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Influenza Challenge Models: Ready for Prime Time?

To the Editor—We wish to thank Dr Memoli and his team for their efforts to revive influenza challenge models. In their recent article they describe a new H3N2 influenza challenge model [1]. We certainly agree that controlled human infection models for influenza and other important infectious diseases can provide critical information for the development of novel and effective drugs and vaccines.

However, we believe controlled human infection models should employ protocols that assess clinical and virological endpoints that mimic the disease target. The current definition of mild to moderate influenza disease (MMID) as requiring only 1 positive viral polymerase chain reaction test (including on Day 1) and the report of only 1 influenza-specific or nonspecific symptom on a single day may not be sufficiently stringent to allow meaningful assessments of novel drugs and/or vaccines [2]. For example, symptoms included in the current MMID definition contain such nonspecific findings as headache, stomachache, nausea, and lack of appetite. Table 2 in their present publication [1] reports that although 6 of 8 (75%) subjects challenged with low doses of H3N2 developed "influenza" symptoms, none shed the virus. This suggests that these symptoms were unrelated to the challenge. In subjects given the higher H3N2 doses, only 55% had a detectable virus by influenza-specific polymerase chain reaction, while the frequencies of clinical symptoms were no higher than the frequencies in the low-dose challenge. Therefore, it is unclear whether the H3N2 challenges resulted in clinically significant influenza infections or whether the symptoms reported truly represent influenza infections. In our experience, subjects housed in challenge units often have headaches, stomachaches, and other nonspecific symptoms even when administered placebos. We are concerned that the use of this illness definition will not provide meaningful assessments of new drugs and/or vaccines or susceptibility to rechallenge [3].

The Memoli group has compared shedders (infected) to nonshedders (no infection) after challenge with their H1N1 challenge strain, and identified differences [4]. However, we suggest that using more stringent definitions of MMID will improve the use of this model. In earlier flu challenge studies, we have required subjects to have a fever (≥100°F) and at least 2 influenza symptoms on 1 or more evaluations, in addition to identifying nasal shedding of the challenge influenza virus strain by culture [5]. Other investigators using influenza challenges have required either multiple symptoms or Grade 2 events for participants to qualify as being ill. Others do not define illness but compare a compilation of signs and symptoms. Similarly, challenge studies of norovirus [6–8] have required specific gastrointestinal symptoms of either diarrhea, vomiting, or more than 1 symptom.

There is a clear need to develop influenza challenge strains that cause more significant illness [9]. However, it is also useful to explore alternate definitions of clinical illness that include multiple symptoms and, perhaps, eliminate nonspecific symptoms. This will increase confidence that the signs and symptoms are secondary to infection and are, indeed, modifiable by new treatments or

prior infections. We recommend that the current data be reevaluated to determine a more stringent definition of MMID that will better differentiate infected from noninfected subjects postchallenge.

Note

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Reply to Bernstein, Atmar, and Hoft

To the Editor—Thank you for the opportunity to respond to the Letter to the Editor submitted by Drs Bernstein, Atmar, and Hoft regarding the endpoints that have been used in our influenza challenge studies. Although we agree that it is important to use high-quality endpoints in human challenge models, we do not agree with their arguments against the use of "mild-to-moderate influenza disease" (MMID) as one of several endpoints in a challenge study [1–3]. This endpoint has been used as the primary endpoint in initial validation studies of new influenza challenge models including this influenza A/H3N2 virus as it encompasses both virologic and clinical measures of disease, giving us an objective measure to characterize the performance of a model in which every participant is exposed to infectious influenza after it is purposefully administered intranasally.

We recognize that all influenza symptoms are nonspecific and are shared with many other respiratory viruses. These symptoms can include fever, but it has been clearly demonstrated that many influenza infections do not result in fever, either in challenge studies [4] or from natural infection [5–7], especially in young adults. Therefore, in evaluating the performance of a challenge virus, we prefer to include a broadly defined list of symptoms to better understand the full spectrum of illness associated with infection. Each participant is assessed daily; if a reasonable explanation for the cause of a symptom other than influenza is found, it is documented and not deemed as meeting MMID criteria as the symptoms must be considered influenza-related. In this setting where participants are carefully screened on admission and tested for 21 different respiratory pathogens daily before and after influenza administration, the symptoms assessed are broad but are known symptoms of influenza [8, 9] and in the absence of other infections, in this context are most plausibly due to influenza.

The authors of the letter point out that several of the H3N2 challenge participants had influenza symptoms without documented viral shedding, indicating that the symptoms were not influenza related. We counter that the detection of viral shedding is not necessary to indicate that a person has been infected with influenza. It is common for individuals to develop influenza infections, including medically attended influenza, without a positive virologic test due to the limitations of sample collection that include: the location and kinetics of the replicating virus, anatomy of the person, variation in sampling techniques (ie, nasal wash, nasopharyngeal wash, nasal swab), and the diagnostic test being performed. This is recognized in many influenza challenge studies [10], including the one referenced in the letter [11] that relied on the isolation of influenza virus and/or a 4-fold or greater increase in serum hemagglutination inhibition (HAI) antibody titers to define influenza infection, recognizing that there are instances when influenza infection occurs without the detection of virus.

Human challenge studies have been used for decades [12] and have allowed for great advances in the development of influenza countermeasures [13]. In using these models for evaluation of novel vaccines or therapeutics it is important to choose endpoints that are apt for the goal of the study. The MMID endpoint is only one of many endpoints we have developed in these challenge models. In all of our challenge studies we include analysis of virologic, immunologic, and clinical endpoints to assess the severity of illness, including the number of days of shedding, the number of days of symptoms, the number of symptoms, and FLU-PRO scores, in addition to many laboratory-based measures such as antibody responses and transcriptomics [14]. All of these endpoints together allow for a more complete picture of the disease to be assessed and any of them could be considered as endpoints in phase II