Reply to "No Clinical Association of Live Attenuated Influenza Vaccine with Nasal Carriage of Bacteria or Acute Otitis Media": Specific Recommendations for Future Studies

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Recently, we reported that vaccinating mice with a live attenuated influenza vaccine (LAIV) led to substantial increases in the dynamics of nasal colonization by important and common bacterial respiratory pathogens, including *Streptococcus pneumoniae* and *Staphylococcus aureus* (1). However, as Coelingh and Belshe point out (2) and as we emphasize both in that paper and again here, in contrast to what occurred when mice were vaccinated against seasonal influenza viruses, mice vaccinated with LAIV did not exhibit increased susceptibility to bacterial disease in the lower respiratory tract, which is of particular concern when considering what is known about interactions between influenza viruses and bacterial pathogens (3).

Our investigation received considerable attention, but unfortunately, a large portion of it came from the antivaccine movement. This is not surprising, as our results—the first to demonstrate an increased level of one pathogen as a result of a vaccine targeted against an unrelated pathogen—can easily, perhaps intentionally, be misinterpreted to seem as though our data advocate against the use of live attenuated influenza vaccines. This is not the case, and we agree firmly with the overwhelming data demonstrating that both LAIV and inactivated influenza vaccines are beneficial in reducing influenza-related disease (4), including from bacterial infections (5, 6). What we demonstrated in our report (1) is that an LAIV induced excess replication of multiple bacterial pathogens in the upper respiratory tracts of mice without causing consequent lower respiratory tract disease.

What do the clinical data show? Citing the abstract (7) of a randomized placebo-controlled study of LAIV ($n = 151$ children) by Thors et al. submitted for presentation at the 2014 International Symposium on Pneumococci and Pneumococcal Disease (ISPPD) in Hyderabad, India, Coelingh and Belshe state that there is no clinical association of LAIV with nasal carriage of bacteria in humans. Based on the initially submitted (and therefore published) abstract detailing the preliminary results of that study, Coelingh and Belshe were correct in their assertion that LAIV was not associated with increased bacterial titers in that study. However, the updated analysis of the results of that trial, which was ultimately presented at the ISPDD in March of 2014 under a title (8) different from that of the published abstract, suggests that the Ann Arbor strain LAIV licensed for use does significantly increase bacterial carriage density.

In the updated and presented analysis, Thors et al. found that the average pneumococcal bacterial density detected in the nasopharyngeal samples of the 151 children was increased by 59% at 7 days postvaccination ($P = 0.16$) and by 200% at 28 days postvaccination ($P = 0.08$). This analysis compared average bacterial titers (negative samples were excluded) measured over the whole cohort of 151 vaccinated children at days 7 and 28 postvaccination to prevaccination titers. While this analysis provides some insight, a more appropriate analysis to determine effects of LAIV on bacterial density would be to assess changes at the individual level and only in those children colonized at baseline (i.e., at the time of vaccination). Indeed, Thors et al. performed this analysis using paired data. Among the 96 children colonized at baseline, vaccination with LAIV increased the bacterial carriage density within an individual, on average, by approximately 1.5-fold (51%; $P = 0.10$) and ~216% ($P = 0.025$) at days 7 and 28 postvaccination, compared to carriage at day 0. Although statistically significant increases (at an alpha of 0.05) were not detected at day 7 versus baseline, as we demonstrate in our recommendations below, this study may have been underpowered to detect a 1.5-fold increase in pneumococcal density.

While we cannot attest to the reasons for the discrepancies between the initial published abstract (7) cited by Coelingh and Belshe and their updated work presented at the meeting (8), the updated analysis by Thors et al. and the discrepancies noted highlight both the need for further investigations and the requirement for multiple investigations before unanimous assertions can be made.

As we pointed out in our initial paper (1) and as Thors et al. concluded in their presented work, the effects of LAIV on bacterial carriage dynamics in humans should be evaluated using larger sample sizes that are better able to detect differences in bacterial carriage density, particularly given the large variance in titers detected.

As baseline colonization rates will be different between study sites, sample size calculations must account for the prevalence of colonization and expected variance in bacterial titers, and a conservative threshold should be used for clinically or ecologically relevant changes in bacterial density. Given these and other complexities and assumptions (e.g., correlated-data structures), such sample size calculations can be difficult to obtain by traditional methods attempting to find a closed-form solution. Using a stochastic-simulation approach, with variance in carriage density parameterized by data previously collected in our lab from 345

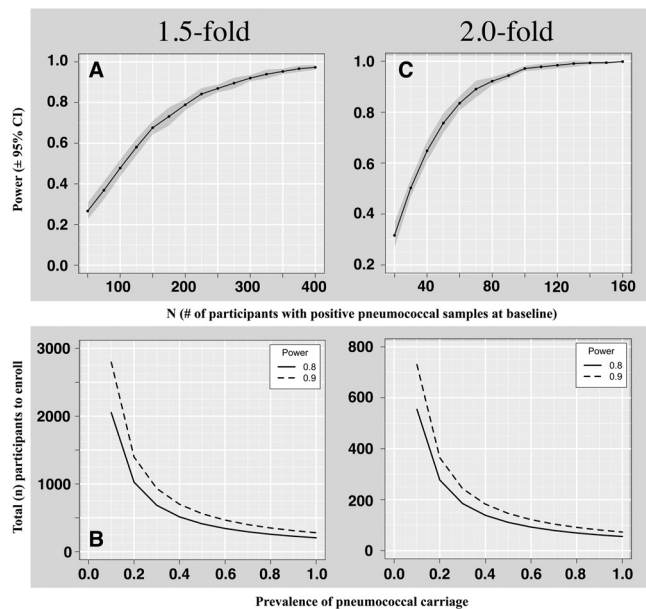Address correspondence to Michael J. Mina, michael.j.mina@gmail.com.

FIG 1 Power and sample size calculations for studies evaluating effects of LAIV on bacterial densities. Ten thousand stochastic simulations were run for each potential sample size interrogated (indicated by filled circles in panels A and C) and for each minimum-effect size (1.5-fold and 2.0-fold), totaling 300,000 unique simulated trials. Variation in simulated bacterial densities was parameterized from data previously collected by our lab from a series of 345 pneumococcus-positive nasal swabs (9). Each series of 10,000 simulations comprised 10 distinct sets of 1,000 unique simulated trials, and each of the trials was tested for statistically significant differences in bacterial titers when data were stochastically simulated for 1.5-fold-increased (A, B) and 2-fold-increased (C, D) bacterial titers from baseline for each individual. Within each of the 10 distinct sets of 1,000 trials, the proportion of trials for which P values were <0.05 (i.e., the power to reject the null hypothesis when the alternative hypothesis is true) was recorded, and the mean of the values from the 10 distinct sets is plotted for each sample size tested (A, C). Because baseline prevalences of pneumococcal carriage will differ between study sites, the final number needed to enroll is plotted against increasing baseline prevalence for powers of 0.8 and 0.9 to detect minimum-effect sizes (i.e., increases in bacterial titers from baseline) of 1.5-fold (C) and 2-fold (D). CI, confidence interval.

pneumococcal nasopharyngeal samples (9), we devised and provide here (Fig. 1) recommendations for sample sizes sufficient to evaluate effects of LAIV on bacterial carriage density. In particular, we provide sample sizes required to detect 1.5-fold changes (50% increases) and 2-fold changes (100% increases) in bacterial density from baseline density, which we feel represent the lower end of what may be clinically relevant, particularly with regard to potential for increased transmission (10; see below).

We detect, for example, a minimum sample size of 92 to achieve 80% power to detect a 2-fold (100%) increase in bacterial density, given a 60% baseline prevalence of pneumococcal carriage (Fig. 1A and B), which is approximately the baseline prevalence for the data presented by Thors et al. (8) (96/151) and which suggests that the study was well designed to detect a 100% increase. Figure 1 also demonstrates that the lack of statistical significance in the 50%-increased density ($P = 0.10$) 7 days after LAIV administration reported by Thors et al. was possibly due to insufficient power to detect only a 1.5-fold increase in bacterial titers, rather than a spurious increase that occurred purely by chance (i.e., the sample size required to achieve 80% power to detect a 50% increase given a baseline prevalence of 64% is 340, over twice the sample size of their study).

These nontrivial power calculations should aid in future investigations by providing a guideline to ensure that studies evaluating effects of LAIV on bacterial densities using individual-level data are not underpowered.

Besides enrolling appropriate sample sizes, future investigations should also consider appropriate sampling intervals when allocating resources. For example, the mouse data reported by us suggest that the time of greatest increased bacterial titers following LAIV (and wild-type virus) administration occurs over a period of days, not weeks. In mice, this excess occurs primarily between days 4 and 12 after LAIV administration (1, 5), but the same timeline cannot be assumed for humans. Indeed, Thors et al. found greater increases in bacterial titers at 28 days (225%) than at 7 days (50%) postvaccination. However, given the lack of sampling within this interval, which spans 3 weeks, a much larger increase in bacterial density may have been missed, in which case, these two samples might represent just the tails of an increased density curve. Thus, an ideal study with unlimited resources and very patient children might sample, at a minimum, at days 0, 7, 14, 21, 28, and 35 after LAIV administration.

This basic study design could be enhanced significantly to simultaneously address effects of LAIV on bacterial acquisition (a common consequence of wild-type influenza virus infections (11)). This can most easily be achieved by separating participants into two study arms, with vaccination between the two arms staggered at least 35 (but preferably more) days apart. Sampling in the lagging arm prior to vaccination should then occur at the same intervals as in the initially vaccinated arm postvaccination. In this design, comparing bacterial acquisition rates between the two arms during the initial 35 days (i.e., one arm receiving LAIV at day 0 and the other arm receiving the vaccine at day 35) can assess whether differences in bacterial acquisition between LAIV-administered subjects and contemporaneous controls exist. Note that in this design, analysis methods must consider correlated data (i.e., at the individual or household levels). Analyses of acquisition can be enhanced considerably further by determination of pneumococcal capsular serotypes, and serotype-specific data will enable detection of interactions between specific serotypes and effects of LAIV on bacterial densities.

As Coelingh and Belshe point out, LAIV has been proven to be safe and effective in numerous trials enrolling tens of thousands of children and even adults. However, given the strong emphasis of vaccine research on individual recipients, no trial has assessed the potential impacts of immunization on contacts of vaccine recipients with regard to a pathogen that is distinct from the vaccine target pathogen. We agree that the LAIV is safe and highly effective at the individual vaccine level, even when altered bacterial carriage densities are considered. Indeed, even within our highly controlled mouse system, increased carriage density following LAIV administration was not associated with increased lower respiratory tract disease and may have even reduced severe bacterial disease (1). However, bacterial carriage begets bacterial transmission, and even relatively small increases in bacterial carriage density may increase bacterial transmission to bystander individuals (10, 12), a finding that has been shown in numerous mouse and ferret models of pneumococcal transmission (10, 13), as well as in studies looking at transmission of other pathogens (14, 15).

In the context of LAIV, one could envision a situation whereby a vaccinated individual with elevated bacterial carriage titers may not himself or herself be susceptible to bacterial disease but in-

stead may act as a reservoir for increased transmission. Such a scenario might be particularly important, for example, between young children and grandparents or other elder individuals already having increased susceptibility to pneumococcal disease (12). Additionally, although LAIV is not recommended for immunocompromised individuals, an unintended LAIV effect of increased bacterial transmission might have an important impact on bacterial disease in immunocompromised contacts.

Studies aiming to determine bacterial transmission among humans are difficult and expensive to perform, requiring longitudinal sampling of contacts surrounding, in this case, the LAIV-administered individual. While bacterial-transmission studies are not without precedent (16, 17), they always require large resources and extensive sampling efforts. Given the immense resources required to properly assess transmission level effects, it is reasonable to hold off on such large studies. However, further evidence of increased bacterial density within the nasopharynx as a consequence of LAIV administration in humans will indicate the need for these types of studies in order to fully understand unintended consequences of LAIV administration on bacterial-pathogen dynamics.

The findings put forth in our initial report for mice (1) bring to light an important topic that should be further evaluated for live influenza vaccines and should lead to additional consideration of the broader aims of vaccine-monitoring programs. Current vaccine safety programs aim to ensure protection at the individual level, in particular, to confirm that vaccination causes no enhanced susceptibility to the pathogens targeted by vaccination and that vaccination has no immunologically relevant adverse events. Given the increased awareness of, and ability to understand, multispecies pathogen interactions within the host (which are often mediated by host immune processes), it is incumbent upon us to consider not only individual-level pathogen-specific vaccine safety and efficacy but also unintended consequences of vaccines on the dynamics of unrelated pathogens. While this task is daunting, the signals to be investigated can be narrowed significantly by focusing on species already known to interact with the naturally circulating pathogen to be vaccinated against. For example, there is perhaps no better-understood multispecies pathogen interaction than that among influenza viruses, pneumococcal bacteria, and the human or animal host. Thus, to determine if a live attenuated influenza vaccine was able to impact a pathogen that is unrelated to the targeted influenza virus, we simply started with the pathogen most known to interact with naturally circulating influenza viruses, i.e., the pneumococcus (1). After the pneumococcus, we then looked at what else has been shown in the literature to interact with influenza virus, namely, *Staphylococcus aureus*. By looking at these interactions in mice, we were able to quickly identify two interactions that can be and, as described above, are being directly evaluated in humans.

The Vaccine Adverse Event Reporting System (VAERS) was set up to monitor postlicensure and postmarketing safety of vaccines and emphasizes sensitivity over specificity to catch unintended and rare events that may occur at the level of the vaccinated individual but that may have gone undetected in premarketing trials (18). Systems such as VAERS are effective tools to detect any number of adverse conditions within a vaccinated individual, be they from adverse immunologic responses, increased incidence of infection, or even potential secondary infections (18). However, by focusing on the individual, most prelicensure and pre- and post-marketing studies and monitoring systems do not detect potential adverse or unintended effects of vaccines that may (i) be subclinical (e.g., increased carriage density without disease) or (ii) occur as a result of increased transmission of the vaccine target pathogen to unvaccinated individuals, much less a pathogen that is phylogenetically distinct from the vaccine target. Thus, such effects would go entirely undetected unless specifically analyzed. Further, if seasonality of the second pathogen coincides with a rollout of vaccination, as is the case with annual influenza vaccinations and the seasonality of pneumococcal disease, special care is required to account for this seasonality of the secondary pathogen so that a potential signal is not lost.

We recognize that this is a new field; indeed, our study is among the very first to suggest the existence of cross-species effects of vaccination on unrelated pathogens. Nevertheless, as we progress into this new millennium, vaccine programs will increase in both size and scope in order to ensure the right to health and wellbeing of all individuals. The growth in these programs must inspire and be equally met by new methods and technological innovations able to ensure that the highest standards of vaccine safety and effectiveness are maintained across the all spectrums of potential effects (19). By all metrics, vaccines are among our most successful and effective tools to prevent disease by infectious pathogens, so let us ensure that our tools to assess them continue to be equally robust and effective.

REFERENCES

1. **Mina MJ, McCullers JA, Klugman KP.** 2014. Live attenuated influenza vaccine enhances colonization of *Streptococcus pneumoniae* and *Staphylococcus aureus* in mice. mBio 5(1):e01040-13. http://dx.doi.org/10.1128/mBio.01040-13.
2. **Coelingh KL, Belshe RB.** 2014. No clinical association of live attenuated influenza vaccine with nasal carriage of bacteria or acute otitis media. mBio 5(2):e01145-14. (Letter to the Editor.) http://dx.doi.org/10.1128/mBio.01145-14.
3. **McCullers JA.** 2014. The co-pathogenesis of influenza viruses with bacteria in the lung. Nat. Rev. Microbiol. 12:252–262. http://dx.doi.org/10.1038/nrmicro3231.
4. **Toback SL, Ambrose CS, Eaton A, Hansen J, Aukes L, Lewis N, Wu X, Baxter R.** 2013. A postlicensure evaluation of the safety of Ann Arbor strain live attenuated influenza vaccine in children 24–59 months of age. Vaccine 31:1812–1818. http://dx.doi.org/10.1016/j.vaccine.2013.01.055.
5. **Mina MJ, Klugman KP, McCullers JA.** 2013. Live attenuated influenza vaccine, but not pneumococcal conjugate vaccine, protects against increased density and duration of pneumococcal carriage after influenza infection in pneumococcal colonized mice. J. Infect. Dis. 208:1281–1285. http://dx.doi.org/10.1093/infdis/jit317.
6. **Tessmer A, Welte T, Schmidt-Ott R, Eberle S, Barten G, Suttorp N, Schaberg T, CAPNETZ Study Group.** 2011. Influenza vaccination is associated with reduced severity of community-acquired pneumonia. Eur. Respir. J. 38: 147–153. http://dx.doi.org/10.1183/09031936.00133510.
7. **Thors V, Morales-Aza B, Christensen H, Vipond B, Muir P, Finn A.** 2014. Live attenuated intranasal influenza vaccine does not increase rates or density of nasal pneumococcal colonisation in healthy children, abstr ISPPD-0514. Pneumonia (Special issue. 9th International Symposium on Pneumococci and Pneumococcal Diseases, Hyderabad, India, 9 to 13 March 2014.) 3:26.
8. **Thors V, Morales-Aza B, Oliver E, Christensen H, Vipond B, Muir P, Finn A.** 2014. Live attenuated intranasal vaccine does not increase rates of nasal pneumococcal colonisation in healthy children but may contribute to increased density, poster P-097. 9th Int. Symp. Pneumococci Pneumococcal Dis., Hyderabad, India, 9 to 13 March 2014. http://isppd.meetingxpert.net/swf/poster_viewer.aspx.
9. **Chien YW, Vidal JE, Grijalva CG, Bozio C, Edwards KM, Williams JV, Griffin MR, Verastegui H, Hartinger SM, Gil AI, Lanata CF, Klugman KP.** 2013. Density interactions among *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Staphylococcus aureus* in the nasopharynx of young

Peruvian children. Pediatr. Infect. Dis. J. **32:**72–77. http://dx.doi.org/10.1097/INF.0b013e318270d850.

10. **Short KR, Reading PC, Wang N, Diavatopoulos DA, Wijburg OL.** 2012. Increased nasopharyngeal bacterial titers and local inflammation facilitate transmission of *Streptococcus pneumoniae*. mBio **3**(5):e00255-12. http://dx.doi.org/10.1128/mBio.00255-12.

11. **Grijalva CG, Griffin MR, Edwards KM, Williams JV, Gil AI, Verastegui H, Hartinger SM, Vidal JE, Klugman KP, Lanata CF.** 12 March 2014. The role of influenza and parainfluenza infections in nasopharyngeal pneumococcal acquisition among young children. Clin. Infect. Dis. http://dx.doi.org/10.1093/cid/ciu148.

12. **Simell B, Auranen K, Käyhty H, Goldblatt D, Dagan R, O'Brien KL, Pneumococcal Carriage Group.** 2012. The fundamental link between pneumococcal carriage and disease. Expert Rev. Vaccines **11:**841–855. http://dx.doi.org/10.1586/erv.12.53.

13. **McCullers JA, McAuley JL, Browall S, Iverson AR, Boyd KL, Henriques Normark B.** 2010. Influenza enhances susceptibility to natural acquisition of and disease due to *Streptococcus pneumoniae* in ferrets. J. Infect. Dis. **202:**1287–1295. http://dx.doi.org/10.1086/656333.

14. **Lowen AC, Mubareka S, Tumpey TM, García-Sastre A, Palese P.** 2006. The guinea pig as a transmission model for human influenza viruses. Proc. Natl. Acad. Sci. U. S. A. **103:**9988–9992. http://dx.doi.org/10.1073/pnas.0604157103.

15. **Mubareka S, Lowen AC, Steel J, Coates AL, García-Sastre A, Palese P.** 2009. Transmission of influenza virus via aerosols and fomites in the guinea pig model. J. Infect. Dis. **199:**858–865. http://dx.doi.org/10.1086/597073.

16. **Melegaro A, Gay NJ, Medley GF.** 2004. Estimating the transmission parameters of pneumococcal carriage in households. Epidemiol. Infect. **132:**433–441. http://dx.doi.org/10.1017/S0950268804001980.

17. **Shiri T, Auranen K, Nunes MC, Adrian PV, van Niekerk N, de Gouveia L, von Gottberg A, Klugman KP, Madhi SA.** 2013. Dynamics of pneumococcal transmission in vaccine-naive children and their HIV-infected or HIV-uninfected mothers during the first 2 years of life. Am. J. Epidemiol. **178:**1629–1637. http://dx.doi.org/10.1093/aje/kwt200.

18. **Zhou W, Pool V, Iskander JK, English-Bullard R, Ball R, Wise RP, Haber P, Pless RP, Mootrey G, Ellenberg SS, Braun MM, Chen RT.** 2003. Surveillance for safety after immunization: Vaccine Adverse Event Reporting System (VAERS)—United States, 1991–2001. MMWR Surveill. Summ. **52:**1–24. http://www.cdc.gov/mmwr/preview/mmwrhtml/ss5201a1.htm.

19. **Ward BJ.** 2000. Vaccine adverse events in the new millennium: is there reason for concern? Bull. World Health Organ. **78:**205–215. http://www.who.int/bulletin/archives/78(2)205.pdf.