination | 8/8 | – | – | 3.9 | – | 0 | 0 |
| | 1 dose | 8/8 | 0 | 0 | 4.6 | 1.2 | 0 | 0 |
| | 2 doses | 8/8 | 0 | 0 | 4.6 | 1.2 | 0 | 0 |
| Placebo A(H5N1) | Pre-vaccination | 6/8 | – | – | 4.5 | 0 | 0 | 0 |
| | 1 dose | 6/8 | 0 | 0 | 4.5 | 1.0 | 0 | 0 |
| | 2 doses | 6/8 | 0 | 0 | 5.6 | 1.3 | 0 | 0 |

GMT, geometric mean titers; HAI, hemagglutination-inhibition.

*Goose erythrocytes.

**The pre-vaccination GMTs to H5N2 antigen in vaccine and placebo group were similar ($P > 0.05$).

***The post-vaccination GMTs after 1 dose were higher than those in placebo group ($P < 0.05$).

†% with ≥fourfold rises higher than that of placebo group ($P = 0.005$) and higher than that after 1 dose ($P < 0.05$).

††The post-vaccination GMTs after 2 doses were higher than those in placebo group ($P = 0.004$) and higher than after 1 dose ($P = 0.003$).

†††% with titers ≥1:20 after 2 doses higher than that of placebo group ($P = 0.01$).

Table 4. Immunogenicity of vaccine strain Len17/H5 at dose of 8.3 log EID$_{50}$/0.5 ml in volunteers (HI-test)*

| Preparation | Doses of vaccine received | GMT | GMT-rise | No. (%) with ≥fourfold rise |
|-------------|---------------------------|-----|----------|----------------------------|
| Vaccine 6.9 log EID$_{50}$/0.5 ml (n = 20) | Pre-vaccination | 5.2 | – | – | 0 (0) |
| | 1 dose | 9.7* | 1.9 | 4 (20) | 5 (25)** | 1 (5) |
| | 2 doses | 15.2*** | 2.9 | 10 (50) | 11 (55)† | 5 (25)†† |
| Vaccine 8.3 log EID$_{50}$/0.5 ml (n = 42) | Pre-vaccination | 6.0 | – | – | 3 (7.1) |
| | 1 dose | 10.2†† | 1.7 | 9 (21.4) | 14 (33.3) | 3 (7.1) |
| | 2 doses | 12.2‡ | 2.1 | 14 (33.3) | 18 (42.8)‡‡ | 7 (16.6) |
| Placebo (n = 8) | Pre-vaccination | 5.9 | – | – | 0 (0) |
| | 1 dose | 7.1 | 1.2 | 0 | 1 (12.5) | 0 (0) |
| | 2 doses | 7.1 | 1.2 | 0 | 1 (12.5) | 0 (0) |

GMT, geometric mean titers; MN, microneutralization.

*The post-vaccination GMTs after 1 dose were higher than the respective pre-vaccination titers ($P = 0.002$).

**After 1 dose percentage with titers ≥1:20 was higher than before vaccination ($P = 0.02$).

***The post-vaccination GMTs after 2 doses were higher than the respective pre-vaccination titers ($P = 0.002$).

†After 2 doses percentage with titers ≥1:40 was higher than before vaccination ($P = 0.001$).

‡After 2 doses percentage with titers ≥1:40 was higher than before vaccination ($P = 0.001$).

††The post-vaccination GMTs after 1 dose were higher than the respective pre-vaccination titers ($P = 0.001$).

‡‡The post-vaccination GMTs after 2 doses were higher than the respective pre-vaccination titers ($P = 0.0001$).

§After 2 doses of vaccine percentage with titers ≥1:20 was higher than before vaccination ($P = 0.0003$) and higher than after 2 doses of placebo ($P < 0.05$).

Table 5. Seroconversions and geometric mean titers (GMTs) of neutralizing antibody to Len17/H5 virus after vaccination with 1 and 2 doses of H5N2 LAIV
shown in Table 6. The immune response after two doses of LAIV demonstrated significant increases of fourfold rise SIgA antibodies (65%), although GMTs (16) was not significantly (1.3 times) greater than that for a single dose.

**Discussion**

Extensive experience over many years with LAIV in Russia has demonstrated its safety and efficacy against drifted virus variants. In clinical trials among adults, children and elderly there was no detectable evidence of genetic reversions to wild type mutations associated with the vaccine attenuation phenotype. LAIV provided a reasonably high level of immediate (first 7 days) protection from stimulated mucosal immunity and increased levels of functional antibodies. Vaccination of schoolchildren with LAIV provided the community with herd immunity. Also there were significant local immune responses (65%) with mean titer 1:16 which is considered to be protective according our previous data. The prevalence of local IgA response after intranasal immunization with LAIV can explain the high protection rate from lethality in the

Phase I and II clinical trials of LAIV based on the ca-re-assortant strain Len17/H5, confirmed previous data about safety of LAIV. The infectivity of LAIV prepared from seasonal A subtypes A(H1N1) and A(H3N2) is usually 7.5 log EID50/0.2 ml. As the first study used LAIV of a new HA subtype to which people in general were not previously exposed, the vaccine for Phase I was prepared at a low virus titer – 6.9 log EID50/0.5 ml. Next year an LAIV with greater infectivity (8.3 log EID50/0.5 ml) was used. Increasing vaccine virus infectivity from 6.9 log EID50/0.5 ml to 8.3 log EID50/0.5 ml led to enhancement of the post-vaccination immune response after the first vaccination to homologous H5N2 antigen from 6 to 31% of fourfold antibody rises according to HI-test. However this effect can be partially explained by decreasing the starting serum dilution from 1:10 to 1:5 thus making the HI test more sensitive to detect fourfold seroconversions.

![Figure 1. Summarized number of seroconversions in volunteers after vaccination with Len17/H5 live cold-adapted influenza vaccine (LAIV) according to both hemagglutination-inhibition (HA) and microneutralization (MN) tests.](image)

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**Table 6. Nasal IgA ELISA antibody response in volunteers after vaccination with Len17/H5 (6.9 log EID50/0.5 ml)**

| Total | No. (%) with ≥fourfold rise SIgA-antibodies | GMTs of SIgA-antibodies | GMTs fold rises |
|-------|---------------------------------------------|--------------------------|-----------------|
|       | 1 dose | 2 doses | Pre-vaccination | 1 dose | 2 doses | 1 dose | 2 doses |
| 20    | 6 (30, 0%) | 13* (65, 0%) | 5.7 | 10.0 | 16.0** | 1.7 | 2.8 |

GMT, geometric mean titers.

*After revaccination % with ≥fourfold rises were higher than that after first vaccination (P = 0.03).

**The post-vaccination GMTs after 2 doses were higher than the respective pre-vaccination titers (P = 0.01).
experimental models. It has also been suggested that local secretory IgA antibody can reduce virus infectivity due to reacting with other viral proteins, including internal proteins.

Recently, we investigated the safety and cross-protective efficacy of the H5N2 LAIV (Len 17/H5) against a genetically distinct variant of a contemporary highly pathogenic avian influenza (HPAI) virus A/Chicken/Kurgan/02/05 (H5N1) in Java macaques. Based on duration and severity of clinical symptoms, antibody response and virus isolation, the vaccination of monkeys with two doses of LAIV (6-9 log EID<sub>50</sub>/0.5 ml) provided 50% cross-protection against challenge with the HPAI and no deaths occurred in the vaccinated monkeys.

Evaluation of our LAIV pandemic vaccine candidate was performed for LAIV development as part of the global influenza pandemic preparation project worked out by WHO. It was considered that LAIV could be produced in greater quantities and more rapidly than inactivated vaccines. All the above together with the generation of herd immunity by LAIV suggest that LAIV implementation during the first wave of a pandemic will provide significant social, economic, and health benefits to the community.

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References

1 Rudenko LG, Slepushkin AN, Monto AS et al. Efficacy of live attenuated and inactivated influenza vaccines in school children and their inactivated contacts in Novgorod, Russia. J Infect Dis 1993; 168: 881–887.
2 Rudenko LG, Alexandrova GI. Current strategies for the prevention of influenza by the Russian cold-adapted live influenza vaccine among different populations; in Osterhaus ADME, Cox N, Hampson AW, (ed): Options for the Control of Influenza IV. Amsterdam: Excerpta Medica, 945–950, 2001.
3 Desheva JA, Lu Kh, Rekstin AR et al. Characterization of an influenza A H5N2 reassortant as a candidate for live-attenuated and inactivated vaccines against highly pathogenic H5N1 viruses with pandemic potential. Vaccine 2006; 24: 6859–6866.
4 Lu X, Edwards LE, Desheva JA et al. Cross-protective immunity in mice induced by live-attenuated or inactivated vaccines against highly pathogenic influenza A (H5N1) viruses. Vaccine 2006; 24: 6588–6593.
5 Subbarao K, Chen H, Swayne D et al. Evaluation of a genetically modified reassortant H5N1 influenza A virus candidate generated by plasmid-based reverse genetics. Virology 2003; 305: 192–200.
6 Treanor JJ, Campbell JD, Zangwill KM, Rowe T, Wolff M. Safety and immunogenicity of an inactivated subvirion influenza A (H5N1) vaccine. N Engl J Med 2006; 354:1343–1351.
7 Suguitan AL, McAuliffe J, Mills KL et al. Live, attenuated Influenza A H5N1 candidate vaccines provide broad cross-protection in mice and ferrets. PLoS Med 2006; 3:1541–1555.
8 Kendal AP, Maassab HF, Alexandrova GI, Ghendon Y. Development of cold-adapted recombinant live, attenuated influenza vaccines in USA and USSR. Antiviral Res 1981; 1:339–365.
9 Klimov AI, Cox NJ. PCR restriction analysis of genome composition and stability of cold-adapted reassortant live influenza vaccines. J Virol Methods 1995; 52:41–49.
10 Gambaryan AS, Tuzikov AB, Bovin NV et al. Differences between influenza virus receptors on target cells duck and chicken and receptor specificity of the 1997 H5N1 chicken and human influenza viruses from Hong Kong. Arch Virol 2002; 147:1107–1208.
11 World Health Organization. WHO manual on animal influenza diagnosis and surveillance. WHO/CDS/CSR/NCS/2002.5 (http://www.who.int/csr/resources/publications/influenza/en/whocdscsrncs20025rev.pdf). [accessed 20 September 2008].
12 Rowe T, Abernathy RA, Hu-Primmer J et al. Detection of antibody to avian influenza A(H5N1) virus in human serum by using a combination of serologic assays. J Clin Microbiol 1999; 37:937–943.
13 Rudenko LG. Live attenuated cold-adapted influenza vaccine in Russia: advantages, further research and development; in Katz JM (ed): Options for the Control of Influenza VI. London, Atlanta: International Medical Press, 122–124, 2008.
14 Palkter T, Kiseleva I, Johnston K et al. Protective efficacy of intranasal cold-adapted influenza A/New Caledonia/20/99 (H1N1) vaccines comprised of egg- or cell culture-derived reassortants. Virus Res 2004; 105:183–194.
15 World Health Organization. Options for live influenza vaccines (LAIV) in the control of epidemic and pandemic influenza, 12-13 June 2007, Rudenko L.G. Fifty years historical experience with live attenuated vaccines in Russia (http://www.who.int/vaccine_research/diseases/influenza/Rudenko_50_years_experience.pdf). [accessed 20 September 2006].
16 Tamura S, Tanimoto T, Kurata T. Mechanisms of broad cross-protection provided by Influenza virus infection and their application to vaccines. Jpn J Infect Dis 2005; 58:195–207.
17 World Health Organization. Global pandemic influenza action plan to increase vaccine supply. WHO/CDS/EPR/GIP/2006.1. September 2006. (http://www.who.int/Vaccines-documents/DocsPDF06/863.pdf) [accessed 20 September 2006].