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A New Determinant of H5N1 Influenza Virus Pathogenesis in Mammals

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In this issue of the *Journal of Virology* (JVI), we publish a paper by Hassan Zaraket, Olga A. Bridges, and Charles J. Russell from St. Jude Children's Research Hospital reporting a sequence polymorphism in the avian H5N1 influenza virus hemagglutinin (HA) protein that regulates the pH optimum of viral membrane fusion and virulence in mice (1). The authors studied four H5N1 viruses in the A/chicken/Vietnam/C58/04 (H5N1) background with progressively lower pH values of HA activation required to trigger fusion. The main finding is that, in comparison to the wild-type virus, a mutant virus with a lysine-to-isoleucine substitution at residue 58 (K58I) in the HA2 subunit, while attenuated in ducks, is more virulent in DBA/2J mice. These data contribute new information about viral determinants of influenza virus virulence and provide additional evidence to support the idea that H5N1 influenza virus pathogenesis in birds and mammals is linked to the pH of HA activation in an opposing fashion. A higher pH optimum of HA activation favors virulence in birds, whereas a lower pH optimum of HA activation favors virulence in mammals.

We think this study makes important contributions. The results define a determinant of influenza virus host range and pinpoint a mechanism for host-specific differences in viral replication. Knowledge of sequence changes that regulate the host range of influenza virus also may provide clues about barriers to interspecies transmission and offer a rationale for enhanced surveillance. In the case of H5N1 influenza virus, several independent sequence polymorphisms are associated with a decreased HA activation pH, including the K58I polymorphism in HA2 studied by Zaraket et al. (1) and a threonine-to-isoleucine substitution at residue 318 (T318I) in the HA1 subunit reported by Imai et al. (2). Thus, based on these studies, surveillance should include phenotypic assessment of the HA activation pH in addition to sequence analysis. Furthermore, introduction of HA2 K58I into seed stocks used to prepare H5N1 influenza vaccines in mammalian cells may enhance replication efficiency and improve vaccine yields.

Given the potential concerns about enhancing the virulence and host range of H5N1 influenza virus, the authors incorporated several features into the experimental design to mitigate risk (1). First, the A/chicken/Vietnam/C58/04 (H5N1) parental strain, while highly pathogenic in ducks, is limited in pathogenicity in mice and ferrets, with virulence similar to that of currently circulating H1N1 viruses. Second, the C58 virus and the mutants used in this study display avian (α2,3-linked sialic acid) and not human (α2,6-linked sialic acid) receptor-binding specificity and express a PB2 polymerase that is inefficient in mammals. Third, the C58 virus is susceptible to oseltamivir and antigenically matched to an A/Vietnam/1203/04 (H5N1) experimental vaccine. Fourth, mutant viruses were not selected during the experimental infections in mice. Fifth, all experiments in this study were conducted in an enhanced animal biosafety level 3 laboratory that is select agent approved and routinely inspected by both institutional biosafety and USDA officials. Finally, and importantly, addition of the K58I mutation in HA2 to the T318I mutation in HA1 is likely to decrease the pH optimum of HA activation even further to a level that would compromise viral fitness (3, 4). Therefore, combining the mutations that alter the pH optimum of HA activation is unlikely to augment virulence.

Prior to submission of this paper to JVI, the study was evaluated for the possibility of dual-use research of concern (DURC) by the St. Jude Children's Research Hospital Institutional Biosafety Committee (St. Jude IBC) and the National Institute of Allergy and Infectious Diseases (NIAID). As described in the United States Government policy for oversight of life sciences (5), DURC is defined as "Life sciences research that, based on current understanding, can be reasonably anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, materiel, or national security" (5). The St. Jude IBC evaluation involved review of the manuscript by the committee and a face-to-face meeting with C. J. Russell. The NIAID evaluation involved review of the manuscript by NIAID influenza program staff, a DURC review group, and institute leadership.

Evaluation by both groups for the possibility of DURC consisted of three sequential steps, each asking a specific question. First, does the work involve one of the 15 agents and toxins specified by the U.S. Government DURC policy? In this case, H5N1 influenza virus is one of the 15 agents and toxins. Second, does the work involve one of the seven listed experiments (or "effects")? Again, in this case, increasing the virulence and host range of H5N1 influenza virus constitutes two of the seven effects. Third, does the resulting knowledge, information, products, or technologies meet the definition of DURC as defined in the policy? Both the St. Jude IBC and NIAID concluded that the study did not meet the definition of DURC, which was relayed to the authors.

The paper was considered for publication by JVI like all others submitted for evaluation. Reviewers were asked to evaluate the

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paper for scientific rigor and significance and to consider whether the research represented DURC. The manuscript also was evaluated for DURC by senior editors at the American Society for Microbiology, which publishes JVI, to determine whether any potential risks of publishing the paper would outweigh the benefits. During this process, the authors, St. Jude IBC leadership, and NIAID representatives were asked for clarification about their adjudication of the DURC issue. Prior to reaching a final decision about publication, ASM convened a teleconference of the authors, editors, St. Jude IBC leadership, and representatives from NIAID and the National Institutes of Health Office of Science Policy to resolve final concerns. Based on the reviews and this discussion, we decided to move forward with publication.

Influenza virus is an exceedingly important human pathogen with the potential to cause worldwide pandemics that threaten hundreds of millions, if not more. Highly pathogenic H5N1 influenza viruses constitute a major health concern, yet significant knowledge gaps exist about these viruses that preclude the deployment of potent therapeutics and vaccines. Reasonable people may disagree about whether the work reported by Zaraket et al. (1) is DURC. We note that a designation of DURC should not necessarily preclude conduct of the research or communication of the findings. The study in this issue of JVI was thoughtfully considered by experts in influenza virus research, biosafety and biosecurity, scientific publication, and the U.S. Government. Following this consideration, we concluded that the benefits of publication outweigh any potential risks. Given the concerns raised about the possibility of DURC in this study, we think that describing the process used to evaluate the manuscript is an important component of communicating the results.

JVI is dedicated to advancing virology by publishing highly significant articles that span the scope of the field. We are committed to responsible publication and will continue to use the editorial DURC review focused on the three-step process to evaluate each manuscript we receive for potential dual-use concerns.

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