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Letter to the Editor

What Are the Risks—Hypothetical and Observed—of Recombination Involving Live Vaccines and Vaccine Vectors Based on Nonsegmented Negative-Strain RNA Viruses?

Newcastle disease virus (NDV) is a nonsegmented negative-strand RNA virus (NNSV) that is being developed as a potential vaccine vector for use in poultry (7, 12, 17) and humans (5, 6). The primary proposed human use would be to express protective antigens of highly pathogenic agents for outbreak control. We noted (1) that one of the advantages of NDV is that "gene exchange seems to be rare for nonsegmented negative strand RNA viruses, with few reported instances. This differs to the frequent gene reassortment observed for segmented viruses, such as influenza virus and rotavirus, and the high frequency of recombination observed for certain viruses, such as coronavirus and poliovirus." In a recent letter to the editor (9), Han et al. criticized our studies, suggesting that we (i) dismissed the possibility of recombination, (ii) failed to recognize the potentially adverse consequences of recombination, and (iii) did not experimentally address the potential for the instability of the inserted foreign gene.

There indeed is evidence consistent with NNSV recombination yielding mosaic virus, but it seems to be much less frequent than for the recombinogenic viruses noted above (4). There have been many attempts to demonstrate genetic exchange between NNSVs in vitro, with only a single reported clear example of a resulting mosaic virus (13, 16). There also is indirect evidence consistent with NNSV recombination in nature, based on the occasional discovery of a virus with sequence discontinuity suggestive of a recombination breakpoint (4, 10, 11, 14, 15, 18, 20). This discovery has involved closely related viruses, has not been reported to involve NNSVs with substantial sequence differences, and seems to be an infrequent event compared to its occurrence in the other viruses noted above.

History provides an extensive safety record supporting the view that NNSV genetic exchange is not an important practical concern for vaccinology. A variety of live, attenuated NNSV vaccines are in veterinary, agriculture, and human use, such as NDV in poultry and mumps and measles viruses in humans. Indeed, the mumps and measles vaccines often are administered in combination, as are certain veterinary NNSV vaccines. We agree that recombination may sometimes occur between a vaccine virus and its circulating wild-type counterpart. There is suggestive evidence that this has occurred between the NDV vaccine virus and a circulating NDV strain (8), although we know of no similar evidence for mumps or measles virus. Recombination yields a virus with a mosaic genome containing sections from the parents. This virus likely would exhibit a virulence phenotype resembling one parent or the other or something in between. It thus does not create a hazard exceeding that of the circulating wild-type virus already present. This is supported by the history of vaccine use: despite long-standing worldwide use of a number of live NNSV vaccines, there have been no reported adverse events attributable to genetic exchange involving an NNSV vaccine. There have been no reports of untoward vaccine-related NNSVs emerging with novel clinical or environmental footprints. Whatever low risk is posed by NNSV genetic exchange, that risk already exists in nature and was not created by the use of live vaccines. Indeed, the restricted infectivity and restricted replication characteristic of an attenuated vaccine strain limit its opportunity to participate in the dual infections necessary for genetic exchange. In addition, the herd immunity induced by an effective vaccine reduces the circulating virus, thereby reducing the opportunity for recombination (15). We believe that these considerations indicate that genetic exchange does not pose a realistic safety concern for the use of live NNSV-based vaccines.

Regarding the specific use of NDV as a vaccine vector in humans, the possibility of recombination is even more remote, and its implications are no more dire. Natural infection of humans with NDV is not common, and NDV is highly restricted for replication in primates (1, 2). This reduced infectivity and reduced replication greatly reduces the opportunity for genetic exchange. Furthermore, even if a recombinant virus is generated, it would remain highly restricted for replication and spread, since both parents, in this case, the NDV vector and a naturally circulating NDV strain, are restricted to humans. Any recombinant virus would also be subject to neutralization by the burgeoning host immune response. In addition, NDV has only a low level of sequence relatedness with potential circulating human NNSVs; as already noted, recombination between dissimilar NNSVs has not been reported and presumably is rare indeed. We note that expressed foreign proteins in NNSVs do not appear to enhance the virulence of the vector or shift its tropism but rather seem to be moderately attenuating (3, 19). Obviously, one must confirm this for each vector/insert combination, using both avian and mammalian experimental species. However, if the foreign inserted gene is indeed silent with regard to virulence, its effect in any recombinants would be neutral or attenuating. Finally, regarding concern about the potential instability of the inserted foreign gene, a number of studies have indicated that inserts borne by NNSVs are surprisingly stable (3). The inserts have been shown to gradually accumulate point mutations that may inactivate the expression of the protein but do not introduce safety concerns. In any event, sequence integrity would be monitored during vaccine production and use in humans.

Situations can be envisioned where potential genetic exchange between certain live vaccines and circulating viruses might be of concern. For example, the use of a live influenza virus vaccine bearing one or more gene segments from a highly pathogenic strain of avian influenza virus has the potential to introduce these genes into other circulating human viruses by reassortment. The use of an attenuated version of the severe acute respiratory syndrome coronavirus as a vaccine might result in recombination with circulating animal or human coronaviruses to create novel mosaic viruses. In contrast, with an NDV vector, the foreign gene is locked in the NDV nucleocapsid and is replicated and expressed by the NDV polymerase. Thus, an inserted avian influenza virus or coronavirus gene in an NDV vector is not situated to participate in influenza virus-mediated reassortment or coronavirus-mediated recombination. This is an important safety advantage.

Vaccines represent one of the great successes of science, have saved countless lives, and have removed one terrible pathogen from circulation and brought others under control. Paradoxically, there is considerable public resistance to vaccines despite their successes and despite the possibility of wider outbreaks of human infections with avian influenza virus and other emerging viruses as well as the potential for bioterrorism. It is imperative to not make the situation worse with vague and unsubstantiated calls of alarm. Genetic instability and genetic exchange are inescapable attributes of viruses, but as argued above, they did not begin with live vaccines and do not necessarily compromise vaccine safety. At this time, there is no realistic basis for suggesting that recombination by NDV is a significant safety problem either for its present use as a live vaccine or for its potential use as a vaccine vector.

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REFERENCES

1. **Bukreyev, A., and P. L. Collins.** 2008. Newcastle disease virus as a vaccine vector for humans. Curr. Opin. Mol. Ther. **10:**46–55.
2. **Bukreyev, A., Z. Huang, L. Yang, S. Elankumaran, M. St. Claire, B. R. Murphy, S. K. Samal, and P. L. Collins.** 2005. Recombinant Newcastle disease virus expressing a foreign viral antigen is attenuated and highly immunogenic in primates. J. Virol. **79:**13275–13284.
3. **Bukreyev, A., M. H. Skiadopoulos, B. R. Murphy, and P. L. Collins.** 2006. Nonsegmented negative-strand viruses as vaccine vectors. J. Virol. **80:**10293–10306.
4. **Chare, E. R., E. A. Gould, and E. C. Holmes.** 2003. Phylogenetic analysis reveals a low rate of homologous recombination in negative-sense RNA viruses. J. Gen. Virol. **84:**2691–2703.
5. **DiNapoli, J. M., A. Kotelkin, L. Yang, S. Elankumaran, B. R. Murphy, S. K. Samal, P. L. Collins, and A. Bukreyev.** 2007. Newcastle disease virus, a host range-restricted virus, as a vaccine vector for intranasal immunization against emerging pathogens. Proc. Natl. Acad. Sci. USA **104:**9788–9793.
6. **DiNapoli, J. M., L. Yang, A. Suguitan, Jr., S. Elankumaran, D. W. Dorward, B. R. Murphy, S. K. Samal, P. L. Collins, and A. Bukreyev.** 2007. Immunization of primates with a Newcastle disease virus-vectored vaccine via the respiratory tract induces a high titer of serum neutralizing antibodies against highly pathogenic avian influenza virus. J. Virol. **81:**11560–11568.
7. **Ge, J., G. Deng, Z. Wen, G. Tian, Y. Wang, J. Shi, X. Wang, Y. Li, S. Hu, Y. Jiang, C. Yang, K. Yu, Z. Bu, and H. Chen.** 2007. Newcastle disease virus-based live attenuated vaccine completely protects chickens and mice from lethal challenge of homologous and heterologous H5N1 avian influenza viruses. J. Virol. **81:**150–158.
8. **Han, G. Z., C. Q. He, N. Z. Ding, and L. Y. Ma.** 2008. Identification of a natural multi-recombinant of Newcastle disease virus. Virology **371:**54–60.
9. **Han, G.-Z., X.-P. Liu, and S.-S. Li.** 2008. Caution about Newcastle disease virus-based live attenuated vaccine. J. Virol. **82:**6782.
10. **Han, G. Z., X. P. Liu, and S. S. Li.** 2008. Cross-species recombination in the haemagglutinin gene of canine distemper virus. Virus Res. **136:**198–201.
11. **McCarthy, A. J., M. A. Shaw, and S. J. Goodman.** 2007. Pathogen evolution and disease emergence in carnivores. Proc. Biol. Sci. **274:**3165–3174.
12. **Park, M. S., J. Steel, A. Garcia-Sastre, D. Swayne, and P. Palese.** 2006. Engineered viral vaccine constructs with dual specificity: avian influenza and Newcastle disease. Proc. Natl. Acad. Sci. USA **103:**8203–8208.
13. **Pringle, C. R.** 1982. The genetics of vesiculoviruses. Arch. Virol. **72:**1–34.
14. **Qin, Z., L. Sun, B. Ma, Z. Cui, Y. Zhu, Y. Kitamura, and W. Liu.** 2008. F gene recombination between genotype II and VII Newcastle disease virus. Virus Res. **131:**299–303.
15. **Schierup, M. H., C. H. Mordhorst, C. P. Muller, and L. S. Christensen.** 2005. Evidence of recombination among early-vaccination era measles virus strains. BMC Evol. Biol. **5:**52.
16. **Spann, K. M., P. L. Collins, and M. N. Teng.** 2003. Genetic recombination during coinfection of two mutants of human respiratory syncytial virus. J. Virol. **77:**11201–11211.
17. **Veits, J., D. Wiesner, W. Fuchs, B. Hoffmann, H. Granzow, E. Starick, E. Mundt, H. Schirrmeier, T. Mebatsion, T. C. Mettenleiter, and A. Romer-Oberdorfer.** 2006. Newcastle disease virus expressing H5 hemagglutinin gene protects chickens against Newcastle disease and avian influenza. Proc. Natl. Acad. Sci. USA **103:**8197–8202.
18. **Wittmann, T. J., R. Biek, A. Hassanin, P. Rouquet, P. Reed, P. Yaba, X. Pourrut, L. A. Real, J. P. Gonzalez, and E. M. Leroy.** 2007. Isolates of Zaire ebolavirus from wild apes reveal genetic lineage and recombinants. Proc. Natl. Acad. Sci. USA **104:**17123–17127.
19. **Yang, L., A. Sanchez, J. M. Ward, B. R. Murphy, P. L. Collins, and A. Bukreyev.** 2008. A paramyxovirus-vectored intranasal vaccine against Ebola virus is immunogenic in vector-immune animals. Virology **377:**255–264.
20. **Zheng, H., G. A. Storch, C. Zang, T. C. Peret, C. S. Park, and L. J. Anderson.** 1999. Genetic variability in envelope-associated protein genes of closely related group A strains of respiratory syncytial virus. Virus Res. **59:**89–99.

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Ed. Note: *There is no reply to this letter.*