Evaluation of a Live-Attenuated Human Parainfluenza Type 1 Vaccine in Adults and Children

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We conducted a phase I clinical trial (clinicaltrials.gov identifier, NCT00641017) of the experimental live-attenuated human parainfluenza virus type 1 (HPIV-1) vaccine rHPIV-1/84/del 170/942A sequentially in 3 groups: adults, HPIV-1–seropositive children, and HPIV-1–seronegative children, the target population for vaccination. rHPIV-1/84/del 170/942A was appropriately restricted in replication in adults and HPIV-1–seropositive children but was overattenuated (ie, insufficiently infectious and immunogenic) for HPIV-1–seronegative children.

Key words. children; HPIV-1 vaccine; lower respiratory tract illness.

Human parainfluenza virus type 1 (HPIV-1) is the principal etiologic agent of croup and an important cause of lower respiratory tract illness (LRI) in children [1–3]. In a recent study in US children younger than 5 years, HPIV-1 accounted for ~2.7% of hospitalizations for fever and/or acute respiratory illness, or ~9000 hospitalizations per year on the basis of US census data [4]. Although the outpatient and emergency department burden of HPIV-1–associated illness is less well characterized, it is likely several-fold higher than the inpatient burden [4]; thus, an effective HPIV-1 vaccine could have an important impact on pediatric respiratory illness. A live-attenuated HPIV-1 vaccine might also be used as a vector to express protective antigens from pathogens such as respiratory syncytial virus. A live-attenuated intranasally administered HPIV-1 vaccine would have the potential advantage of inducing a full spectrum of immune responses (humoral and cellular, local and systemic) and could replicate in the upper respiratory tract of infants even in the presence of maternally derived antibodies [3, 5].

A live-attenuated HPIV-1 vaccine, designated rHPIV-1/84/del 170/942A, was previously developed by reverse genetics and contains attenuating mutations involving the accessory C protein, phosphoprotein P, the hemagglutinin-neuraminidase (HN) protein, and polymerase L protein [6]. This experimental vaccine candidate is temperature sensitive (shut-off temperature, 38°C) and attenuated and protective in nonhuman primates [6]. Here, we report its evaluation in a phase I clinical trial in adults, HPIV-1–seropositive children, and HPIV-1–seronegative children.

METHODS

Vaccine
rHPIV-1/84/del 170/942A was derived from complementary DNA as previously described [7]. Clinical trial material was manufactured in qualified Vero cells at Charles River Laboratories (Malvern, PA), stored at ~70°C, and diluted on site using qualified Leibovitz L15 medium. L15 medium was also used as the placebo.

Study Population, Study Design, and Clinical Trial Oversight
The trial (clinicaltrials.gov identifier, NCT00641017) was conducted at the Center for Immunization Research at Johns Hopkins Bloomberg School of Public Health. The vaccine was evaluated sequentially in (i) adults who were not screened for HPIV-1 serostatus (but who all proved to be HPIV-1 seropositive), (ii) HPIV-1–seropositive children aged 15 to 59 months, and (iii) HPIV-1–seronegative children aged 6 to 36 months. For the purpose of this study, HPIV-1 seropositive was defined as a hemagglutination-inhibition (HAI) antibody titer of >1:8, and HPIV-1 seronegative was defined as a HAI antibody titer of ≤1:8.

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seronegative was defined as an HAI titer of \( \leq 1:8 \). The age groups for HPIV-1-seropositive and HPIV-1-seronegative children differed and therefore eliminated the possibility of enrolling HPIV-1-naïve infants whose sera contained maternally derived antibodies. The studies in adults were open label, with all subjects receiving vaccine. The studies in children were randomized, double-blinded, and placebo controlled, with subjects randomly assigned 2:1 to receive vaccine or placebo, respectively [8]. Vaccines and placebo were each administered as nose drops (0.25 mL per nostril). The dose of vaccine was \( 10^{6.0} \) 50% tissue culture infectious doses (TCID\(_{50}\)) for adults and HPIV-1-seropositive children; \( 10^{5.0} \) TCID\(_{50}\) and \( 10^{6.0} \) TCID\(_{50}\) doses were evaluated sequentially in HPIV-1-seronegative children.

Written informed consent was obtained from the study participants (adults) or from parents or guardians of the study participants (children) before enrollment. These studies were conducted in accordance with the standards of good clinical practice as defined by the International Conference on Harmonization. The clinical protocol, consent forms, and Investigators’ Brochure were reviewed and approved by the Western Institutional Review Board (approval number 20080536) and the trial sponsors, the National Institute of Allergy, Immunology, and Infectious Diseases (NIAID) Regulatory Compliance and Human Subjects Protection Branch. Clinical data were reviewed by the Center for Immunization Research and NIAID investigators and by the Data Safety Monitoring Board of the NIAID Division of Clinical Research.

For adults and HPIV-1-seropositive children, clinical assessments and nasal washes (NWs) were performed on study day 0 (NW performed before inoculation) and days 3 to 7 and day 10 after inoculation. After day 10, illness data (adverse events and reactogenicity events) were collected through day 28, with additional physical examinations performed and NW specimens obtained in the event of LRI. For seronegative children, clinical assessments and NWs were performed on days 0, 3 to 7, and 10, 12, 14, 17, 19, and 21 (±1 day for each visit). After the last scheduled NW, illness data were obtained for seronegative children through day 56, with physical examinations performed and additional NW specimens obtained in the event of LRI. The titers of vaccine virus in the NW specimens were determined as described below. Fever, upper respiratory tract illness (rhinorrhea or pharyngitis), cough, LRI, and otitis media were defined as previously described [8]. When illnesses occurred, NW specimens were tested for adventitious respiratory viruses by realtime reverse transcriptase polymerase chain reaction (FTD Respiratory Pathogens 21; Fast-Track Diagnostics, Junglinster, Luxembourg).

Sera were obtained to measure antibody levels to HPIV-1 before inoculation, ~1 month after inoculation in adults and seropositive children, and ~2 months after inoculation in seronegative children.

**Isolation, Quantitation, and Characterization of Virus**

NW specimens were obtained, snap frozen, and stored as previously described [9, 10]. An aliquot of each NW specimen was rapidly thawed, inoculated onto LLC-MK2 cells, and incubated at 32°C for primary virus isolation. For all specimens that showed evidence of infection, a second aliquot was titered by an immunoplaque assay with a methylcellulose overlay on LLC-MK2 cells [9, 10] using serum from a rabbit hyperimmunized to HPIV-1. Titers of the vaccine virus are expressed as the number of plaque-forming units (PFU) mL of NW fluid. The lower limit of detection was 0.6 log\(_{10}\) PFU/mL.

**Immunologic Assays**

Sera were tested for antibodies to HPIV-1 by the HAI antibody test [10] using the HPIV-1 Washington/1964 strain as the reference virus.

**Data Analysis**

Infection with vaccine virus was defined as isolation of the vaccine virus and/or a ≥ 4-fold rise in antibody titer [8]. The mean peak titer of vaccine virus shed (log\(_{10}\) PFU/mL) was calculated for infected vaccine recipients. HAI reciprocal titers were transformed to log\(_{2}\) values for the calculation of mean log\(_{2}\) titers. Rates of illness among vaccine recipients and placebo recipients were compared by the 2-tailed Fisher’s exact test.

**RESULTS**

The rHPIV-1/84/del 170/942A vaccine was initially evaluated in 15 adults at a dose of \( 10^{6.0} \) TCID\(_{50}\). rHPIV-1/84/del 170/942A was highly restricted in replication, with shedding only detected for 2 days in a single vaccine recipient (peak titer, \( 10^{1.75} \) PFU/mL). Sore throat and hoarseness occurred in a vaccine recipient who shed rhinovirus but not the vaccine virus. Sequential bilateral facial paralysis (Bell palsy) resulting in hospitalization occurred in a vaccine recipient beginning on day 4. There was no evidence of infection with rHPIV-1/84/del 170/942A as assessed by viral culture, polymerase chain reaction, or rise in antibody titer, and this serious adverse event was judged by the clinical investigator and the sponsor to be unlikely related to the vaccine. Because of this serious adverse event, rHPIV-1/84/del 170/942A was evaluated in an additional 20 adults. Two of these 20 subjects shed vaccine virus on day 10; the peak titer of vaccine virus shed was \( 10^{0.75} \) PFU/mL. Both of these subjects were asymptomatic. Two subjects had rhinorrhea that was not associated with...
vaccine virus shedding. A fourfold rise in HAI antibody titer was detected in 1 of the 35 vaccinated adults (Table 1). On the basis of the high degree of attenuation of rHPIV-1/84/del 170/942A in adults, an evaluation of a 10^{6.0}TCID_{50} dose was next performed in 15 HPIV-1–seropositive children (10 vaccine recipients, 5 placebo recipients). Of the 10 vaccinated children, one experienced 2 days of fever (peak 101°F rectally) on days 9 and 10, and 2 others experienced rhinorrhea (Table 1), one on days 0 to 2 and the other on days 7 to 10. None of the other vaccinated children or the placebo recipients experienced any illness. None of the children shed vaccine virus or developed an antibody response.

rHPIV-1/84/del 170/942A was next evaluated at a dose of 10^{5.0}TCID_{50} in 22 HPIV-1–seronegative children (14 vaccine recipients, 8 placebo recipients). Shedding of vaccine virus and antibody responses were detected in 2 subjects (Table 1). One subject was asymptomatic and shed virus on days 7 and 12, and one subject had rhinorrhea on days 6, 7, 25, and 26, during which time rhinovirus was detected; the vaccine virus was detected on day 12 only. Mild respiratory or febrile illnesses occurred in 6 vaccine recipients and 3 placebo recipients, and infection with adventitious viral agents (human metapneumovirus, rhinovirus, bocavirus, influenza, or HPIV-3) was detected in 8 of these subjects.

Because the 10^{5.0}TCID_{50} dose was minimally infectious, a 10^{6.0}TCID_{50} dose was next evaluated in 15 HPIV-1–seronegative children (10 vaccine recipients, 5 placebo recipients). None of the vaccine recipients shed vaccine virus, but 3 developed an antibody response to HPIV-1. Respiratory or febrile illnesses were observed in 5 vaccine recipients and 2 placebo recipients: fever (1 vaccine recipient), rhinorrhea (3 vaccine recipients, 2 placebo recipients), and cough (2 vaccine recipients, 1 placebo recipient). The illnesses occurred in association with rhinovirus (4 subjects) or parechovirus (1 subject) infection; again, the rates of illness did not differ significantly between the treatment groups (Table 1). Wheezing in association with rhinovirus infection occurred in a vaccine recipient starting on day 56; however, there was no evidence of infection with the vaccine virus. On the basis of the low infectivity and apparent overattenuation of rHPIV-1/84/del 170/942A, the study was terminated.

**DISCUSSION**

An effective HPIV-1 vaccine could prevent a substantial amount of respiratory tract illness in young children, and an attenuated HPIV-1 might be used as a vector for antigens of respiratory syncytial virus or other pathogens. Although the rHPIV-1/84/del 170/942A was immunogenic
and protective against HPIV-1 challenge in nonhuman primates [6], this experimental vaccine was overattenuated in HPIV-1–seronegative children. Thus, although preclinical studies can identify appropriate vaccine candidates, careful stepwise assessment in clinical trials is essential for determining the characteristics of these empirically derived vaccines in target populations. The results of this study also highlight the need for placebo-controlled trials with assessment for infection with adventitious viral agents, because respiratory illnesses occur frequently in young children [11]. Future efforts to develop a live-attenuated HPIV-1 vaccine are warranted.

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