

Live Respiratory Syncytial Virus Attenuated by M2-2 Deletion and Stabilized Temperature Sensitivity Mutation 1030s Is a Promising Vaccine Candidate in Children

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(See the Editorial commentary by Teng et al, on pages 501–3.)

Background. The safety and immunogenicity of live respiratory syncytial virus (RSV) candidate vaccine, LID/ΔM2-2/1030s, with deletion of RSV ribonucleic acid synthesis regulatory protein M2-2 and genetically stabilized temperature-sensitivity mutation 1030s in the RSV polymerase protein was evaluated in RSV-seronegative children.

Methods. Respiratory syncytial virus-seronegative children ages 6–24 months received 1 intranasal dose of 10⁵ plaque-forming units (PFU) of LID/ΔM2-2/1030s (n = 21) or placebo (n = 11). The RSV serum antibodies, vaccine shedding, and reactogenicity were assessed. During the following RSV season, medically attended acute respiratory illness (MAARI) and pre- and postsurveillance serum antibody titers were monitored.

Results. Eighty-five percent of vaccinees shed LID/ΔM2-2/1030s vaccine (median peak nasal wash titers: 3.1 log₁₀ PFU/mL by immunoplaque assay; 5.1 log₁₀ copies/mL by reverse-transcription quantitative polymerase chain reaction) and had ≥4-fold rise in serum-neutralizing antibodies. Respiratory symptoms and fever were common (60% vaccinees and 27% placebo recipients). One vaccinee had grade 2 wheezing with rhinovirus but without concurrent LID/ΔM2-2/1030s shedding. Five of 19 vaccinees had ≥4-fold increases in antibody titers postsurveillance without RSV-MAARI, indicating anamnestic responses without significant illness after infection with community-acquired RSV.

Conclusions. LID/ΔM2-2/1030s had excellent infectivity without evidence of genetic instability, induced durable immunity, and primed for anamnestic antibody responses, making it an attractive candidate for further evaluation.

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

Respiratory syncytial virus (RSV), a major cause of lower respiratory illness (LRI) in children <5 years, results in significant

morbidity and mortality worldwide [1, 2]. It is the most frequent cause of mortality among postneonatal infants in low-income countries where it mainly impacts term infants, whereas in high-income countries, mortality risk is associated with prematurity and cardiopulmonary disease [3]. However, 50% to 70% of RSV hospitalizations occur in healthy, full-term children [4]. An RSV vaccine could significantly reduce the burden of RSV [5].

The pipeline of RSV vaccine candidates has expanded [6], and among these are several live-attenuated candidates in development. A live-attenuated RSV vaccine is of particular importance for active immunization of infants and children [7, 8] because this strategy has been shown to avoid the RSV disease enhancement previously observed in RSV-naïve recipients of

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formalin-inactivated RSV vaccines [9–11] and subunit RSV vaccines [12]. In addition, live-attenuated RSV vaccines administered by the intranasal route should stimulate innate, cellular, and antibody responses systemically as well as in the respiratory tract, the latter location being important for restricting a respiratory pathogen [13–16].

A salient challenge for live-attenuated RSV vaccines is achieving a satisfactory balance between safety and immunogenicity. The use of reverse genetics [17] and an improved understanding of RSV gene function [18] have allowed for rational design of attenuated RSV candidate vaccines, whereby well characterized attenuating mutations are combined to achieve desired levels of attenuation, immunogenicity, and genetic stability. Several complementary deoxyribonucleic acid (cDNA)-derived candidate vaccines have been evaluated in children and infants [8, 19–23]. A promising attenuation strategy involves deletion of most of the open reading frame (ORF) encoding the ribonucleic acid (RNA) synthesis regulatory protein M2-2 [21]. 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 [24]. Loss of M2-2 results in increased viral RNA gene transcription and antigen expression, but decreased genome replication [25]. The increased antigen expression results in greater immunogenicity despite lower replication [21]. Attenuating gene-deletion mutations typically are refractory to deattenuation that has been a problem for candidates with point mutations [20, 26]. Reverse genetics also have been used to generate point mutations that are stabilized against deattenuation by the choice of codons, such as the “1030s” temperature-sensitivity attenuating mutation that consists of K(AAA) and S(TCA) at codons 1321 and 1313 in the L polymerase gene [27].

Two candidate vaccines attenuated by M2-2 deletion, MEDI/ΔM2-2 and LID/ΔM2-2, have been evaluated [21, 22]. These candidates were derived from 2 recombinant parental cDNAs that differ by 21 nucleotide (nt) assignments scattered through the genome. The candidates also differ in the design of the M2-2 deletion [22, 25], and LID/ΔM2-2 has silent mutations in the small hydrophobic (SH) gene and the SH ORF introduced to improve the stability of the cDNA during growth in bacteria [28]. Despite these differences, the 2 candidate vaccines had similar phenotypes in vitro and in animals. However, when administered to RSV-seronegative children (ages 6–24 months), MEDI/ΔM2-2 exhibited substantial neutralizing antibody responses with very low peak titers of vaccine virus recovered from nasal washes (NWs), whereas LID/ΔM2-2 had peak titers of vaccine virus in NWs approximately 100-fold higher than MEDI/ΔM2-2 [22]. Thus, modest genetic differences apparently contributed to a different replication phenotype in children.

Although LID/ΔM2-2 was well tolerated in the Phase I study, we were concerned that its higher replication might make it poorly tolerated in some recipients when administered to

large populations. Therefore, for the present study, we developed LID/ΔM2-2/1030s, which contains the mutations present in LID/ΔM2-2 plus 1030s, the above-mentioned, genetically stabilized, temperature-sensitivity attenuating mutation in the polymerase protein L [27]. We describe the evaluation of this new intranasal vaccine candidate in RSV-seronegative children aged 6–24 months.

METHODS

Vaccine

The vaccine, LID/ΔM2-2/1030s, is a cDNA-derived version of RSV subgroup A, strain A2 (the recombinant wild-type [wt] parent is GenBank [KT992094](#)), with 241 nts deleted from the M2-2 ORF (nt 8189–8429 relative to GenBank [KT992094](#)) and the 3 potential translation initiation codons of the M2-2 ORF silenced (ATG to ACG; the T > C mutations were positions 8161, 8167, and 8179) by site-directed mutagenesis. In addition, 112 nts of the downstream nontranslated region of the SH gene (nt 4499–4610 relative to GenBank [KT992094](#)) were deleted, and 5 translationally silent nt changes were present in the 3' end of the SH ORF (4489C>T, 4492C>T, 4495A>T; 4497A>G; 4498G>A). These changes in the SH gene, described previously, were designed to stabilize RSV full-length cDNA plasmids during propagation in bacteria and appeared phenotypically inconsequential based on replication in mice [28]. Site-directed mutagenesis was used to add the “1030s” mutation in the polymerase protein L, which consists of the temperature-sensitivity attenuating mutation Y1321>K1321(AAA) combined with the stabilizing mutation S1313(AGC)>S1313(TCA) [27], which was genetically stable in preclinical studies as well as in a prior clinical trial [23]. LID/ΔM2-2/1030s has a 40°C shut-off temperature (the lowest restrictive temperature at which the reduction in replication in vitro compared with 32°C was 100-fold or greater than that of wt RSV at the 2 temperatures) and a 38°C small plaque temperature (the lowest restrictive temperature at which the small plaque phenotype was observed), compared with values of >40°C for wt RSV.

LID/ΔM2-2/1030s was recovered from cDNA in qualified Vero cells, and clinical trial material (CTM) was manufactured (Charles River Laboratories, Malvern, PA). Sequence analysis confirmed that the seed virus and final drug product were identical. The CTM was diluted onsite before dosing using Leibovitz L15 medium to a dose of 10⁵ plaque-forming units (PFU) in a 0.5-mL volume. This was administered intranasally as a single dose divided between nostrils. Leibovitz L15 medium was used as placebo.

Study Design

This randomized (2:1 vaccine to placebo), double-blind, placebo-controlled study (ClinicalTrials.gov identifiers: NCT02952339 and NCT02794870) was conducted at US clinical trials sites (Johns Hopkins Center for Immunization Research [Baltimore,

MD] and 7 domestic International Maternal Pediatric Adolescent AIDS Clinical Trials [IMPAACT] sites), with accrual between July 15, 2016 and October 5, 2016 and surveillance for RSV-medically attended acute respiratory illness (MAARI) during the ensuing RSV season from November 1, 2016 through March 31, 2017. Eligible children were aged ≥ 6 and < 25 months, healthy, and with no history of current or past lung disease. Children born to women living with human immunodeficiency virus (HIV) but proven to be HIV-uninfected were permitted to enroll. Eligible children were RSV seronegative at screening, defined as having a complement-enhanced serum RSV 60% plaque reduction neutralizing titer (PRNT₆₀) $< 1:40$.

Clinical assessments and NWs were performed on study days 0, 3, 5, 7, 10, 12, 14, 17, and 28 (± 1), with telephone contact on all intervening days. Additional examinations and NWs were obtained in the event of respiratory illness: namely, upper respiratory illness (URI) defined as rhinorrhea, pharyngitis, or hoarseness; cough; acute otitis media (OM); fever; and LRI, defined as wheezing, rhonchi, or rales, or a diagnosis of pneumonia or laryngotracheobronchitis (croup). All adverse events were collected through day 28; serious adverse events (SAEs) were collected until day 56. During RSV season surveillance, weekly communication identified instances of MAARI, defined as fever, URI, LRI, or OM, and, within 3 days, a NW was obtained. Sera to measure antibodies to RSV were obtained before inoculation, 56 days after inoculation, presurveillance (October 1–31 or at day 56 if that date was after October 1), and postsurveillance (April 1–30).

Written informed consent was obtained from the parents/guardians 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

Nasal wash specimens collected during illness were tested for adventitious respiratory agents by reverse-transcription quantitative polymerase chain reaction ([RT-qPCR] Respiratory Pathogens21 multiplex kit; Fast Track Diagnostics, Luxembourg). Vaccine virus in NWs was quantified by immunoplaque assay on Vero cells and by RT-qPCR specific for the RSV matrix (M) protein gene as described [21]. Genetic stability of vaccine isolates was determined as described [22]. Serum RSV PRNT₆₀ were determined by complement-enhanced 60% plaque reduction neutralization assay [29]. Serum immunoglobulin (Ig)G antibody titers to the RSV F glycoprotein (anti-RSV F IgG) were determined by IgG-specific enzyme-linked immunosorbent assay using purified baculovirus-expressed F protein [30, 31],

provided by Novavax, Inc. (Gaithersburg, MD), as described [21, 31, 32].

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 for testing for statistical differences. Medians and interquartile ranges (IQRs) were used to summarize peak NW titers and serum antibody titers to RSV. Mean and standard deviation values were also presented to allow descriptive comparisons with prior studies (Supplemental Tables 1 and 2). The summaries of vaccine virus shed in NW detected by immunoplaque assay and RT-qPCR were restricted to the 18 (of 20 evaluable) vaccine recipients who were infected with vaccine. Infection was defined as the detection of vaccine virus (by plaque assay and/or RT-qPCR) and/or a ≥ 4 -fold rise in either serum RSV PRNT₆₀ or anti-RSV F IgG titer. When comparing the vaccinated and placebo groups, 1-tailed tests were used when there was a clear biological prediction for directionality, and 2-tailed tests were used when testing for differences with no predicted 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, version 9.4 (SAS Institute Inc., Cary, NC).

RESULTS

Accrual and Participant Characteristics

The study accrued 22 vaccine and 11 placebo recipients. Two children in the vaccine group discontinued study after randomization: 1 after inoculation (before assessments) and 1 without inoculation. Of these 2, only the child who was inoculated is included in the demographics table. Baseline characteristics were similar for vaccine and placebo recipients (Table 1).

Safety and Adverse Events

During the 28 days postinoculation, respiratory and/or febrile illnesses were common in both vaccine and placebo recipients, with 60% (90% CI, 39%–78%) and 27% (90% CI, 8%–56%) having 1 or more illness episodes, respectively (Table 2). Among the 12 vaccinees with illness, Fast Track RT-qPCR of NW detected vaccine virus alone in 3, vaccine virus plus ≥ 1 other adventitious agent in 4, and no vaccine virus but ≥ 1 other agent in 5. Among the 3 placebo recipients with illness, rhinovirus was detected in 1 and no agent was detected in 2. Grade 3 fever occurred in 2 participants, 1 in each group. The vaccinee with grade 3 fever (104°F, day 3) and respiratory symptoms (days 5–22) did not have detectable vaccine virus shedding or a serum RSV antibody response (thus no evidence of infection by vaccine virus), but he/she did have 4 other viruses detected in NW collected between days 5 and 22. All other events in both groups were grade 2 or lower in severity. One vaccinee had a grade 2

Table 1. Baseline Characteristics of Vaccine and Placebo Recipients

Characteristics	Number (%)		
	Vaccine N = 21	Placebo N = 11	Total N = 32
Gender			
Female	7 (33%)	7 (64%)	14 (44%)
Ethnicity			
Hispanic or Latino	10 (48%)	4 (36%)	14 (44%)
Not Hispanic or Latino	11 (52%)	7 (64%)	18 (56%)
Race			
African American	10 (48%)	5 (45%)	15 (47%)
White	8 (38%)	5 (45%)	13 (41%)
American Indian	1 (5%)	0 (0%)	1 (3%)
More than one race	0 (0%)	1 (9%)	1 (3%)
Unknown	2 (10%)	0 (0%)	2 (6%)
Residence^a			
California	6 (29%)	3 (27%)	9 (28%)
Colorado	6 (29%)	3 (27%)	9 (28%)
Illinois	9 (43%)	2 (18%)	11 (34%)
Maryland	0 (0%)	1 (9%)	1 (3%)
New York	0 (0%)	2 (18%)	2 (6%)
HIV exposed, uninfected	14 (67%)	6 (55%)	20 (63%)
Age in months ^b	9 (7–14)	11 (8–16)	9 (7–16)

Abbreviations: HIV, human immunodeficiency virus.

^aOf the 8 sites, 2 each were in California, Illinois, and New York.

^bAge expressed as median (interquartile range).

LRI on day 21 after inoculation, characterized by wheezing, cough, and URI symptoms. This vaccinee had vaccine virus detectable in NW only on day 9. At the time of his LRI, vaccine virus was no longer detected in the NW, but rhinovirus was present. There were no SAEs through day 56.

Infectivity and Immunogenicity

Eighteen of the 20 vaccinees (90%) were infected with vaccine virus (ie, shed vaccine virus and/or had a ≥ 4 -fold rise in serum

RSV antibodies). Vaccine virus was detected by immunoplaque assay and/or RT-qPCR for 17 of the 20 vaccinees (85%) (90% CI, 78%–99.7%) and in none of the placebo recipients (Table 2). Vaccine virus was detected for a median duration of 10 days (IQR, 9–12) by immunoplaque assay and 12 days (IQR, 10–14) by RT-qPCR. Daily median viral shedding was highest on study days 7 to 10 (Figure 1). Medians of the peak titers from each vaccinee, irrespective of study day (Figure 1, open diamonds), were 3.1 log₁₀ PFU/mL and 5.1 log₁₀ copies/mL for the 18 infected vaccinees (Table 2). Titers exceeding 4 log₁₀ PFU/mL were detected in 4 (20%) vaccinees (Figure 1). Genetic stability of vaccine isolates was demonstrated by sequence analysis for the $\Delta M2-2$ deletion and the 1030s mutation in the 15 and 14 vaccinees, respectively, comprising all vaccinees in whom the sequences could be determined for the respective regions.

Serum RSV antibody responses were assessed at day 56 postinoculation (Table 3, Figure 2). Eighteen (90%) vaccinees had a ≥ 4 -fold increase in 1 or both serum RSV antibody assays. Four-fold or greater rises in serum RSV PRNT₆₀ were detected in 17 (85%) vaccinees (all the vaccinees with vaccine virus detected in NW) and no placebo recipients ($P < .001$). Four-fold or greater rises in serum IgG anti-RSV F titers were detected in 17 (85%) vaccinees and 2 (18%) placebo recipients ($P < .001$). The 2 placebo recipients with increase in anti-RSV IgG, both enrolled in August, were not ill and did not shed RSV in NW during the 28 days postinoculation.

Respiratory Syncytial Virus Surveillance

During the surveillance period (the RSV season after vaccination), rates of any MAARI were similar between vaccine and placebo groups: 9 of 20 (45%; 90% CI, 26%–65%) vs 7 of 11 (64%; 90% CI, 35%–86%). The MAARI associated with RSV (all RSV-B) occurred in 1 vaccinee (an LRI) and 2 placebo recipients (1 LRI, 1 URI). The vaccinee with RSV-associated MAARI was 1 of the 2 vaccinees without evidence of infection with vaccine virus.

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

Group	No. of Children	% Shedding Vaccine Virus ^c	Viral Detection ^a		Number (%) With Indicated Symptom ^b					
			Plaque Assay Log ₁₀ PFU/mL ^d	RT-qPCR Log ₁₀ copies/mL ^e	Fever	URI	LRI	Cough	OM	Respiratory or Febrile Illness
Vaccine	20	85	3.1 (2.1, 3.7)	5.1 (4.4, 6.0)	4 (20)	10 (50)	1 (5)	4 (20)	2 (10)	12 (60)
Placebo	11	0	0.5 (0.5, 0.5)	1.7 (1.7, 1.7)	1 (9)	2 (18)	0 (0)	1 (9)	0 (0)	3 (27)

Abbreviations and definitions: LRI, lower respiratory illness (defined as wheezing, rhonchi, or rales, or having been diagnosed with pneumonia or laryngotracheobronchitis [croup]); OM, acute otitis media; PFU, plaque-forming units; RT-qPCR, reverse-transcription quantitative polymerase chain reaction; URI, upper respiratory illness (defined as rhinorrhea, pharyngitis, or hoarseness).

^aMedian (25th, 75th percentile) peak viral titers detected in nasal washes (NWs). For the vaccine group, these summaries were calculated only for the 18 children who were infected with vaccine virus; infection was defined as the detection of vaccine virus by immunoplaque assay and/or RT-qPCR and/or a ≥ 4 -fold rise in respiratory syncytial virus (RSV) serum-neutralizing antibody titer and/or serum anti-RSV F antibody titer.

^bNumber (percentage) of children with indicated respiratory symptoms occurring in the 28 days after inoculation.

^cPercentage of children with vaccine virus detected in NW by immunoplaque assay and/or RT-qPCR; 15 children had vaccine virus detected by both immunoplaque assay and PCR, and 2 only by RT-qPCR.

^dFor each child, the individual peak (highest) titer, irrespective of day, was selected from among all titers measured in the NW by immunoplaque assay and expressed as log₁₀ PFU/mL. The lower limit of detection was 0.5 log₁₀ PFU/mL.

^eFor each participant, the individual peak (highest) titer, irrespective of day, was selected from among all titers measured in NW by RT-qPCR and expressed as log₁₀ copies/mL. The lower limit of detection was 1.7 log₁₀ copies/mL.

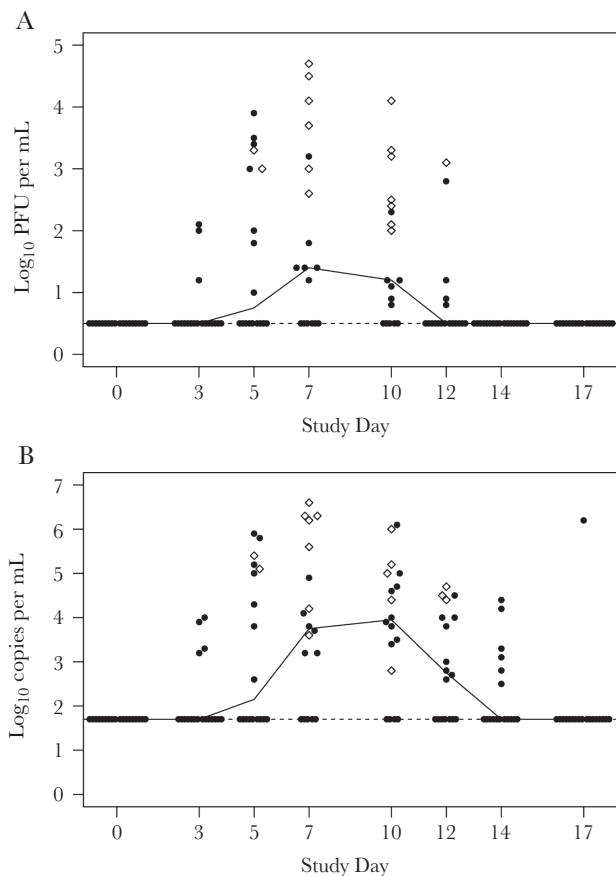


Figure 1. Vaccine virus shed in nasal washes (NWs) in vaccinees. Titers from individual participants (closed circles and open diamonds) and median titers (solid line) of vaccine virus detected in NWs collected from vaccinees during study visits (indicated study day \pm 1 day) after inoculation on day 0, determined by immunoplaque assay (A) \log_{10} plaque-forming units (PFU) per mL and reverse-transcription quantitative polymerase chain reaction (RT-qPCR) (B) \log_{10} copies per mL. Peak titers for each participant are indicated by open diamonds; nonpeak titers are indicated by closed circles. Lower limits of detection indicated by dashed lines were $0.5 \log_{10}$ PFU/mL and $1.7 \log_{10}$ copies/mL for immunoplaque assay and RT-qPCR, respectively.

Comparison of serum RSV antibody titers pre- and postsurveillance allowed evaluation of anamnestic responses after presumed infection with wt RSV. A postsurveillance increase of ≥ 4 -fold in PRNT₆₀ occurred in 6 of 19 (32%) vaccinees and 8 of 10 (80%) placebo recipients, including all 3 participants with an RSV-associated MAARI. Two of the 6 vaccinees with wt RSV infection did not have a ≥ 4 -fold increase in RSV PRNT₆₀ at day 56 postvaccine. The median postsurveillance RSV PRNT₆₀ titer among these 6 vaccinees was 6.5-fold higher than the median postsurveillance titer observed in the placebo recipients (median \log_2 [Q1, Q3] vaccine 9.4 [8.8, 10.1] vs placebo 6.7 [5.8, 8.0]) (Figure 3). The median postsurveillance RSV PRNT₆₀ titer in the placebo recipients (6.7 [5.8, 8.0]), which represents a primary response to wt RSV, was similar to the median day 56 titer in the vaccine recipients (6.4 [5.7, 7.1]), which represents a primary response to the vaccine (Table 3).

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

Group	No. of Children	Serum RSV-Neutralizing Antibodies ^a				Serum IgG ELISA RSV F Antibodies ^a							
		Inoculation		Surveillance		Inoculation		Surveillance					
		Pre ^b	Post ^c	Pre ^d	Post ^d	Pre ^b	Post ^d	Pre ^d	Post ^d				
Vaccine	20	2.3 (2.3–2.3)	6.4 (5.7–7.1)	6.2 (5.6–7.1)	6.6 ^g (5.6–8.8)	71 (5.9–8.9)	14.0 (12.7–14.8)	13.8 (12.7–14.8)	13.7 ^g (12.6–15.6)	17 (85)	17 (85)	5 ^g (26)	5 ^g (26)
Placebo	11	2.9 (2.3–2.3)	2.3 (2.3–2.3)	2.3 ^g (2.3–2.3)	6.2 ^g (5.6–7.9)	72 (5.9–8.8)	6.7 (4.6–8.9)	6.8 ^g (5.2–8.9)	15.2 ^g (14.8–15.6)	2 (18)	2 (18)	8 ^g (80)	8 ^g (80)

Abbreviations: ELISA, enzyme-linked immunosorbent assay; Ig, immunoglobulin.

^aSerum RSV 60% plaque reduction neutralizing titer (PRNT₆₀) was determined by complement-enhanced 60% plaque reduction neutralization assay; serum IgG titers to RSV F were determined by ELISA to baculovirus-expressed RSV F protein. Titer results are expressed as median reciprocal \log_2 with the 25th and 75th percentiles indicated in parenthesis, determined for all participants in each group. Specimens with titers below the limit of detection were assigned reciprocal titers of $2.3 \log_2$ (PRNT₆₀) and $4.6 \log_2$ (ELISA).

^bBefore inoculation.

^cPostinoculation at study day 56.

^dPresurveillance, collected October 1–31 or on day 56 if on or after October 1; postsurveillance, collected April 1 to 30 at approximately 6 months postinoculation (1 vaccine recipient had the last visit in July).

^eNumber and percentage of vaccine and placebo recipients with ≥ 4 -fold increase in antibody titers between preinoculation and postinoculation.

^fNumber and percentage of vaccine and placebo recipients with ≥ 4 -fold increase in antibody titers between presurveillance and postsurveillance.

^gOne participant had missing data at this time point.

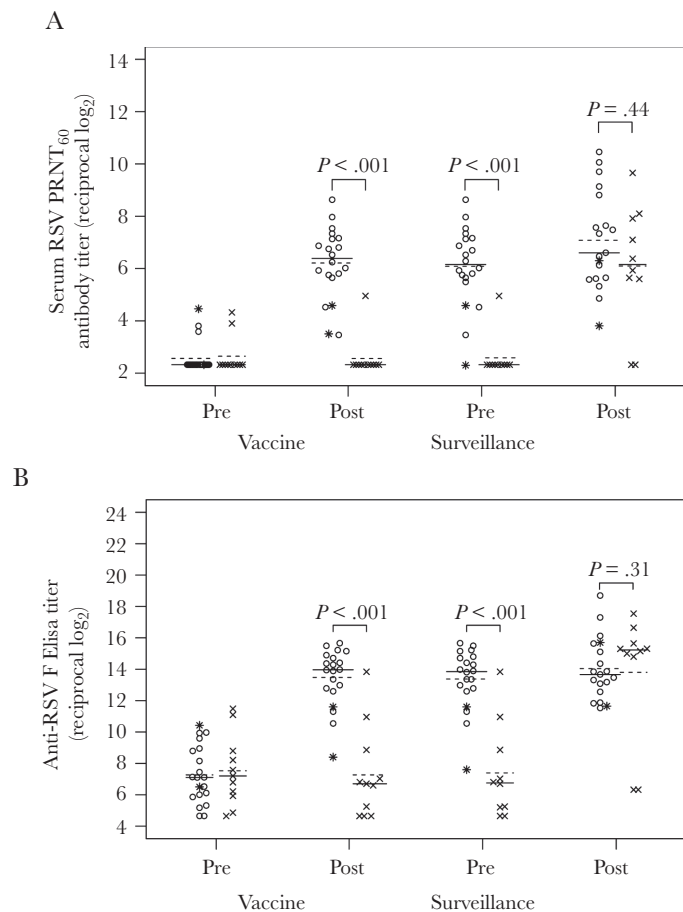


Figure 2. Serum respiratory syncytial virus (RSV) antibody titers in vaccine and placebo recipients. Serum RSV 60% plaque reduction neutralizing antibody titers (PRNT₆₀) (A) and anti-RSV F immunoglobulin (Ig)G enzyme-linked immunosorbent assay titers (ELISA) (B) were determined by complement-enhanced 60% plaque reduction neutralization assay and IgG-specific ELISA against purified RSV F protein, respectively, for vaccine (open circles) and placebo (x) recipients in sera collected at preinoculation (screening), postinoculation (study day 56), presurveillance (October 1–31), and postsurveillance (April 1–30, after the RSV season). Two vaccinees who did not shed vaccine virus and did not have a ≥4-fold increase in either antibody response are indicated by asterisks instead of open circles. The lines indicate median (solid line) and mean (dashed line) values. Serum antibody titers are expressed as the reciprocal log₂. P values were determined by Wilcoxon rank-sum test. Postsurveillance data are missing for 1 vaccine recipient, and pre- and postsurveillance data are missing for 1 placebo recipient.

Postsurveillance serology also allowed evaluation of the durability of primary vaccine-induced antibody responses in recipients who did not have a 4-fold increase in antibody titer and thus were presumed not to have been infected with RSV during the surveillance. In the 14 vaccinees who did not have boosted responses, the pre- and postsurveillance median RSV PRNT₆₀ was minimally changed (median 6.7 [IQR, 5.8–7.3] log₂ vs 6.1 [IQR, 5.6–7.3] log₂). Similar results were observed for the pre- and postsurveillance anti-RSV F IgG titer (median 14.1 [IQR, 13.4–15.2] log₂ vs 13.2 [IQR, 11.9–13.7] log₂).

DISCUSSION

Respiratory syncytial virus vaccine candidate LID/ΔM2-2/1030s was well tolerated in seronegative children ages 6–24 months, with no LRIs associated with vaccine shedding, nor with other concerning safety signals. LID/ΔM2-2/1030s demonstrated excellent immunogenicity, with serum RSV-neutralizing and/or anti-F IgG responses in 90% of the vaccinees. Titers of RSV

antibodies remained robust when evaluated at 6 to 9 months after vaccination. The postvaccination neutralizing antibody response in vaccinees was essentially the same magnitude as in placebo recipients with primary wt RSV infection after RSV season. Rates of induction of neutralizing antibody and anti-RSV F IgG were comparable to those observed in previous studies in similar populations vaccinated with 2 alternative RSV vaccine candidates attenuated by deletions in M2-2 (MEDI/ΔM2-2 and LID/ΔM2-2) [21, 22], but higher than one third of ΔM2-2-based vaccine (LID/cp/ΔM2-2) that was poorly infectious and highly restricted [33], and higher than vaccine candidates with combinations of cold-passaged, temperature-sensitivity (cpts), and RSV SH protein deletion mutations [20]. Although not measured in the present study, a live vaccine would be expected to induce a variety of immune responses such as local and systemic innate and cellular immunity and mucosal antibody responses [13–16], in addition to the measured serum antibody responses, which would be expected to

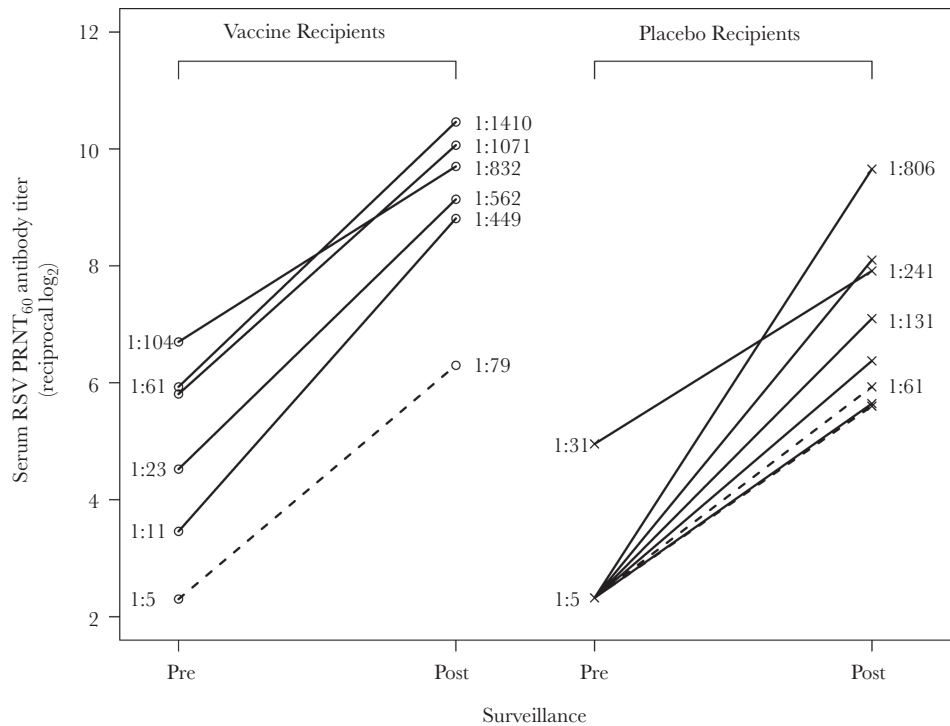


Figure 3. Rises in serum respiratory syncytial virus (RSV)-neutralizing antibody titers, and incidences of medically attended acute RSV illness (RSV-MAARI), during the RSV season surveillance. Serum RSV-neutralizing antibody titer (PRNT₆₀) in sera collected pre- and postsurveillance are shown for vaccine (left) and placebo (right) recipients who had a postsurveillance ≥ 4 -fold increase in either serum RSV PRNT₆₀ or anti-RSV F immunoglobulin G enzyme-linked immunosorbent assay titer. Dashed and solid lines indicate participants with and without RSV-MAARI during surveillance, respectively. Titers are expressed as the reciprocal log₂, but for ease of interpretation, titers corresponding to the arithmetic values are indicated for several participants.

contribute to preventing and restricting subsequent wt RSV infection. The $\Delta M2-2$ and 1030s mutations were found to be stable, which confirms results from recent preclinical and clinical studies [22, 23, 27] and removes a significant obstacle to developing a live RSV vaccine.

During RSV season, 32% and 80% of the vaccinees and placebo recipients, respectively, demonstrated ≥ 4 -fold increases in their RSV-specific serum antibody titers, indicating that they had been infected with wt RSV. For children with serologic evidence of wt RSV, the postsurveillance PRNT₆₀ was substantially higher for most vaccinees than for the placebo recipients, indicating that the vaccinees experienced anamnestic antibody responses compared with primary responses for the placebo recipients. Evidence of anamnestic responses after RSV vaccination has been observed in prior studies of RSV candidates attenuated by the M2-2 deletion [21, 22]. The 1 vaccinee with an RSV-MAARI was among the minority of vaccinees who had neither a postvaccine serologic response nor detectable vaccine virus shedding.

Based on the frequency of boosted serum RSV antibody responses, infection with wt RSV during the surveillance period was frequent among the placebo recipients (80%) in this study, compared with only 32% among vaccinees. The RSV infections were usually asymptomatic because RSV-MAARI was uncommon in both placebo and vaccine recipients, which may be

related to a mild RSV season and/or may reflect that the eligibility criteria for this study selected for healthy children. Given that the 2 groups should have had similar risk of exposure to RSV during the season, the lower rate of wt RSV infection in vaccinees suggests that the vaccine may have provided protection against RSV infection. In previous studies, an increase in RSV-neutralizing antibodies has emerged as a possible correlate of protection against RSV-MAARI/medically attended acute lower respiratory infection [34, 35]. Two of the 6 vaccinees with a 4-fold increase in serum RSV antibody responses during surveillance did not have a vaccine-induced serum RSV-neutralizing antibody response, which would be consistent with this concept.

The median peak titer of vaccine virus shed by vaccinees in this study was slightly lower than observed in a study of LID/ $\Delta M2-2$ (3.1 vs 3.8 log₁₀ PFU/mL) [22]. Although this comparison is limited by sample size and because the vaccines were not studied concurrently, the studies shared the same study design, research sites, laboratory assays, and participant baseline characteristics [22]. Because these vaccines had the same nt and amino acid sequences except for the 2 codons of the 1030s mutation in LID/ $\Delta M2-2$ /1030s, the 1030s mutation likely accounts for the lower peak titers of vaccine shedding titer, thereby achieving the goal of further attenuation of the parent LID/ $\Delta M2-2$. Although the optimal peak shedding titer for an RSV vaccine has not been

determined, experience with live-attenuated RSV vaccines suggests that median peak vaccine shedding should be $<3.0 \log_{10}$ PFU/mL, with peak titers in almost all recipients of $\leq 4.0 \log_{10}$ PFU/mL. In support of these values, a prior study of a different vaccine virus (cpts 248/404), in which titers were 4.0–4.9 \log_{10} PFU/mL, had high rates of nasal congestion in young infants, resulting in difficulty feeding and sleeping [19]. By contrast, a derivative of that vaccine virus with additional attenuating mutations (cpts 248/202/1030 Δ SH), in which titers were 2.4–3.5 \log_{10} PFU/mL, was well tolerated in infants [20]. In the current study, the observations that the median peak titer was 3.1 \log_{10} PFU/mL and $<4.0 \log_{10}$ PFU/mL in 80% of vaccinees suggest that LID/ Δ M2-2/1030s may have an appropriate level of attenuation. Because the rate of mild respiratory illness is frequent in both vaccine and placebo recipients, tolerability of this vaccine can only be determined by studies that enroll larger numbers of children.

The present study has several limitations. The small sample size precludes firm estimates of rates of vaccine-associated events, infectivity, immunogenicity, and viral replication. The immune assessment included the most established correlate of protection against RSV disease, namely, the serum RSV-neutralizing antibody titer. We also measured serum anti-RSV F IgG titers. Future studies could include measurements such as NW RSV-neutralizing antibodies, serum, and NW antibodies specific to RSV prefusion protein, and cellular immunity.

CONCLUSIONS

In summary, in seronegative young children, the LID/ Δ M2-2/1030s vaccine had high rates of vaccine virus shedding and serum RSV antibody responses. The vaccine-induced titers of serum RSV-neutralizing antibodies were essentially equivalent to primary wt RSV infection and were durable across the subsequent RSV season. In some individuals, these titers were strongly boosted during the subsequent RSV season, suggesting that the vaccine primed for potent anamnestic responses upon RSV exposure. The LID/ Δ M2-2/1030s vaccine was more attenuated than its LID/ Δ M2-2 parent, indicating that addition of the temperature sensitivity mutation (1030s) had the desired effect of further reducing viral replication. This study demonstrates that incremental modifications by rational design of a candidate RSV vaccine generated a version with increased attenuation. The LID/ Δ M2-2/1030s vaccine virus is a very attractive candidate for further development as a live-attenuated intranasal pediatric RSV vaccine.

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. U. J. B., C. L., and P. C. 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. 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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