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BRIEF REPORT

Evaluation of a Live Attenuated Human Metapneumovirus Vaccine in Adults and Children

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We conducted a phase I clinical trial of an experimental live attenuated recombinant human metapneumovirus (HMPV) vaccine (rHMPV-Pa) sequentially in adults, HMPV-seropositive children, and HMPV-seronegative children, the target population for vaccination. rHMPV-Pa was appropriately restricted in replication in adults and HMPV-seropositive children but was overattenuated for HMPV-seronegative children.

Human metapneumovirus (HMPV) was first reported in 2001, although serologic studies have confirmed its global circulation in humans for at least 50 years [1–5]. Like respiratory syncytial virus (RSV), HMPV causes upper respiratory illness, otitis media, and lower respiratory illness (LRI), including bronchiolitis, croup, pneumonia, and exacerbations of asthma in infants and young children [6, 7], although the mean age of HMPV-infected infants is typically older than that of RSV-infected infants [8]. In the United States, HMPV is associated with 5% to 13% of pediatric hospitalizations for respiratory illness and up to 12% of medically attended LRIs in children [7, 9]. HMPV is the second or third leading cause of viral LRI in children [6–8] and can also cause severe disease in premature infants and in elderly and immunocompromised people [8].

An HMPV vaccine could reduce the global burden of LRI in infants and children. A live attenuated intranasally administered HMPV vaccine would have the potential advantage of inducing a full spectrum of local and systemic immune responses and should replicate in the upper respiratory tract of infants even in the presence of maternally derived antibodies [10, 11].

We previously developed a live attenuated HMPV, designated rHMPV-Pa, which is a chimeric virus that expresses the P protein from the related avian metapneumovirus (AMPV) subtype C and all other proteins from HMPV [12]. This experimental vaccine candidate replicates efficiently in vitro and is attenuated and highly immunogenic in hamsters and nonhuman primates (NHPs) [12]. Here, we report its evaluation in a phase I clinical trial in adults, HMPV-seropositive children, and HMPV-seronegative children.

METHODS

rHMPV-Pa was derived from complementary DNA (cDNA), as previously described [12]. 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 a placebo.

Study Population, Study Design, and Clinical Trial Oversight

This trial was conducted at the Center for Immunization Research, Johns Hopkins Bloomberg School of Public Health, from 2011–2014, and between March and December of each year. The vaccine was evaluated sequentially in adults who were not screened for HMPV serostatus (but who all proved to be HMPV-seropositive), HMPV-seropositive children aged 12 to 59 months, and HMPV-seronegative children aged 6 to 59 months. Studies in the adults were open label; all of the subjects received the vaccine. The studies in children were randomized, double-blind, and placebo-controlled; the subjects were assigned randomly 2:1 to receive vaccine or placebo, administered as nose drops (0.25 mL per nostril). The dose of vaccine was $10^{6.0}$ plaque-forming units (PFU) for adults and HMPV-seropositive children, and $10^{5.0}$ and $10^{6.0}$ PFU doses were evaluated sequentially in HMPV-seronegative children.

Written informed consent was obtained from the study participants (adults) or the parents or guardians of study participants (children) before enrollment. The studies were conducted in accordance with the Standards of Good Clinical Practice as defined by the International Conference on Harmonization (ClinicalTrials.gov identifier NCT01255410). The clinical protocol, consent forms, and Investigator's Brochure were reviewed and approved by the Western Institutional Review Board and the National Institute of Allergy and Infectious Diseases (NIAID) Regulatory Compliance and Human Subjects Protection Branch. Clinical data were reviewed by the Center for Immunization Research, NIAID investigators, and the Data and Safety Monitoring Board of the NIAID Division of Clinical Research.

For adults and HMPV-seropositive children, clinical assessments and nasal washes (NWs) were performed on study day 0

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Table 1. Clinical Responses, Vaccine Virus Shedding, and Antibody Responses After Administration of rHMPV-Pa or Placebo to Adults and HMPV-Seropositive and -Seronegative Children

Subject	Dose (log_{10} $TCID_{50}$/mL)	No. of Subjects	Subjects With Indicated Illness (%)						Viral Shedding[b]			Serum PRN Ab Response			Serum HMPV IgG Ab Response		
			Infected (%)[a]	Fever	URI	LRI	Cough	OM	Respiratory or Febrile Illness	% Shedding Virus	Peak Titer (log_{10} PFU/mL) (mean [SD])	Peak Titer (log_{10} copies/mL) (mean [SD])	Mean of $1/log_2$ (SD)			Mean of $1/log_2$ (SD)		≥4-Fold Rise (%)
													Before Vaccine	After Vaccine	≥4-Fold Rise (%)	Before Vaccine	After Vaccine	
Adults																		
Vaccine recipients[c]	6.0	15	0	0	13	7	13	0	13	0	0.6 (0.0)	2.1	7.7 (0.9)	7.8 (0.9)	0	13.3 (1.0)	12.9 (1.4)	0
Seropositive children																		
Vaccine recipients	6.0	10	20	0	10	0	0	0	10	0	0.6 (0.0)	2.1	7.8 (1.0)	7.8 (1.0)	0	12.0 (1.6)	12.4 (1.7)	20
Placebo recipients	—	5	0	20	20	0	0	0	40	0	0.6 (0.0)	2.1	7.7 (0.8)	7.9 (0.9)	0	11.6 (1.4)	11.2 (0.9)	0
Seronegative children																		
Vaccine recipients	5.0	10	30	0	40	0	10	10	50	0	0.6 (0.0)	2.1	2.3 (0.0)	2.9 (1.2)	20	4.6 (0.0)	5.4 (2.2)	10
	6.0	10	50	10	30	10	20	0	50	20	0.6 (0.0)	3.1 (0.6)	2.9 (0.9)	4.1 (1.7)	30	6.3 (2.1)	8.3 (1.9)	50
Placebo recipients	—	10	0	10	20	0	0	10	30	0	0.6 (0.0)	2.1	2.8 (0.7)	2.8 (0.7)	0	6.1 (2.4)	6.3 (2.3)	0

Abbreviations: Ab, antibody; HMPV, human metapneumovirus; LRI, lower respiratory tract illness; OM, otitis media; PFU, plaque-forming units; PR, placebo recipient; PRN, plaque-reduction neutralization; rHMPV-Pa, live attenuated recombinant HMPV vaccine; SD, standard deviation; $TCID_{50}$, 50% tissue culture infective dose; URI, upper respiratory tract illness; VR, vaccine recipient.

[a]Infection was defined as a ≥4-fold rise in neutralizing antibody or HMPV F immunoglobulin G antibody titer and/or detection of vaccine virus by culture or RT-qPCR.

[b]The limit of detection of vaccine virus by culture was 0.6 log_{10} PFU/mL, and the limit of detection by real-time quantitative polymerase chain reaction (RT-qPCR) was 2.1 log_{10} copies/mL. The percentage shedding virus was as detected by culture or RT-qPCR.

[c]One VR had wheezing (LRI) associated with recovery of respiratory syncytial virus from her nasal wash fluid sample.

(NWs were performed before inoculation), and on days 3 through 7, 10, and 12 ± 1 day after inoculation. Illness data (adverse and reactogenicity events) were collected through day 28, and additional physical examinations were performed and an NW was obtained in the event of lower respiratory tract illness (LRI). For seronegative children, a clinical assessment and NW were performed on days 0, 3, 5, 7, 10, 12, 14, 17, 19, 21, and 28 ± 1 day. Illness data for seronegative children were obtained through day 56, and physical examinations were performed and additional NWs were obtained in the event of LRI. Titers of vaccine virus in NW fluid were determined as described below. Fever, upper respiratory illness (rhinorrhea or pharyngitis), cough, LRI, and otitis media were defined as described previously [13]. When illnesses occurred, NW fluid specimens were tested for adventitious agents by real-time reverse-transcription polymerase chain reaction (rRT-PCR) (Fast-Track Diagnostics, Luxembourg).

Sera were obtained from all participants before and after inoculation to measure antibodies to HMPV. In adults and seropositive children, specimens were obtained ~1 month after inoculation, and in seronegative children, specimens were obtained ~2 months after inoculation.

Isolation, Quantitation, and Characterization of Virus

NWs were performed, and NW fluid was snap-frozen and stored as described previously [13]. Viral cultures and quantification were performed as described previously [14]; the lower limit of detection was 0.6 log_{10} PFU/mL. Shedding of vaccine virus was also quantified by real-time quantitative PCR as described previously [14]. The lower limit of detection was 2.1 log_{10} copies/mL.

Immunologic Assays

We tested sera for antibodies to HMPV by using a plaque-reduction neutralization assay and an enzyme-linked immunosorbent assay that quantitated immunoglobulin G (IgG) antibodies to the HMPV fusion (F) protein [14].

Data Analysis

Infection with vaccine virus was defined as the isolation of vaccine virus and/or a ≥4-fold rise in antibody titer [14]. The mean peak titers of vaccine virus shed (log_{10} PFU/mL and copy number per mL) were calculated for infected vaccine recipients. Plaque-reduction neutralization and enzyme-linked immunosorbent assay reciprocal titers were transformed to log_2 values for calculating mean log_2 titers. Rates of illness among vaccine recipients and placebo recipients were compared with the 2-tailed Fisher exact test.

RESULTS

rHMPV-Pa was evaluated in 15 adults at a dose of $10^{6.0}$ PFU. Vaccine virus was not detected in any of the subjects, nor were neutralizing or HMPV F IgG antibody responses. Four subjects had respiratory or systemic illnesses that were judged to be

unrelated to the vaccine (rhinorrhea, pharyngitis, cough, muscle soreness, and hoarseness associated with shedding of human coronavirus OC43 [1 subject]; nasal congestion, rhinorrhea, pharyngitis, cough, hoarseness, wheezing, headache, and fatigue associated with detection of RSV type A [1 subject]; transient mild chills and myalgia on study day 0 [1 subject]; and emesis associated with the detection of coronavirus OC43 [1 subject]). These findings reveal that rHMPV-Pa was highly attenuated in adults.

Next, a $10^{6.0}$ PFU dose was evaluated in 15 HMPV-seropositive children (10 vaccine recipients, 5 placebo recipients). Vaccine virus was not detected in any of the subjects, nor were neutralizing antibody responses detected. HMPV F IgG antibody responses were detected in 2 asymptomatic vaccine recipients (Table 1). The following mild illnesses occurred, all of which were judged to be unrelated to vaccine: rhinorrhea on days 5 through 11 in a vaccine recipient (rhinovirus was detected on days 0, 4, and 7); rhinorrhea on days 3 through 10 in 1 placebo recipient (rhinovirus and enterovirus were detected on days 0, 4, and 5); epistaxis after an NW in a vaccine recipient and a placebo recipient; 1 episode of emesis in a vaccine recipient; and emesis and a rectal temperature of 100.8°F in a placebo recipient.

rHMPV-Pa was evaluated next at a dose of $10^{5.0}$ PFU in 15 HMPV-seronegative children (10 vaccine recipients, 5 placebo recipients). Shedding of vaccine virus was not detected, but antibody responses were detected in 3 vaccine recipients (Table 1). Illnesses occurred in 5 vaccine recipients: rhinorrhea (2), rhinorrhea and cough (1), rhinorrhea and otitis media (1), and hoarseness (1). Rhinovirus was detected in 2 of these subjects. Illness also occurred in 3 placebo recipients: rash and emesis (1), nasal congestion, rhinorrhea and otitis media (1), and nasal congestion alone (1). Rhinovirus was detected in NW fluid from the child with nasal congestion, rhinorrhea, and otitis media, and coronavirus NL63 was detected in the child with nasal congestion alone.

Because the $10^{5.0}$ PFU dose was minimally infectious, a $10^{6.0}$ PFU dose was evaluated next in 15 HMPV-seronegative children (10 vaccine recipients, 5 placebo recipients). Shedding of vaccine virus was not detected by culture, but it was detected by rRT-qPCR in 2 vaccine recipients on days 10 and 12 and days 9 and 11. Each of these vaccine recipients was asymptomatic. Neutralizing antibody responses were detected in 3 vaccine recipients, and HMPV F IgG responses were detected in 5 vaccine recipients, including those with neutralizing antibody responses and those who shed virus. In all, 50% of recipients of the $10^{6.0}$ PFU dose were infected with the vaccine virus. We observed respiratory or febrile illnesses in 2 vaccine recipients and 2 placebo recipients: fever (1 vaccine recipient, parainfluenza virus type 3 infection was detected; 1 placebo recipient, associated with a urinary tract infection), fever, rhinorrhea, and cough (1 vaccine recipient, parainfluenza virus type 3 and bocavirus were detected), and rhinorrhea (1 placebo recipient, adenovirus and bocavirus were detected). The rates of illness did not differ significantly between treatment groups (Table 1). However, on the basis of the low infectivity rate and apparent overattenuation of rHMPV-Pa, the study was terminated.

DISCUSSION

An effective HMPV vaccine could prevent a substantial number of respiratory tract illnesses in young children. To our knowledge, rHMPV-Pa remains the only HMPV vaccine that has been evaluated in clinical trials. Although rHMPV-Pa was immunogenic and protective against HMPV challenge in NHPs [12], this experimental vaccine was overattenuated (i.e., insufficiently infectious and immunogenic in HMPV-seronegative children). Thus, although preclinical studies can identify appropriate vaccine candidates, careful stepwise assessment in clinical trials is essential. The results of this study also highlight the need for placebo-controlled trials and assessment for adventitious viral infections in young children, because respiratory illnesses occur frequently in this population [15]. Future efforts to develop a live attenuated HMPV vaccine are warranted. Our data on rHMPV-Pa provide preclinical benchmarks that will help identify other vaccine candidates that are less attenuated. These candidates might include, for example, rHMPV-Na, which bears the AMPV N gene rather than the AMPV P gene and was shown to be less attenuated than rHMPV-Pa in both the upper and lower respiratory tracts of NHPs [12]; HMPV with deletion of the SH or G gene, which was shown to be less attenuated in the upper (ΔSH and ΔG) and lower (ΔSH) respiratory tracts of NHPs than rHMPV-Pa [16]; and HMPV bearing point mutations designed from comparable mutations in RSV, which have not yet been evaluated in NHPs.

Notes

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