Live Attenuated Vaccine With a Stabilized Mutation and Gene Deletion for Prevention of Respiratory Syncytial Virus Disease in Young Children

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(See the Major Article by Costagliola et al, on pages 516–22.)

Respiratory syncytial virus (RSV) remains the leading cause of hospitalization in infants worldwide and is associated with significant acute and long-term respiratory morbidity [1]. Identification of a safe and immunogenic RSV vaccine has been an important but elusive initiative for more than 60 years. In recent years, however, there has been an explosion of passive and active immunization strategies moving through the drug discovery pipeline (<https://path.org/resources/rsv-vaccine-and-mab-snapshot/>).

Live attenuated vaccines (LAVs) represent an attractive approach for children at least 4–6 months old, because they provide active immunization that mimics natural infection without causing enhanced RSV disease. LAVs have been at the forefront of development since the 1960s, after children who had received the formalin-inactivated vaccine in a clinical trial experienced enhanced disease on subsequent natural infection [2]. The main challenge for the development of LAVs has not been enhanced disease, but rather balancing attenuation

with immunogenicity, particularly given the young age of the target population. The first attempts at developing LAVs were based on virus mutants selected by growing the virus at low temperature (cold passage) and/or by mutagenizing and selecting virus that could not grow at high temperature (temperature sensitive mutants), so that they would be able to replicate in the upper but not lower respiratory tract [3]. The advent of a reverse genetics system for introducing specific changes and even deletions into the RSV genome has greatly enhanced LAV development. More recently, attenuated LAVs combining deletions of nonessential viral genes and temperature-sensitive mutations have progressed to clinical trials.

One gene deletion of particular interest is that of the M2-2 transcription regulatory factor [4]. The initial phase 1 study of an RSV vaccine candidate lacking M2-2 (MEDI/ΔM2-2) showed that the vaccine was both safe and immunogenic [5]. However, an independently derived M2-2 deletion candidate (LID/ΔM2-2) with a slightly different genetic background was found to be underattenuated, causing objectionable levels of symptoms in a subsequent clinical study [6], despite the lack of phenotypic differences between MEDI/ΔM2-2 and LID/ΔM2-2 in tissue culture and in mice. An attempt to increase attenuation of LID/ΔM2-2 by inclusion of a suite of mutations associated with cold-passage (LIDcpΔM2-2) resulted in

overattenuation of the vaccine candidate, inducing too weak of an immune response to be protective [7]. These differences in clinical phenotype attributed to the vaccine viruses underscore the need for more robust preclinical models for testing.

In this issue of *The Journal of Infectious Diseases*, McFarland et al [8] report the results of a phase 1 trial with the LID/ΔM2-2/1030s vaccine candidate in 20 seronegative children, 6–24 months of age. The 1030 mutation was originally identified as a temperature-sensitive point mutation of the RSV polymerase (L) that confers a reduced (38°C) shutoff temperature to RSV [9]. This mutation has also been previously studied in the context of an SH gene deletion (MEDI-559) [10]. However, the 1030 mutation was found to be genetically unstable, both in culture and in vaccinees [10, 11]. Further study identified a mutation at a second site in L that stabilized the 1030 change [12]. This combination of 2 L polymerase gene mutations, the temperature-sensitive and stabilizing mutations, are termed “1030s” and were added to the M2-2 gene deletion virus.

In the current study, 20 children were vaccinated intranasally with 10⁵ plaque-forming units of the LID/ΔM2-2/1030s vaccine candidate. Eleven additional children (placebo recipients) were mock-vaccinated. The attenuating phenotypes of the ΔM2-2 and 1030s mutations have been reported individually or in

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combination with other mutations, in cell culture and in animals [5, 12], but their combination in a single virus has not. Before vaccination, none of these children had serum neutralizing antibodies to RSV. To determine whether vaccinees had been infected by the vaccine virus, nasal wash samples were obtained every other day from 3 to 14 days after inoculation. Immunoplaque assay and/or reverse-transcription polymerase chain reaction demonstrated virus in 17 of 20 vaccinees (85%). Serum samples obtained 56 days after inoculation also displayed a ≥ 4 -fold rise in neutralizing antibody titer in 17 of 20 vaccinees. Antibody was detected in 1 vaccinee in whom no vaccine virus was detected, increasing the total to 18 of 20 (90%). However, it is possible that the vaccinee who did not shed vaccine virus but had antibody was infected with community-acquired RSV during the 56 days after vaccination. In any case, a “take” rate of 85% (17 of 20) or 90% (18 of 20) for such a vaccine is excellent.

The development of severe symptoms after LID/ Δ M2-2/1030s administration in these young seronegative children would have represented a hard stop for the development of this vaccine. Of the 20 vaccine recipients, 12 (60%) displayed a nonsevere respiratory or febrile illness within 28 days after vaccination. Many other respiratory pathogens can cause infections in young children during the winter months, and some of these clinical manifestations could have been caused by viruses other than the vaccine virus. However, the 60% rate of respiratory or febrile illness in vaccinees is higher than that in the placebo recipients, 27% (3 of 11). This difference is a cause for concern that will require additional assessments in future studies.

In nasal wash samples obtained at the time of illness, the vaccine virus was detected in 7 vaccinees (35%): in 3 (15%) as the sole detected virus and in 4 (20%) in combination with ≥ 1 other respiratory virus. A simple calculation that subtracts these 7 vaccinees from the 12 with respiratory or febrile illness yields 5 vaccinees (25%) with a respiratory or

febrile illness caused by viruses other than RSV, similar to the 27% in placebo recipients. Alternatively, the respiratory or febrile illness in the 4 vaccinees with another respiratory virus identified along with the vaccine virus could have been caused by the other virus. The sample sizes (20 and 11 children) are too small to draw firm quantitative conclusions, and safety concerns should be addressed in future, larger trials. Nevertheless, none of the vaccinees had evidence of lower respiratory tract infection, a critical consideration in assessing whether a vaccine is appropriately attenuated, and these results are encouraging.

Stable maintenance of the vaccine mutations without reversion to a wild-type (WT) pathogenic phenotype is also essential for an LAV. One of the attenuating mutations included the deletion of the M2-2 gene, precluding reversion to WT RSV at that site. In addition, the authors have previously demonstrated that the combination of 1 attenuating and 1 stabilizing mutation, the 1030s mutations, are stable during replication in cell culture [12]. To determine whether these mutations would also be stable in vaccinated children, nasal wash specimens collected from the 17 vaccinees who had been infected by the vaccine virus were examined using reverse-transcription polymerase chain reaction and sequencing of the M2-2 and the 1030 gene regions.

Of the 17 vaccinees who shed vaccine virus, no reversions were found in 15 and 14 viruses that could be sequenced at the M2-2 and 1030s sites, respectively. It is not clear when these virus samples were obtained during the infection—that is, early, at the peak of virus production, or late. Reversion would probably be more prominent late in the infection because there is additional time to propagate and because of the selective growth advantage a revertant would have. Furthermore, this approach/procedure only determines the dominant consensus sequence. Revertants may have been generated but not yet become dominant in this small

sample size and perhaps short replication period. Analysis by next-generation sequencing might answer this question more stringently and convincingly.

The response of vaccinees and placebo recipients during the subsequent RSV season is also important; in most cases, this season began soon after the convalescent blood sample was obtained, 58 days after inoculation. The rates of medically attended acute respiratory infections were lower for the vaccinees, 9 of 20 (45%), than for the placebo recipients, 7 of 11 (64%), consistent with vaccine-induced protection from RSV. The 5 of the 20 vaccinees (25%) who were infected by WT RSV, as determined by the boost in their serum RSV antibody levels, did not experience medically attended acute respiratory infections, indicating that in their second encounter with RSV they were protected. The other 9 vaccinees who were not protected from such infections were likely infected with a different virus, because their antibody responses to RSV were not boosted. Four remaining vaccinees either did not encounter RSV or were completely protected from RSV infection.

Finally, 8 of the 11 placebo recipients generated antibodies to RSV over the RSV season, as did 1 of the 2 vaccinees who had not responded to the vaccine. During this period, natural infection of the children from the placebo group induced antibody titers that, on average, were roughly similar in magnitude to the response the vaccinees had mounted to the vaccine virus. This result indicates that the potency of the vaccine candidate was similar to that of natural RSV infection, which again is encouraging. All in all, the LID/ Δ M2-2/1030s vaccine candidate has potential for infants and young children, and future studies are warranted. LAVs, in general, continue to show promise for reducing the burden of RSV disease.

Note

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References

1. Mejias A, Wu B, Tandon N, et al. Risk of childhood wheeze and asthma after respiratory syncytial virus infection in full-term infants. *Pediatr Allergy Immunol* **2019**; 00:1–10. doi:10.1111/pai.13131.
2. Kapikian AZ, Mitchell RH, Chanock RM, Shvedoff RA, Stewart CE. An epidemiologic study of altered clinical reactivity to respiratory syncytial (RS) virus infection in children previously vaccinated with an inactivated RS virus vaccine. *Am J Epidemiol* **1969**; 89:405–21.
3. Gharpure MA, Wright PF, Chanock RM. Temperature-sensitive mutants of respiratory syncytial virus. *J Virol* **1969**; 3:414–21.
4. Bermingham A, Collins PL. The M2-2 protein of human respiratory syncytial virus is a regulatory factor involved in the balance between RNA replication and transcription. *Proc Natl Acad Sci USA* **1999**; 96:11259–64.
5. Karron RA, Luongo C, Thumar B, et al. A gene deletion that up-regulates viral gene expression yields an attenuated RSV vaccine with improved antibody responses in children. *Sci Transl Med* **2015**; 7:312ra175.
6. McFarland EJ, Karron RA, Muresan P, et al; International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT) 2000 Study Team. Live-attenuated respiratory syncytial virus vaccine candidate with deletion of RNA synthesis regulatory protein M2-2 is highly immunogenic in children. *J Infect Dis* **2018**; 217:1347–55.
7. Cunningham CK, Karron R, Muresan P, et al; International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT) 2012 Study Team. Live-attenuated respiratory syncytial virus vaccine with deletion of RNA synthesis regulatory protein M2-2 and cold passage mutations is overattenuated. *Open Forum Infect Dis* **2019**; 6:ofz212.
8. McFarland EJ, Karron RA, Muresan P, et al; International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT) 2011 Study Team. Live respiratory syncytial virus attenuated by M2-2 deletion and stabilized temperature sensitivity mutation 1030s is a promising vaccine candidate in children. *J Infect Dis* **2019**; jiz603. [Epub ahead of print]
9. Whitehead SS, Firestone CY, Karron RA, et al. Addition of a missense mutation present in the L gene of respiratory syncytial virus (RSV) cpts530/1030 to RSV vaccine candidate cpts248/404 increases its attenuation and temperature sensitivity. *J Virol* **1999**; 73:871–7.
10. Malkin E, Yogev R, Abughali N, et al. Safety and immunogenicity of a live attenuated RSV vaccine in healthy RSV-seronegative children 5 to 24 months of age. *PLoS One* **2013**; 8:e77104.
11. Schickli JH, Kaur J, Tang RS. Nonclinical phenotypic and genotypic analyses of a Phase 1 pediatric respiratory syncytial virus vaccine candidate MEDI-559 (rA2cp248/404/1030ΔSH) at permissive and non-permissive temperatures. *Virus Res* **2012**; 169:38–47.
12. Luongo C, Winter CC, Collins PL, Buchholz UJ. Increased genetic and phenotypic stability of a promising live-attenuated respiratory syncytial virus vaccine candidate by reverse genetics. *J Virol* **2012**; 86:10792–804.