COMMENTARY

NS1 is the fluid for "flu-transmission"

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The development of modern medicine has allowed us to conquer numerous infectious diseases; however, we human beings constantly face threats from novel infectious diseases that have been previously unrecognized. These so-called "emerging infectious diseases" are often caused by zoonotic pathogens, which mostly originate in wild animals (1, 2). Human diseases, such as AIDS, severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), Ebola viral disease, and pandemic influenza are all caused by such pathogens. To cause zoonosis, the pathogens that originate in animals must cross the species barrier and transmit to humans. If these pathogens are able to efficiently transmit from human to human, a pandemic would result, endangering the lives of humans globally.

Aquatic wild birds harbor a large gene pool of influenza A viruses that have been the source of influenza pandemics. Although influenza A viruses can infect a wide range of species, host restriction usually constrains their interspecies transmission; however, some mammalian-adaptive mutations have been identified in hemagglutinin (HA) and Polymerase Basic 2 (PB2) that allow avian influenza viruses to overcome the species barrier and become transmissible via the airborne route among ferrets (3). In addition, PB1 has been shown to confer to airborne transmission to H5N1 viruses (4). For over 25 y, Webster's group has conducted surveillance of avian viruses at Delaware Bay, New Jersey, and has investigated the biological properties of the isolated H1N1 avian viruses in mammalian models (5, 6). Surprisingly, some of the H1N1 avian isolates transmitted via the airborne route in a ferret model without prior adaptation (5–7), suggesting no adaptive mutations were required for these viruses to become transmissible. By comparing the genomes of the transmissible and nontransmissible viruses, Zanin et al. (7) identify differences in the PB2, PB1, PB1-F2, PA-X, NS1, and NEP genes that are potentially associated with airborne transmissibility. A loss-of-function study revealed the potential role of an amino acid substitution at position 213 of the NS1 protein in the airborne transmission, although this substitution alone was not sufficient for airborne transmissibility (Fig. 1). The findings of Zanin et al. that avian H1N1 viruses isolated from wild birds can be airborne-transmissible in mammals without prior adaptation raises an alarm over the pandemic potential of avian H1N1 viruses circulating in wild birds in North America, and highlights the importance of continuous surveillance of avian viruses to monitor genetic markers of transmissibility. Understanding of the molecular mechanisms of pathogenicity and transmissibility of avian viruses is important for preventing future pandemics by proactively mitigating risk through identification and control of pandemic potential-bearing influenza viruses in nonhuman animals.

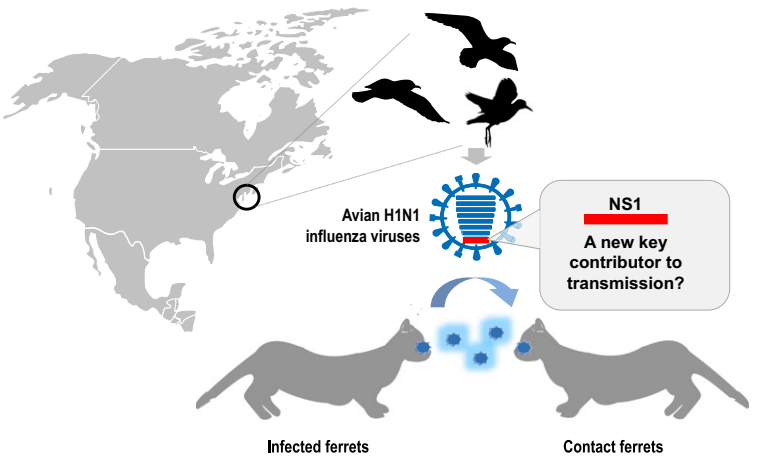

Fig. 1. Aquatic birds are a major natural reservoir for influenza A viruses. Some avian H1N1 influenza viruses isolated from aquatic wild birds in Delaware Bay, NJ, were transmitted via the airborne route among ferrets without prior adaptation. NS1 was found to play a key role in the airborne transmission of the viruses in mammals.

When the extent of virus replication in the upper respiratory tracts of mammals is low, transmission

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generally does not occur. NS1 protein functions as a major antagonist of the antiviral host responses and is required for efficient viral replication in IFN-competent cells. Zanin et al. (7) generated two recombinant viruses, which possess serine or proline at position 213 in NS1 in the background of an avian H1N1 isolate, which can be transmitted via the airborne route among ferrets. The authors show that the recombinant virus carrying NS1-213S transmitted via the airborne route among ferrets, whereas the recombinant virus carrying NS1-213P failed to transmit. They found that the airborne-transmissible virus carrying NS1-213S replicated more efficiently than nontransmissible viruses in the upper respiratory tract of ferrets. Interestingly, the NS1-213S of the airborne-transmissible virus was less able to prevent retinoic acid-inducible gene 1-mediated IFN-β promoter activation than the NS1-213P of the nontransmissible virus. These findings suggest that the efficient replication of the airborne transmissible virus in the upper respiratory tract of mammals may be unrelated to the suppression of IFN-β induction, which is one of the established functions of NS1. NS1 exhibits multiple functions in infected cells during the life cycle of the virus (8); one of these other known functions, or perhaps an as yet uncharacterized function of NS1, may be responsible for the enhanced viral replication associated with the airborne transmissibility of avian influenza viruses among mammals.

Although Zanin et al. (7) focus on the role of NS1 in transmissibility in their study, other viral proteins may also contribute to transmissibility in concert with NS1 given that the authors' data suggested that NS1 is important, but not sufficient, for airborne transmission. The receptor-binding specificity of HA is thought to be a key determinant for efficient transmission in humans. HAs from human strains preferentially bind glycans that terminate with a sialic acid linked to galactose by α2,6-linkages ("human-type" receptors) on the cell surface; in contrast, avian virus HAs preferentially bind to glycans that terminate with sialic acid linked to galactose by α2,3-linkages ("avian-type" receptors) (9). Therefore, a shift in HA from avian- to human-type receptor specificity is thought to be critical for avian viruses to have enhanced replication and efficient transmission in humans. The airborne-transmissible viruses used in the study by Zanin et al. (7) do not have E190D and D225G (H3 numbering) mutations, which are associated with the shift from avian- to human-type receptor recognition of H1 HAs. However, the authors' previous work demonstrated that the airborne-transmissible avian H1N1 viruses have dual avian- and human-type receptor specificity, yet viruses recovered from contact ferrets bound mainly to human-type receptors even though they acquired no further mutations, as determined by Sanger sequencing (6). It would be interesting to deep sequence viruses collected from contact ferrets to investigate viral quasi-species, and identify novel amino acids responsible for receptor specificity.

Recent studies suggest that the airborne transmission of influenza viruses in mammals requires that HA proteins develop a certain level of tolerance to changes in pH to maintain viral infectivity. Viral particles bound to the cell surface are transported to endosomes. The low pH inside the endosomes triggers an irreversible conformational change in the HA (10) to induce viral fusion with the endosomal membranes, leading to the release of the ribonucleoprotein complexes into the cytoplasm. Premature exposure of the virus to the low pH in the extracellular environment results in a loss of viral infectivity because this irreversible conformational change in HA occurs too soon, leading to the generation of nonfunctional HA. Similarly, heat treatment at neutral pH also promotes the acidic form of HA and serves as a surrogate for HA stability (11). In the HA of avian H5N1 viruses, mutations conferring human-type receptor recognition have been shown to raise the pH threshold for membrane fusion activity and reduce thermal stability (4, 12, 13). Notably, H5N1 viruses carrying such mutations failed to transmit among ferrets, likely due to inactivation of the HA protein in the environment. However, mammalian-adaptive mutations in HA identified in airborne-transmissible H5N1 viruses increased HA stability while accommodating mutations that allow binding to human-type receptors. Similar findings have been reported with H7N9 viruses (14). These observations suggest that, in some subtypes of avian influenza virus, viral fitness is markedly reduced by the acquisition of human-type receptor-binding specificity and that its restoration requires compensatory mutations.

The findings of Zanin et al. that avian H1N1 viruses isolated from wild birds can be airborne-transmissible in mammals without prior adaptation raises an alarm over the pandemic potential of avian H1N1 viruses circulating in wild birds in North America.

Many questions must be answered before we can fully understand the molecular mechanisms of transmission of avian influenza viruses in mammals. For example, is the NS1 requirement identified in this study applicable to other viruses? Moreover, which viral factors are required for airborne transmission in addition to NS1? Furthermore, when mutations, such as E190D and D225G, are introduced into the HA of the airborne-transmissible viruses with dual receptor specificity that were found in this study (7), how do the mutated HA alter the biological properties of these viruses (e.g., receptor-binding specificity, HA stability, and transmissibility in ferrets)?

However, despite the importance of such studies, influenza researchers are prevented from performing them by a pause on gain-of-function (GOF) research by the United States government, as explained in the Zanin et al. article (7). A debate began in 2011 before the publication of two H5N1 GOF ferret transmission studies by Herfst et al. (15) and Imai et al. (12). These two independent groups identified specific amino acid substitutions in HA that, in combination with lysine at position 627 of PB2, were required for transmission via the airborne route in a ferret model, by utilizing a GOF strategy (i.e., testing the transmissibility of genetically modified H5N1 viruses possessing the amino acid residues responsible for transmission). These H5N1 GOF transmission studies created controversy: some argued that the benefits obtained from such GOF studies outweighed the potential risk (16–18), while others felt that the benefits for public health did not justify this type of research (19, 20). The outcome has been a moratorium on funding certain types of GOF research on influenza, SARS, and MERS viruses in the United States since 2014 (21). However, we, as researchers must stress the importance of GOF research: results from GOF studies would almost certainly help in understanding the pandemic potential of influenza viruses and produce public health benefits, such as the prioritization and development of prepandemic vaccines and antiviral drugs. Fundamental GOF research on transmissibility, host-range restriction, drug resistance, immunogenicity, pathogenicity, and replicative ability would also benefit global public health. Therefore, we hope that these essential GOF projects can resume soon.

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