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Live-Attenuated Respiratory Syncytial Virus Vaccine With M2-2 Deletion and With Small Hydrophobic Noncoding Region Is Highly Immunogenic in Children

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Background. Respiratory syncytial virus (RSV) is the leading viral cause of severe pediatric respiratory illness, and vaccines are needed. Live RSV vaccine D46/NS2/N/ΔM2-2-HindIII, attenuated by deletion of the RSV RNA regulatory protein M2-2, is based on previous candidate LID/ΔM2-2 but incorporates prominent differences from MEDI/ΔM2-2, which was more restricted in replication in phase 1.

Methods. RSV-seronegative children aged 6–24 months received 1 intranasal dose (10^5 plaque-forming units [PFUs] of D46/NS2/N/ΔM2-2-HindIII [$n = 21$] or placebo [$n = 11$]) and were monitored for vaccine shedding, reactogenicity, RSV-antibody responses and RSV-associated medically attended acute respiratory illness (RSV-MAARI) and antibody responses during the following RSV season.

Results. All 21 vaccinees were infected with vaccine; 20 (95%) shed vaccine (median peak titer, $3.5 \log_{10}$ PFUs/mL with immunoplaque assay and $6.1 \log_{10}$ copies/mL with polymerase chain reaction). Serum RSV-neutralizing antibodies and anti-RSV fusion immunoglobulin G increased ≥ 4 -fold in 95% and 100% of vaccinees, respectively. Mild upper respiratory tract symptoms and/or fever occurred in vaccinees (76%) and placebo recipients (18%). Over the RSV season, RSV-MAARI occurred in 2 vaccinees and 4 placebo recipients. Three vaccinees had ≥ 4 -fold increases in serum RSV-neutralizing antibody titers after the RSV season without RSV-MAARI.

Conclusions. D46/NS2/N/ΔM2-2-HindIII had excellent infectivity and immunogenicity and primed vaccine recipients for anamnestic responses, encouraging further evaluation of this attenuation strategy.

Clinical Trials Registration. NCT03102034 and NCT03099291.

Keywords. respiratory syncytial virus; live-attenuated viral vaccine; pediatric RSV vaccine; neutralizing antibodies; immunogenicity; RNA regulatory protein M2-2.

Respiratory illness due to respiratory syncytial virus (RSV) is a major cause of disease and death, especially among infants and children <5 years of age [1, 2]. An effective vaccine for RSV has the potential for major impact on infant and child health.

In recent years, the pipeline of RSV vaccine candidates has expanded [3], and several live-attenuated candidates are in development. Live-attenuated vaccines for intranasal administration have potential advantages in that they can induce a full spectrum of innate, antibody, and cell-mediated responses [4], including mucosal responses protecting the upper respiratory tract, the site of natural infection. In addition, live-attenuated RSV vaccines will express the RSV fusion (F) glycoprotein largely in its prefusion form, resulting in effective induction of neutralizing antibodies [5, 6]. Key to success for live-attenuated RSV vaccines is achieving high rates of immune response while maintaining attenuation. Expanded understanding of RSV viral pathogenesis factors

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[7] and techniques of reverse genetics [8] are powerful tools for rational design of live-attenuated vaccine candidates. Several recombinant candidate vaccines have recently been evaluated in children and infants [4, 9–13].

A promising attenuation strategy involves deletion of most of the open reading frame (ORF) encoding the RNA synthesis regulatory protein M2-2 [11, 14]. The RSV M2-2 protein is a small, nonabundant protein encoded by the second, downstream ORF in the M2 messenger RNA, which slightly overlaps the 5'-proximal, upstream M2-1 ORF [15]. Deletion of M2-2 results in increased viral RNA gene transcription and antigen expression but decreased genome replication [14]. In RSV-seronegative children, the increased antigen expression seems to result in greater immunogenicity despite lower replication [11]. Attenuating gene-deletion mutations typically are refractory to deattenuation that has been a problem for live vaccines attenuated by point mutations [10, 16].

Two candidate vaccines attenuated by M2-2 deletion, MEDI/ΔM2-2 and LID/ΔM2-2, have recently been evaluated [11, 12]. These 2 candidates were derived from 2 different recombinant parental complementary DNAs (cDNAs) that differ by 21 nucleotide assignments scattered through the genome, including 2 coding changes in the N2 and N ORFs. The candidates also differ in the design of the M2-2 deletion [12, 17], and LID/ΔM2-2 has a 112-nucleotide deletion and silent mutations in the small hydrophobic (SH) gene and the SH ORF introduced to improve the stability of the cDNA during growth in bacteria [18]. The 2 vaccines had similar phenotypes *in vitro* and in animals. When administered to RSV-seronegative children (ages 6–24 months), both vaccines induced strong RSV-neutralizing antibody responses in a large proportion of vaccinees and demonstrated anamnestic antibody responses after wild-type RSV exposure; however, the peak titer of vaccine virus shed in nasal wash (NW) specimens was approximately 100-fold lower for MEDI/ΔM2-2 [12, 14]. Thus, these modest genetic differences apparently contributed to a different replication phenotype in children.

Although LID/ΔM2-2 was well tolerated in the phase I study, the higher replication might make it poorly tolerated in some recipients when administered to large populations. Therefore, D46/NS2/N/ΔM2-2-HindIII was built on the LID backbone but with several modifications that are expected to generate a phenotype similar to MEDI/ΔM2-2. We describe the evaluation of this new intranasal vaccine candidate in RSV-seronegative children aged 6–24 months.

METHODS

Vaccine

The vaccine, D46/NS2/N/ΔM2-2-HindIII, is a cDNA-derived version of RSV subgroup A, strain A2 (the recombinant wild-type parent is GenBank KT992094). The vaccine uses the

backbone of the previously studied LID/ΔM2-2 vaccine [12] but has a 234-nucleotide M2-2 deletion with the same structure as in MEDI/ΔM2-2 [11, 17]. In addition, D46/NS2/N/ΔM2-2-HindIII has the MEDI/ΔM2-2 assignments at the only 2 amino acid positions that differ between MEDI/ΔM2-2 and LID/ΔM2-2 (K51R in the NS2 ORF, T24A in the N ORF; all nucleotide and amino acid numbering based on recombinant RSV A2, Genbank accession no. KT992094 [19]). The 2 viruses retain 19-nucleotide differences from their parental viruses, scattered throughout the genome. Finally, similarly to MEDI/ΔM2-2, D46/NS2/N/ΔM2-2-HindIII contains the complete 112-nucleotide 3' noncoding region of the SH gene that is present in biological RSV A2 but was deleted in LID/ΔM2-2. D46/NS2/N/ΔM2-2-HindIII was recovered from cDNA in qualified Vero cells, and clinical trial material was manufactured (Charles River Laboratories).

Sequence analysis confirmed that the seed virus and final drug product were identical, except for 3 polymorphisms which were considered biologically inconsequential: (1) a nucleotide dimorphism at nucleotide 2485 (about 20% of the virus population containing a G2485A mutation at this site), which would result in a 20% subpopulation with a D47N amino acid change in the P ORF; (2) a subpopulation of about 30% with a nucleotide insertion of an additional thymidine nucleotide in a polythymidine stretch (nucleotides 4537–39), located in the 3' noncoding region of the SH gene; and (3) a subpopulation of about 70% with an nucleotide insertion of 2 adenosine (A) residues in a polyadenosine stretch (nucleotides 15 064–15 068) of the L gene end signal (+1A [insertion of 1 adenosine] in about 30% of the population, +2A [insertion of 2 adenosines] in about 70%). Vaccine was diluted on site before administration to a dose of 10⁵ plaque-forming units (PFUs) in a 0.5-mL volume. This was administered intranasally as a single dose divided between nostrils. The placebo and vaccine diluent was Leibovitz L15 medium during the first 5 months; it was then changed to the more readily available lactated Ringer's solution for injection, USP for the final 2 months of enrollment. The stability and *in vitro* infectivity of vaccine diluted in the 2 media were confirmed to be indistinguishable.

Study Design

This randomized (2:1 vaccine to placebo), double-blind, placebo-controlled study (ClinicalTrials.gov identifiers NCT03102034 and NCT03099291; <https://clinicaltrials.gov>) was conducted at 10 clinical trial sites (9 domestic International Maternal Pediatric Adolescent AIDS Clinical Trials [IMPAACT] sites and the Johns Hopkins Center for Immunization Research [CIR], Baltimore, Maryland), with accrual between 6 April and 1 October 2017 and surveillance for RSV-like illness from 1 November 2017 through 31 March 2018. Eligibility was determined as described elsewhere [12]; the study included children ≥6 and <25 months of age who were healthy, had no current

or past lung disease, and were RSV seronegative at screening (defined as serum RSV 60% plaque reduction neutralizing titer [PRNT₆₀] <1:40).

Clinical assessments were performed and NW specimens obtained on study days 0 (before intranasal inoculation), 3, 5, 7, 10, 12, 14, 17, and 28, with telephone contact to collect symptom information on all the intervening days. Additional clinical assessments and NW specimens were obtained in the event of respiratory illness (including upper respiratory tract illness [URTI] (defined as rhinorrhea, pharyngitis, or hoarseness), cough, acute otitis media, and lower respiratory tract illness [LRTI]) or fever. Data for all adverse events and reactogenicity events were collected through day 28. After day 28, children were monitored until day 56 for serious adverse events. During the RSV surveillance period (1 November through 31 March), families were contacted weekly to determine whether medically attended acute respiratory illnesses (MAARIs) had occurred, which were defined as fever, URTI, LRTI, or otitis media. Within 3 days of each illness episode, a clinical assessment and NW was obtained. Serum samples to measure RSV antibodies were obtained before inoculation, 56 days after inoculation (this sample also served as the pre-RSV surveillance sample when collected after 1 October), and before (1–31 October) and after the RSV winter surveillance period (1–30 April; 1 vaccinee's postsurveillance visit occurred in May).

Written informed consent was obtained from the parents or guardians of participants before enrollment. These studies were approved by each site's institutional review board, conducted in accordance with the principles of the Declaration of Helsinki and the Standards of Good Clinical Practice, as defined by the International Conference on Harmonization, and monitored by the independent data safety and monitoring board of the National Institute of Allergy and Infectious Diseases, Division of Clinical Research.

Laboratory Assays

NW specimens from days of illness were tested for common adventitious respiratory agents by means of reverse-transcription (RT) quantitative polymerase chain reaction (qPCR) Respiratory pathogens 21 multiplex kit (Fast Track Diagnostics). Vaccine virus in NW specimens was quantified by immunoplaque assay on Vero cells and by RT-qPCR specific for the RSV matrix (M) protein, as described elsewhere [11]. Genetic stability of vaccine isolates was determined as described elsewhere; briefly, genome regions encompassing 2 amino acid differences to LID/ΔM2-2 in the NS2 and N genes and the M2-2 deletion were amplified by RT-PCR, followed by sequence analysis [12].

Serum RSV PRNT₆₀ values were determined by complement-enhanced 60% plaque reduction neutralization assay [20]. Serum immunoglobulin G (IgG) antibody titers to the RSV

F glycoprotein (anti-RSV F IgG) were determined by an IgG-specific enzyme-linked immunosorbent assay using a purified baculovirus-expressed F protein [21, 22], provided by Novavax, as described elsewhere [11, 22, 23].

Statistical Analysis

Reciprocal serum PRNT₆₀ and anti-RSV F IgG titers were transformed to log₂ values. Even though log-transformed, some data deviated from normality; thus, nonparametric methods were used to test for statistical differences and assessing correlations. Medians and interquartile ranges (IQRs) were used to summarize peak NW specimen titers and serum antibody titers to RSV. Mean and standard deviation values were presented to allow descriptive comparisons with other studies (Supplementary Tables 1 and 2). The summaries of vaccine virus shed in NW specimens, detected by means of immunoplaque assay and RT-qPCR, include vaccine recipients who were infected with vaccine. Infection was defined as detection of vaccine virus with immunoplaque assay and/or RT-qPCR and/or a ≥4-fold rise in serum RSV PRNT₆₀ or anti-RSV F IgG titer. For comparing vaccinated and placebo groups, 1-tailed tests were used when there was a clear biological prediction for directionality, and 2-tailed tests when differences were tested for but there was no definite directional hypothesis. The Wilcoxon rank sum test was used to compare peak viral titers and antibody titers between vaccine and placebo recipients. All analyses were performed using SAS software, version 9.4 (SAS Institute), and the graphs were produced using R software version 3.2.2.

RESULTS

Accrual and Participant Characteristics

The study accrued 21 vaccine and 11 placebo recipients. The distributions of sex, age, ethnicity and racial characteristics were similar for vaccine and placebo recipients (Table 1). All children received their assigned study treatment. One vaccine recipient discontinued the study after the day 56 evaluation owing to parental time constraints. This participant is not included in the summary of RSV-MAARI during RSV surveillance. Another vaccinee did not have serum obtained at either the pre- or post-RSV surveillance visits.

Safety and Adverse Events

During the 28 days after inoculation, mild upper respiratory tract and/or febrile events occurred in both vaccine and placebo recipients, with 76% (90% confidence interval [CI], 56%–90%) and 18% (3%–47%) having ≥1 illness episode, respectively (Table 2). All respiratory symptoms in both groups were grade 1. Grade 2 fever occurred in 1 vaccinee and 1 placebo recipient. There were no LRTIs, serious adverse events, or grade 3 or 4 events. Of the 16 vaccinees with respiratory or febrile illness, illness was concurrent with vaccine alone detected in NW

Table 1. Baseline Characteristics of Vaccine and Placebo Recipients

| Characteristic | Recipients, No. (%) | | |
|------------------------------|---------------------|------------------|----------------|
| | Vaccine (n = 21) | Placebo (n = 11) | Total (n = 32) |
| Sex | | | |
| Female | 13 (62) | 8 (73) | 21 (66) |
| Male | 8 (38) | 3 (27) | 11 (34) |
| Ethnicity | | | |
| Hispanic or Latino | 12 (57) | 6 (55) | 18 (56) |
| Not Hispanic or Latino | 9 (43) | 5 (45) | 14 (44) |
| Race | | | |
| African American | 6 (29) | 4 (36) | 10 (31) |
| White | 14 (67) | 6 (55) | 20 (63) |
| Unknown | 1 (5) | 1 (9) | 2 (6) |
| Residence^a | | | |
| California | 5 (24) | 3 (27) | 8 (25) |
| Colorado | 2 (10) | 1 (9) | 3 (9) |
| Georgia and Tennessee | 2 (10) | 0 (0) | 2 (6) |
| Illinois | 6 (29) | 3 (27) | 9 (28) |
| Maryland | 4 (19) | 2 (18) | 6 (19) |
| New York | 2 (10) | 2 (18) | 4 (13) |
| HIV-exposed, uninfected | 9 (43) | 5 (45) | 14 (44) |
| Age, median (IQR) mo | 9 (7–13) | 9 (6–15) | 9.0 (7–14) |

Abbreviations: HIV, human immunodeficiency virus; IQR, interquartile range.

^aOf the 10 sites, 2 each were in California, New York, and Illinois, and 1 each in Colorado, Georgia, Tennessee, and Maryland.

specimens in 12, vaccine plus rhinovirus in 2, vaccine plus rhinovirus and adenovirus in 1, and parainfluenza type 4 and no vaccine virus in 1. Of the 4 vaccinees with fever, vaccine virus alone was detected in NW specimens in 2, vaccine virus plus rhinovirus in 1, and parainfluenza type 3 virus without concurrent vaccine virus in 1. One of the 4 had a second episode of fever after vaccine shedding and concurrent with adenovirus. Among the 2 placebo recipients with illness, rhinovirus was detected in 1 and no agent in 1.

Generally, the rhinorrhea and cough symptoms seemed to cluster around days 8–14 after inoculation, suggesting that D46/NS2/N/ΔM2-2-HindIII shedding might be associated with mild rhinorrhea and/or mild cough. However, the 5 vaccinees without any respiratory or febrile illness during the 28-day period after study product administration included the vaccinee with the highest peak titer by PCR; 2 of these 5 nonsymptomatic vaccinees had the highest overall levels of vaccine shedding assessed by area under the curve for PCR titers, and 4 of the 5 had vaccine areas under the curve above the median for the vaccinees (data not shown). There was no association between high magnitude of vaccine shedding and respiratory symptoms.

Infectivity and Immunogenicity

All 21 vaccinees were infected with vaccine virus (ie, shed vaccine and/or had a rise in serum RSV antibodies). Twenty of 21 vaccinees (95%; 90% CI, 79%–99.7%) had vaccine virus detected with both immunoplaque assay and RT-qPCR (Table 2). Vaccine virus was shed for a median of 10 days (range and IQR, 0–17 and 7–12 days, respectively) by immunoplaque assay and 14 days (0–29 and 12–17 days) by RT-qPCR. Median viral titers were highest on study days 5–7 (Figure 1). Medians of the peak titers from each vaccinee, irrespective of study day, were 3.5 log₁₀ PFUs/mL and 6.1 log₁₀ copies/mL (Table 2). Of note, 4 (19%) of the 21 had peak titers >4 log₁₀ PFUs/mL by immunoplaque assay and of those, 1 had a peak titer >5 log₁₀ PFUs/mL (Figure 1). Sequence analyses were performed on vaccine isolates from NW specimens to investigate the stability of the NS2[K51R] and N[T24A] amino acid assignments and the M2-2 deletion. The NS2/N and M2-2 genome regions (PCR amplicons nucleotides 539–2384 and 7974–8863, respectively) were successfully analyzed from virus from 16 and 19 participants, respectively, and in each case the mutations were

Table 2. Vaccine Virus Shedding, Peak Virus Titers, and Clinical Assessment During the First 28 Days After Inoculation

| Group | Children, No. | Shedding Vaccine Virus, % ^a | Peak Viral Titer in NW Specimens, Median (IQR) | | Children With Symptom, No. (%) ^d | | | | | |
|---------|---------------|--|--|---|---|---------|-------|---------|-------|--------------------------------|
| | | | Plaque Assay, Log ₁₀ PFUs/mL ^b | RT-qPCR, Log ₁₀ Copies/mL ^c | Fever | URTI | LRTI | Cough | OM | Respiratory or Febrile Illness |
| Vaccine | 21 | 95 | 3.5 (2.9–3.8) | 6.1 (5.9–6.6) | 4 (19) | 15 (71) | 0 (0) | 12 (57) | 0 (0) | 16 (76) |
| Placebo | 11 | 0 | 0.5 (0.5–0.5) | 1.7 (1.7–1.7) | 2 (18) | 1 (9) | 0 (0) | 0 (0) | 0 (0) | 2 (18) |

Abbreviations: LRTI, lower respiratory tract infection; NW, nasal wash; OM, otitis media; PFUs, plaque-forming units; RT-qPCR, reverse-transcription quantitative polymerase chain reaction; URTI, upper respiratory tract infection.

^aPercentage of children with vaccine virus detected in NW specimens by immunoplaque assay and/or RT-qPCR. In all cases, vaccine shedding was detected by both immunoplaque assay and PCR.

^bFor each child, the individual peak (highest) NW specimen titer, irrespective of day, measured by immunoplaque assay and expressed as log₁₀ PFUs per milliliter. The lower limit of detection was 0.5 log₁₀ PFUs/mL.

^cFor each participant, the individual peak (highest) NW specimen titer, irrespective of day, measured by RT-qPCR and expressed as log₁₀ copies per milliliter. The lower limit of detection was 1.7 log₁₀ copies/mL.

^dChildren with indicated respiratory symptoms occurring in the 28 days after inoculation. LRTI was defined as wheezing, rhonchi, or rales or a diagnosis of pneumonia or laryngotracheobronchitis (croup). URTI was defined as rhinorrhea, pharyngitis, or hoarseness.

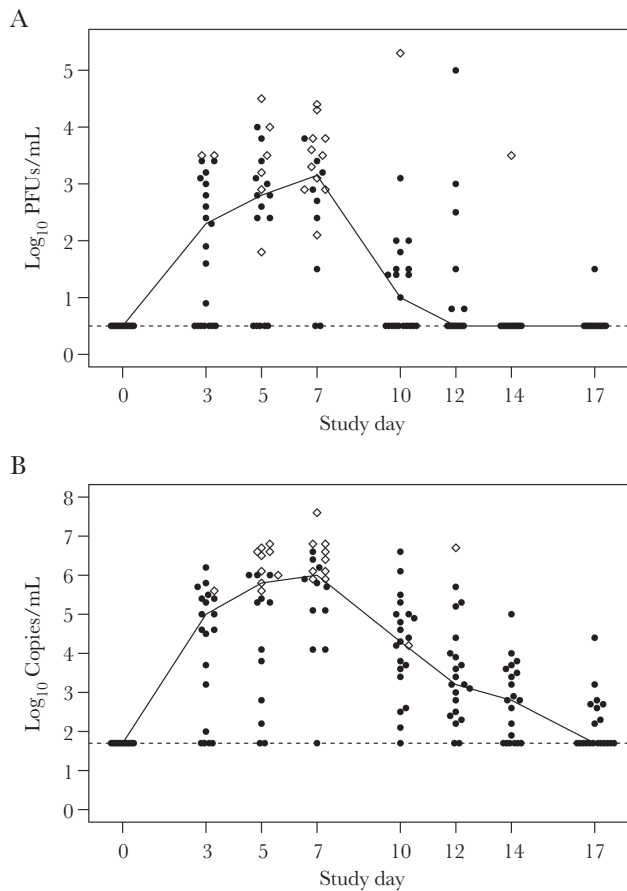


Figure 1. Vaccine virus shed in nasal wash (NW) specimens from vaccinees. Titers (closed circles) and peak titers (open diamonds) for individual participants are shown along with median titers (solid line). NW specimens were collected from vaccinees during acute-phase study visits (window: indicated study day \pm 1 d) after inoculation on day 0, and titers were determined by immunoplaque assay (A) and reverse-transcription quantitative polymerase chain reaction (RT-qPCR) (B). The lower limits of detection (dashed lines) were 0.5 log₁₀ plaque-forming units (PFUs)/mL and 1.7 log₁₀ copies/mL for immunoplaque assay and RT-qPCR, respectively.

retained. This included specimens from 3 participants isolated on days 28–29, confirming stability over time.

Serum antibody responses to the vaccine were assessed approximately 2 months after inoculation (Table 3 and Figure 2). Increases of ≥ 4 -fold in serum RSV PRNT₆₀ and anti-RSV F IgG titers were detected in 20 (95%) and 21 (100%) of vaccinees and none of the placebo recipients ($P < .001$). The serum RSV PRNT₆₀ achieved by 85% of vaccinees was ≥ 5.5 log₂ ($\geq 1:45$).

RSV Surveillance

During the RSV surveillance period (the RSV season after vaccination), rates of all-cause MAARI were similar in the vaccine and placebo recipient groups (55% [90% CI, 35%–74%] vs 73% [44%–92%], respectively). Six of the 19 vaccinees who completed RSV surveillance and 4 of the 11 placebo recipients experienced natural RSV infection during the RSV surveillance period as determined by either an RSV-associated MAARI

and/or ≥ 4 -fold increase in either RSV PRNT₆₀ or anti-RSV F IgG titers in serum samples obtained before and after RSV surveillance. Of the 6 vaccinees, only 2 had an RSV-associated MAARI: 1 with fever and URTI (RSV-A) and 1 with fever, otitis media, and cough (RSV-B). Interestingly, the vaccinee with RSV-B infection did not have a ≥ 4 -fold increase in either RSV PRNT₆₀ or RSV F IgG antibody titers during the RSV season. All 4 placebo recipients with RSV infection during surveillance had an associated MAARI; 1 had rhinorrhea alone (RSV-B), 1 had rhinorrhea with fever (RSV-B), 1 had otitis media (RSV-B), and 1 had an LRTI (bronchiolitis; RSV-A). Among the participants with natural RSV infection, the post-RSV surveillance serum RSV PRNT₆₀ tended to be higher for vaccinees than for placebo recipients (median [IQR], 10.4 [6.4–11.0] vs 7.1 [5.9–8.8] log₂) (Figure 3), indicative of an anamnestic response to wild-type RSV.

Assessment of antibody titers in vaccinees who did not have a boosted response after the RSV surveillance period, and thus were presumed not to have been exposed to RSV during the surveillance period, provided an opportunity to evaluate the durability of the primary response. In these 14 vaccinees, the pre- and post-RSV surveillance median RSV PRNT₆₀ was minimally changed (median [IQR], 6.7 [5.4–8.4] vs 6.1 [5.8–7.9] log₂). Similar results were observed for the pre- and post-RSV surveillance anti-RSV F IgG titer (median [IQR], 14.4 [13.5–15.7] vs 13.7 [11.9–15.6] log₂).

DISCUSSION

When administered to RSV-naive 6–24 month-old infants and children, the RSV D46/NS2/N/ΔM2-2-HindIII candidate vaccine was well tolerated, was highly infectious (100% of participants had evidence of vaccine shedding and/or a serum RSV antibody response), and resulted in excellent induction of serum RSV-specific antibodies, including neutralizing antibodies. Vaccination induced neutralizing antibody responses in 95% of participants, a frequency matching or exceeding that observed in previously studied live-attenuated RSV vaccine candidates [12, 13, 24]. Importantly, vaccinees demonstrated anamnestic serum RSV antibody responses after natural RSV infection during the RSV season, often without associated medically attended illnesses. In vaccinees without anamnestic responses or other evidence of RSV infection (presumably not exposed to RSV during the surveillance period), serum RSV antibody responses were durable, with minimal changes in titer 6–9 months after vaccination.

The vaccine was well tolerated, with no LRTIs or other concerning safety signals. Although there was a slightly greater incidence of URTIs among vaccinees than among placebo recipients, all respiratory symptoms were mild (grade 1), and there was only 1 participant with fever with vaccine shedding and no other adventitious respiratory agent. Although the respiratory symptoms seemed to cluster during the period of vaccine

Table 3. Respiratory Syncytial Virus (RSV)-Specific Serum Antibody Responses Before and After Inoculation and After RSV Surveillance

| Group | Serum RSV-Neutralizing Antibodies ^a | | | | | Serum IgG ELISA RSV F Antibodies ^a | | | | |
|-----------------------------|--|-------------------|---------------------------|------------------------------------|----------------------------------|---|-------------------|-------------------------------|------------------------------------|-------------------------------|
| | Vaccination | | RSV Surveillance | | | Vaccination | | RSV Surveillance | | |
| | Pre ^c | Post ^d | Median (IQR) ^b | ≥4-Fold Rise, No. (%) ^e | ≥4-Fold Rise, N (%) ^f | Pre ^c | Post ^d | Median (IQR) ^b | ≥4-Fold Rise, No. (%) ^e | Post ^d |
| Vaccine recipients (n = 21) | 2.3 (2.3–2.3) | 7.2 (6.1–8.3) | 6.5 (5.2–8.4) | 20 (95) | 4 ¹ (21) | 9.4 (7.3–10.2) | 15.7 (13.9–15.7) | 13.9 ¹ (12.5–15.7) | 21 (100) | 13.9 ¹ (13.0–15.7) |
| Placebo recipients (n = 11) | 2.3 (2.3–2.3) | 2.3 (2.3–2.3) | 2.3 (2.3–2.3) | 0 (0) | 4 (36) | 8.5 (7.2–10.1) | 8.1 (6.0–9.1) | 7.6 (6.0–8.5) | 0 (0) | 8.6 (6.2–15.7) |

Abbreviations: ELISA, enzyme-linked immunosorbent assay; F, fusion; IgG, immunoglobulin G; IQR, interquartile range; RSV, respiratory syncytial virus.

^aSerum RSV 60% plaque reduction neutralizing titer (PRNT₆₀) was determined with complement-enhanced 60% plaque reduction neutralization assay; serum IgG titers to RSV F, with ELISA.

^bTiter results are expressed as median reciprocal log₂ values (with IQRs), determined for all participants in each group. Specimens with titers below the limit of detection were assigned reciprocal titers of 2.3 log₂ (PRNT₆₀) and 4.6 log₂ (ELISA).

^cBefore inoculation.

^dAfter inoculation at study day 56.

^eNo (%) of vaccine and placebo recipients with ≥4-fold increase between preinoculation and postinoculation antibody titers.

^fBefore RSV surveillance, collected 1–31 October or on day 56 if on or after 1 October.

¹After RSV surveillance, collected approximately 6–12 months after inoculation (1–30 April except in 1 vaccine recipient, who had serum collected in May).

²No. (%) of vaccine and placebo recipients with a ≥4-fold increase between pre- and post-RSV surveillance antibody titers.

³Two vaccine recipients had missing data at these time points.

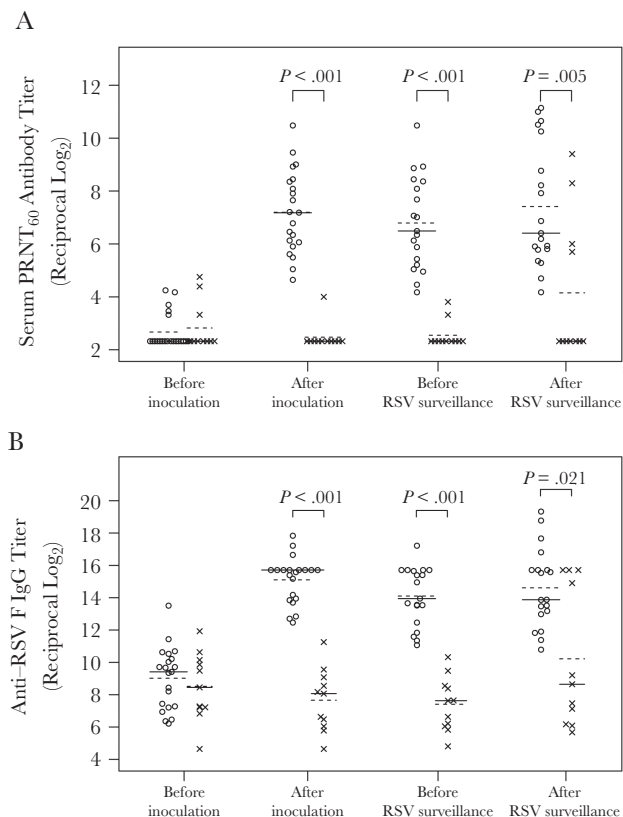


Figure 2. Serum respiratory syncytial virus (RSV) antibody titers in vaccine and placebo recipients. Serum RSV 60% plaque reduction neutralizing titers (PRNT₆₀) (A) and anti-RSV fusion (F) immunoglobulin G (IgG) titers (B) were determined by means of complement-enhanced 60% plaque reduction neutralization assay and IgG-specific enzyme-linked immunosorbent assay against purified RSV F protein, respectively, for vaccine (open circles) and placebo (x's) recipients in serum samples collected before inoculation (screening), after inoculation (study day 56), before surveillance (October of the enrollment year), and after surveillance (usually April after the RSV season). Titers are expressed as the reciprocal log₂ values. Lines indicate median (solid lines) and mean (dashed lines) values. *P* values were determined by means of Wilcoxon rank sum test. Data for the presurveillance and postsurveillance visits are missing for 2 vaccine recipients.

shedding, there was no evidence for an association of symptoms with the magnitude of vaccine shedding. Most notably, 2 vaccinees with the highest overall level of vaccine shedding were among the vaccinees with no solicited signs and symptoms, and only 1 of the asymptomatic vaccinees had an overall level of vaccine shedding below the median. Larger studies are needed to determine the precise frequency of vaccine-related adverse events, especially because URIs are frequent in this age group. Nevertheless, the symptoms were of a mild grade and type that might be acceptable for an efficacious vaccine.

This study was 1 of 3 trials with the same study design, each examining a different vaccine candidate constructed by the addition of attenuating mutations to the vaccine LID/ΔM2-2 [24, 25]. The level of vaccine shedding is an important measure of attenuation: the optimal level is unknown, but earlier studies indicated that titers of 4.0–4.9 log₁₀ PFUs/mL were associated

with a high rate of nasal congestion in young infants, resulting in difficulty feeding and sleeping [9]. One of the present series of vaccines, LID/cp/ΔM2-2, designed with the insertion of a set of 5 defined point mutations originally derived from serial cold passage, resulted in an overattenuated vaccine that had low infectivity and low-titer antibodies in only a fraction of the participants [24]. A second vaccine constructed by the addition of the genetically stabilized mutation 1030s, LID/ΔM2-2/1030s, resulted in a vaccine that was more attenuated than the parent vaccine, with a median peak virus titer of 3.1 log₁₀ PFUs/mL, and none of the 20 vaccinees had a peak titer ≥5 log₁₀ PFUs/mL [25].

The vaccine candidate in the current report, D46/NS2/N/ΔM2-2-HindIII, was designed by incorporating elements from MEDI/ΔM2-2 into LID/ΔM2-2 with the goal to generate a vaccine with high infectivity and immunogenicity, comparable to both the parent viruses, and with low peak vaccine virus shedding in NW specimens, comparable to the MEDI/ΔM2-2. The new construct did achieve the goals for infectivity and immunogenicity with robust neutralizing antibody responses observed in almost every vaccinee. The median peak vaccine virus titer for D46/NS2/N/ΔM2-2-HindIII was similar to that for LID/ΔM2-2 (both 3.5 log₁₀ PFUs/mL). However, D46/NS2/N/ΔM2-2-HindIII had a narrower range and less variability in peak titers, with only 5% with a peak titer ≥5 log₁₀ PFUs/mL versus 15% for LID/ΔM2-2. This suggests greater attenuation than LID/ΔM2-2, but considerably higher peak viral titers than MEDI/ΔM2-2, for which the highest peak viral titer was 3.8 log₁₀ PFUs (mean peak titer [standard deviation], 1.5 [0.9] log₁₀ PFUs/mL) [11]. Thus, LID/ΔM2-2 and its 3 derivatives seem to exhibit a range of attenuation.

This study has several limitations. The small sample size precludes firm conclusions regarding rates of vaccine-associated events. The immune assessment included only the most established correlates of protection against RSV disease, namely serum RSV-neutralizing and serum anti-RSV F IgG titers. Future studies could include measurements such as serum antibodies specific to the prefusion version of the RSV F protein and measures of cellular immunity.

In conclusion, the D46/NS2/N/ΔM2-2-HindIII vaccine had excellent infectivity and generated robust neutralizing antibody and anti-RSV F protein IgG responses. An anamnestic response was observed after the subsequent RSV season in several vaccinees, most of whom lacked medically attended RSV disease. Because this is a live vaccine administered intranasally, it is likely to induce local and systemic innate and cellular as well as humoral responses. This study provides further evidence of the safety and immunogenicity of live-attenuated RSV vaccines using a deletion mutation in M2-2 as the primary attenuating factor. Although the respiratory symptoms observed in this study were mild, the level of shedding of D46/NS2/N/ΔM2-2-HindIII suggests that this vaccine may not be sufficiently

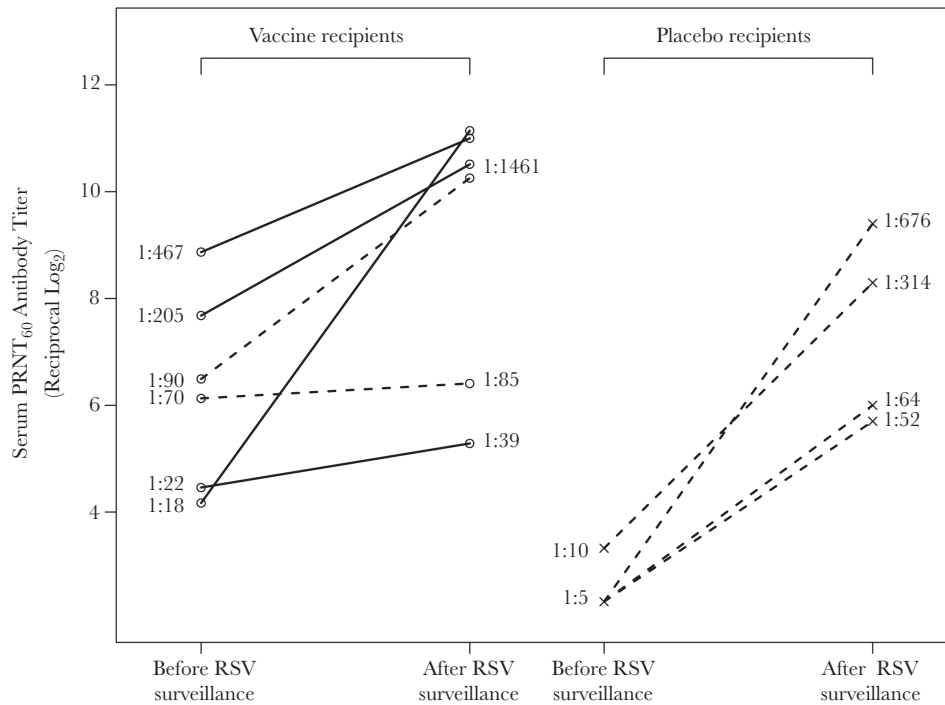


Figure 3. Serum respiratory syncytial virus (RSV)–neutralizing antibody titers among participants with wild-type RSV during RSV surveillance. Serum RSV 60% plaque reduction neutralizing titers (PRNT₆₀) in serum samples collected before and after RSV surveillance are shown for vaccine and placebo recipients who had RSV-associated medically attended respiratory illness (MAARI) or had a ≥ 4 -fold increase in either serum RSV PRNT₆₀ or anti-RSV fusion (F) immunoglobulin G (IgG) titer. Dashed and solid lines indicate subjects with or without RSV-associated MAARI during surveillance. Note that 1 vaccine recipient with a ≥ 4 -fold increase in anti-RSV F IgG titer had a smaller increase in serum neutralizing antibody titer, and 1 vaccine recipient with RSV-associated MAARI did not have a 4-fold increase in either antibody titer. Titers are expressed as reciprocal log₂ values, but for ease of interpretation, some arithmetic values are indicated.

attenuated for further development. However, the results demonstrate the feasibility and the promise of rational design for the iterative process to create a live-attenuated RSV vaccine. Larger studies will be needed to evaluate vaccine safety, as well as the efficacy of protection from RSV disease in infants and children.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential conflicts of interest. C. L., P. L. C., and U. J. B. are listed as inventors on patents related to live-attenuated RSV vaccines, including vaccines made by ablating expression of the M2-2 open reading frame and vaccines containing genetically stabilized attenuating mutations and received research support and royalties paid by Sanofi Pasteur. R. A. K. and L. Y. report research support from Sanofi Pasteur. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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