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Genetic stability of live attenuated vaccines against potentially pandemic influenza viruses

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ABSTRACT

Background: Ensuring genetic stability is a prerequisite for live attenuated influenza vaccine (LAIV). This study describes the results of virus shedding and clinical isolates' testing of Phase I clinical trials of Russian LAIVs against potentially pandemic influenza viruses in healthy adults.
Methods: Three live attenuated vaccines against potentially pandemic influenza viruses, H2N2 LAIV, H5N2 LAIV and H7N3 LAIV, generated by classical reassortment in eggs, were studied. For each vaccine tested, subjects were randomly distributed into two groups to receive two doses of either LAIV or placebo at a 3:1 vaccine/placebo ratio. Nasal swabs were examined for vaccine virus shedding by culturing in eggs and by PCR. Vaccine isolates were tested for temperature sensitivity and cold-adaptation (*ts/ca* phenotypes) and for nucleotide sequence.
Results: The majority of nasal wash positive specimens were detected on the first day following vaccination. PCR method demonstrated higher sensitivity than routine virus isolation in eggs. None of the placebo recipients had detectable vaccine virus replication.

All viruses isolated from the immunized subjects retained the *ts/ca* phenotypic characteristics of the master donor virus (MDV) and were shown to preserve all attenuating mutations described for the MDV. These data suggest high level of vaccine virus genetic stability after replication in humans.

During manufacture process, no additional mutations occurred in the genome of H2N2 LAIV. In contrast, one amino acid change in the HA of H7N3 LAIV and two additional mutations in the HA of H5N2 LAIV manufactured vaccine lot were detected, however, they did not affect their *ts/ca* phenotypes.
Conclusions: Our clinical trials revealed phenotypic and genetic stability of the LAIV viruses recovered from the immunized volunteers. In addition, no vaccine virus was detected in the placebo groups indicating the lack of person-to-person transmission.

LAIV TRIAL REGISTRATION at ClinicalTrials.gov: H7N3-NCT01511419; H5N2-NCT01719783; H2N2-NCT01982331.

1. Introduction

Pigs and some avian species are the main reservoir of newly emerging pandemic influenza viruses [1–3]. Global circulation of all influenza A virus subtypes in avian species pose a constant threat to human public health. As of January 6, 2015, 694 cases of H5N1 influenza virus were reported by World Health Organization (WHO), 402 (58%) of which were fatal [4]. A total of 450 laboratory-confirmed cases of human infection with avian influenza H7N9 virus, including 165 (37%) deaths, have been reported to WHO on June 27, 2014 [5]. Influenza H2N2 viruses are also considered as a probable subtype to cause future pandemic because these viruses have not circulated in the human population since 1968. Therefore, people born after the H2N2 pandemic in 1968 more likely have no immunity to these viruses and may be more vulnerable if the H2N2 subtype returns to circulation [6,7].

Vaccination remains the principal strategy against both seasonal and pandemic influenza. Over the last years, the interest in the live cold-adapted reassortment influenza vaccine (LAIV) has increased considerably. To a certain degree, it is because WHO recognized

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the advantages of LAIV in comparison with inactivated vaccine in the event of pandemic situation. These advantages include needle-free administration, higher vaccine virus yield, easier down-stream processing, cross-reactivity of immune responses and the induction of herd immunity [8].

Two types of LAIVs are now available commercially. The first, licensed in 1987 for the prevention of influenza in persons 3 years and older as Ultravac (Microgen, Russia), is based on cold-adapted MDVs, A/Leningrad/134/17/57 (H2N2) and B/USSR/60/69 [9–14]. Pre-master seed vaccine viruses for LAIV production are developing by IEM (Institute of Experimental Medicine, St Petersburg, Russia).

The second, licensed as FluMist in 2003 (MedImmune, Inc, USA), is based on cold-adapted MDVs, A/Ann Arbor/6/60ca (H2N2) and B/Ann Arbor/1/66ca and uses for the prevention of influenza in persons younger than 49 and older than 2 years of age [15–19].

Seasonal influenza vaccination does not appear to protect against pandemic or H5N1 influenza viruses [20–22]. Therefore, in the face of pandemic development of appropriate potentially pandemic LAIVs is of primary strategic importance. A number of potentially pandemic LAIVs already generated by MedImmune [23–26] and IEM [14,27–29]. IEM is preparing a National collection of vaccine strains against potentially pandemic influenza viruses, which may cause serious and fatal disease.

Stability of genotype and phenotype, together with the absence of transmission potency are the main properties of live attenuated vaccines, which ensure their safety profile [30]. Confirmation of genetic and phenotypic stability of an LAIV is especially important in a pandemic situation to guarantee its safety during large-scale immunization campaigns. The lack of person-to-person transmission of Russian live attenuated vaccine was demonstrated in several clinical trials [27,28,31]. To our knowledge, there was only a single documented case of an LAIV virus transmission to an unvaccinated child during clinical trials of seasonal LAIVs prepared by MedImmune [32].

The safety profile and immunogenicity of Russian H2N2 [28], H5N2 [29] and H7N3 [27] potentially pandemic LAIVs were already well documented and published. In this paper, we presented the results of molecular genetics and virological studies conducted as a part of three clinical trials. The goal of this paper was to evaluate shedding, transmission and genetic stability of H7N3, H5N2 and H2N2 LAIVs against potentially pandemic influenza viruses developed by IEM (Russia) and to compare our results with published data by MedImmune (USA).

2. Material and methods

2.1. Vaccines tested

Three live attenuated vaccine strains against potentially pandemic influenza viruses, A/17/mallard/Netherlands/00/95 (H7N3) [33], A/17/California/66/395 (H2N2) [34] or A/17/turkey/Turkey/2005/133 (H5N2) [35] were generated in Institute of Experimental Medicine (IEM, St. Petersburg, Russia) by classical reassortment in eggs as pre-master seed viruses (pre-MSVs). Cold-adapted A/Leningrad/134/17/57 (H2N2) virus was used as MDV. H2N2 and H7N3 LAIVs displayed 6:2 genomic composition (six MDV genes and wild-type hemagglutinin and neuraminidase genes) and H5N2 LAIV candidate was 7:1 reassortant (seven MDV genes and wild-type hemagglutinin gene). Microgen (Irkutsk, Russia) manufactured the clinical lots for the three reassortants, H2N2, H7N3 and H5N2 from pre-MSVs.

2.2. Study design and study procedures

The described molecular genetics and virological studies were conducted on samples derived from three randomized, double-blinded, placebo-controlled phase I trials in healthy adults conducted at the St. Petersburg Institute of Influenza (registered on ClinicalTrials.gov as H7N3: NCT01511419; H5N2: NCT01719783 and H2N2: NCT01982331). In each of those studies, 38–40 18–49-years old participants (male and female) were randomly assigned to receive two doses of vaccine or placebo at a 3:1 vaccine:placebo ratio. Vaccine and placebo recipients were not isolated from each other, two to four recipients were housed in one room. They were discharged from the Isolation Unit on day 6 (H2N2 and H5N2 LAIV clinical trials) or on day 7 (H7N3 LAIV clinical trial) after vaccination/revaccination.

Vaccine/placebo were administrated intranasally (0.25 mL into each nostril) with a single-use nasal sprayer.

2.3. PCR-based virus detection

Nasal swabs for detection of LAIV virus shedding by PCR were obtained from each nostril and mixed with transport medium. Collections were made on the day of vaccination prior to vaccine administration, (days 0 and 28) and on days 1 through 6 and 29 through 34, after the first and second vaccination, respectively. RNA was extracted from 100 μl of the nasal swabs using "RIBO–sorb" reagent kit (InterLabService, Moscow, Russia). Real-time PCR testing was performed using SuperScript III Platinum One-step qRT-PCR System (Invitrogen). Primers and probes to detect and subtype virus RNA were kindly provided by the US Centers for Disease Control (CDC, Atlanta, USA).

2.4. Vaccine virus isolation in embryonated chicken eggs

Detection of vaccine virus shedding and recovery of viruses from nasal swabs obtained after vaccination (days 1, 2, 3, 5, 6 after the first vaccine dose and days 29 and 31 after the second) was carried out by culture of 0.2 mL of clinical samples in 10–11-day-old embryonated chicken eggs ("Nazia" poultry plant, St Petersburg, Russia) according to WHO standard procedure [36].

2.5. Genomic analysis

RNA was extracted from allantoic fluid of HA-positive eggs inoculated with the nasal swab specimens using a QIAamp Viral RNA mini kit following the manufacturer's instructions (Qiagen GmbH, Hilden, Germany). The origin of the RNA segments of each isolate and the presence of attenuating mutations specific for the MDV virus was determined by partial sequencing strategy using a universal primer set described in Isakova-Sivak et al. [34]. The presence of attenuating mutations specific for the MDV virus was confirmed by nucleotide sequencing of regions adjacent to the mutations using a set of primers listed in Table S1. The partial sequencing strategy covered on average 300−500 nucleotides of each gene segment [34], and additional sequencing of the regions adjacent to the mutations of interest resulted in approximately 50% coverage of the virus genome. The HA and NA genes of the clinical isolates were fully sequenced using sequence-specific primers. RT-PCR products were generated using SuperScript®III One-Step RT-PCR with Platinum®*Taq* reagents (Invitrogen) according to the manufacturer's instructions. Sequence analysis was performed by using a BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems) and a 3130xl Genetic Analyzer (Applied Biosystems) according to the manufacturer's instructions.

2.6. Determining temperature sensitivity and cold−adaptation (ts/ca *phenotypes)*

The parental viruses and clinical isolates were cultured in 10–11-day-old embryonated chicken eggs at the optimal