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Potentially Pandemic Live Influenza Vaccines Based on Russian Master Donor Virus are Genetically Stable after Replication in Humans

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Abstract

Stability of attenuating mutations is an important premise for live attenuated influenza vaccine (LAIV). The presence of multiple mutations in internal gene segments of live attenuated viruses contributes to the stability of their genomes and consistent phenotypic properties. This study describes the evaluation of results of clinical isolates obtained in Phase I clinical trials of three Russian LAIVs against potentially pandemic influenza viruses which may cause serious and fatal disease in humans. As part of clinical trials, nasal swabs were tested for vaccine virus shedding, for temperature sensitivity and cold–adaptation and for nucleotide sequence. Vaccine viruses isolated from the vaccinated subjects were shown to retain phenotypic characteristics and attenuating mutations. These data suggest genetic stability of vaccine virus after replication in humans. In addition, no vaccine virus was detected in the placebo groups indicating the lack of person–to–person transmission.

Keywords: Influenza viruses of pandemic potential; Live influenza vaccines; Genetic stability

Description

Birds and Mammals are the main reservoir of newly emerging pandemic influenza viruses [1-3]. Global circulation of influenza A viruses in avian species poses a constant threat to human public health. Vaccination remains the main strategy against influenza. Over the last decade, the interest in the live attenuated cold-adapted reassortant influenza vaccine (LAIV) has increased dramatically. To a large degree, it is because World Health Organization (WHO) recognized the advantages of LAIV in the event of pandemic situation. For instance, needle–free administration, high vaccine virus yield, easy down–stream processing, cross–reactivity of immune responses etc. make LAIV very attractive preparation in the face of pandemic situation [4].

Genetic stability and the absence of transmission potency are the main properties of LAIVs, which guarantee their safety [5]. Confirmation of genetic and phenotypic stability is one of key points of characterisation of LAIV, which is especially important in pandemic situation to guarantee its safety profile during large–scale immunization campaigns.

Three pre–pandemic LAIVs tested were generated in the Institute of Experimental Medicine (IEM, St Petersburg, Russia) by classical reassortment in embryonated chicken eggs [6-8]. Clinical lots were manufactured by Microgen (Irkutsk, Russia). Randomized, double–blinded, placebo–controlled Phase I trials of A/17/mallard/Netherlands/00/95 (H7N3) (registered on ClinicalTrials.gov as H7N3: NCT01511419), A/17/California/66/395 (H2N2) (registered on ClinicalTrials.gov as H2N2: NCT01982331) and A/17/turkey/Turkey/2005/133 (H5N2) (registered on ClinicalTrials.gov as H5N2: NCT01719783) were conducted at the Research Institute of Influenza (St Petersburg, Russia). In each of those studies, 38-40, 18-49 years old participants both sexes were randomly assigned to receive two doses of vaccine or placebo at a 3:1 vaccine:placebo ratio. Vaccine and placebo were administrated intranasally. The preclinical studies, as well as safety and immunogenicity of these three LAIVs for use in the pandemic situations were earlier well documented and published [6-11]. In this paper, we presented the results of molecular genetics and virological studies conducted as a part of phase I clinical trials. In particular, shedding, transmission and genetic stability of H7N3, H5N2 and H2N2 LAIVs against pre–pandemic influenza viruses were studied.

Detection of vaccine virus shedding and recovery of viruses from nasal swabs obtained after vaccination and revaccination was carried out by culture of clinical samples in 10-11 day old embryonated chicken eggs according to WHO standard procedure [12]. Sequence analysis was performed by using a 3130 × l Genetic Analyzer (Applied Biosystems) according to the manufacturer's instructions. Determining temperature sensitivity and cold-adaptation (*ts/ca* phenotypes) was performed by culture of clinical isolates in embryonated chicken eggs at the optimal (2 days at 33°C) and non–permissive temperatures (6 days at 26°C, and 2 days at 38°C, respectively). Viruses were considered as *ts* if their titer at elevated temperature of 38°C was ≤ 4.2 log10 EID_{50}/mL and as *ca* if their titer at low temperature of 26°C was ≥ 5.7 log10 EID_{50}/mL.

Vaccine virus isolation

Following the first dose of H7N3 LAIV, vaccine virus was detected by PCR in 87% (26/30) subjects following the first dose of vaccine and recovered in eggs from 4 of 30 (13%). After the second dose of H7N3 vaccine, 82% subjects (24/29) were positive by RT-PCR, however no virus could be recovered by culturing in eggs.

For H5N2 LAIV, virus was detected by PCR on day 1 in 93% (28/30) and recovered by culture in 33% subjects (10 of 30) on day 1 after the first dose. After the second dose, RNA was detected by RT-PCR in 69% subjects (20/29) and recovered by culture in 21% (6/29), predominantly on the day after vaccination.

For H2N2 LAIV, virus was detected by PCR in 74% subjects (20/27) after the first dose of vaccine, primarily on days 1 through 3. Virus was recovered by culture in 43% subjects (12/28), all on day 1. After the second dose of the vaccine virus was detected by PCR in 74% subjects (20/27) on day 1 only and virus was recovered in eggs from 30% of vaccinees (8/27).

A total of 40 isolates were recovered in three clinical trials in embryonated chicken eggs, 20 isolates from the H2N2 LAIV recipients, 16 from the H5N2 LAIV recipients and 4 from the H7N3 LAIV recipients.

From the moment the first LAIV was developed, concerns about its potential transmissibility to close contacts have been raised. Nevertheless, there was the only single documented case of MedImmune LAIV virus transmission to an unvaccinated child [13]. In our studies no viral RNA was detected in any placebo recipients over the 6 days follow-up after the first and/or the second vaccine dose for any of the three LAIVs reported here, which supports, we believe, the lack of vaccine transmissibility of pre-pandemic LAIVs.

Genetic stability of clinical isolates

A/Leningrad/134/17/57 (H2N2), master donor virus (MDV) for Russian LAIV, contains following mutations in the internal genes which are responsible for its attenuation: Val-478-Leu in PB2, Lys-265-Asn and Val-591-Ile in PB1, Leu-28-Pro and Val-341-Leu in PA, Ile-15-Val and Phe-144-Leu in M1 and Met-100-Ile in NS2 [14]. Following the WHO recommendation for the production of LAIVs [5] a complete genetic sequence of the attenuated vaccine strains should be performed. Nucleotide sequences from all isolates confirmed their vaccine genotype. Besides that, they did not revert at nucleotides known to confer an attenuating phenotype retaining all attenuating mutations in internal genes described for MDV.

Retention of key phenotypic features of clinical isolates

H5, H7 and H2 parental viruses exhibited typical for the wild type viruses' non-*ts*/non-*ca* phenotype. In contrast, all 40 H2N2, H5N2 and H7N3 vaccine isolates retained the phenotypic characteristics (cold adaptation and temperature sensitivity) of the MDV. Mean log10 reduction of virus titer (EID_{50}/mL) at 33°C/26°C exceeded 3.0. Mean log10 reduction of virus titer (EID_{50}/mL) at 33°C/38°C was more than 9.0.

Thus, it was confirmed that during replication in humans the vaccine viruses did not revert to wild–type phenotype.

Conclusion

Phenotypic and genotypic analyses conducted on the potentially pandemic H2N2, H5N2 and H7N3 vaccine viruses recovered from the volunteers suggest that Russian pre-pandemic LAIVs are genetically stable after replication in humans. The ability to replicate efficiently at low temperature of 26°C (*ca* phenotype) and inability to replicate at elevated temperature of 38°C (*ts* phenotype) were demonstrated consistently for all clinical isolates tested. This was confirmed by the fact that phenotypic markers were mapped genetically-vaccine virus isolates retained all attenuating mutations described for MDV.

Thus, clinical isolates were shown to preserve the temperature sensitivity and cold-adaptation properties of the MDV. In addition, vaccine virus retained all attenuating mutations described for MDV and was not detected in placebo groups supporting the concept that transmission of pre-pandemic LAIVs may not occur after vaccination. Therefore, our Phase I clinical trials have demonstrated key phenotypic features and genetic stability of the potentially pandemic LAIVs' genome.

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Competing Interest

The authors have declared that no competing interests exist.

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