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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References

1. Han A, Czajkowski LM, Donaldson A, et al. A dose-finding study of a wild-type influenza A(H3N2) virus in a healthy volunteer human challenge model. Clin Infect Dis **2019**; 69:2082–90.
2. Memoli MJ, Czajkowski L, Reed S, et al. Validation of the wild-type influenza A human challenge model H1N1pdMIST: an A(H1N1)pdm09 dose-finding investigational new drug study. Clin Infect Dis **2015**; 60:693–702.
3. Memoli MJ, Han A, Walters KA, et al. Influenza A reinfection in sequential human challenge: implications for protective immunity and "universal" vaccine development. Clin Infect Dis **2020**; 70:748–53.
4. Han A, Poon JL, Powers JH 3rd, Leidy NK, Yu R, Memoli MJ. Using the influenza patient-reported outcome (FLU-PRO) diary to evaluate symptoms of influenza viral infection in a healthy human challenge model. BMC Infect Dis **2018**; 18:353.
5. Reuman PD, Bernstein DI, Keefer MC, Young EC, Sherwood JR, Schiff GM. Efficacy and safety of low dosage amantadine hydrochloride as prophylaxis for influenza A. Antiviral Res **1989**; 11:27–40.
6. Bernstein DI, Atmar RL, Lyon GM, et al. Norovirus vaccine against experimental human GII.4 virus illness: a challenge study in healthy adults. J Infect Dis **2015**; 211:870–8.
7. Frenck R, Bernstein DI, Xia M, et al. Predicting susceptibility to norovirus GII.4 by use of a challenge model involving humans. J Infect Dis **2012**; 206:1386–93.
8. Atmar RL, Bernstein DI, Harro CD, et al. Norovirus vaccine against experimental human Norwalk virus illness. N Engl J Med **2011**; 365:2178–87.
9. Fry AM, Zhong W, Gubareva LV. Advancing treatment options for influenza: challenges with the human influenza challenge. J Infect Dis **2015**; 211:1033–5.

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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

trials. Through careful development of novel influenza challenge models with endpoints like MMID, these validated models can be applied in various ways and at various stages in the development of novel broadly protective influenza vaccines to assess efficacy as well as breadth of protection, and they will play a key role in the development of the next generation of influenza vaccines and countermeasures.

Note

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References

1. Han A, Czajkowski LM, Donaldson A, et al. A Dose-finding study of a wild-type influenza A(H3N2) virus in a healthy volunteer human challenge model. Clin Infect Dis **2019**; 69:2082–90.
2. Memoli MJ, Czajkowski L, Reed S, et al. Validation of the wild-type influenza a human challenge model H1N1pdMIST: an A(H1N1)pdm09 dose-finding investigational new drug study. Clin Infect Dis **2015**; 60:693–702.
3. Memoli MJ, Shaw PA, Han A, et al. Evaluation of antihemagglutinin and antineuraminidase antibodies as correlates of protection in an influenza A/H1N1 virus healthy human challenge model. mBio **2016**; 7:e00417–16.
4. Carrat F, Vergu E, Ferguson NM, et al. Time lines of infection and disease in human influenza: a review of volunteer challenge studies. Am J Epidemiol **2008**; 167:775–85.
5. Ridgway JP, Bartlett AH, Garcia-Houchins S, et al. Influenza among afebrile and vaccinated healthcare workers. Clin Infect Dis **2015**; 60:1591–5.
6. Chughtai AA, Wang Q, Dung TC, Macintyre CR. The presence of fever in adults with influenza and other viral respiratory infections. Epidemiol Infect **2017**; 145:148–55.
7. Jeong I, Lee CH, Kim DK, Chung HS, Park SW. Mild form of 2009 H1N1 influenza infection detected by active surveillance: implications for infection control. Am J Infect Control **2010**; 38:482–5.
8. Monto AS, Gravenstein S, Elliott M, Colopy M, Schweinle J. Clinical signs and symptoms predicting influenza infection. Arch Intern Med **2000**; 160:3243–7.
9. To KK, Wong SS, Li IW, et al. Concurrent comparison of epidemiology, clinical presentation and outcome between adult patients suffering from the pandemic influenza A (H1N1) 2009 virus and the seasonal influenza A virus infection. Postgrad Med J **2010**; 86:515–21.
10. Hayden FG, Treanor JJ, Fritz RS, et al. Use of the oral neuraminidase inhibitor oseltamivir in experimental human influenza: randomized controlled trials for prevention and treatment. JAMA **1999**; 282:1240–6.
11. Reuman PD, Bernstein DI, Keefer MC, Young EC, Sherwood JR, Schiff GM. Efficacy and safety of low dosage amantadine hydrochloride as prophylaxis for influenza A. Antiviral Res **1989**; 11:27–40.
12. Darton TC, Blohmke CJ, Moorthy VS, et al. Design, recruitment, and microbiological considerations in human challenge studies. Lancet Infect Dis **2015**; 15:840–51.
13. Hayden FG. Experimental human influenza: observations from studies of influenza antivirals. Antivir Ther **2012**; 17:133–41.
14. Walters KA, Zhu R, Welge M, et al. Differential effects of influenza virus NA, HA head, and HA stalk antibodies on peripheral blood leukocyte gene expression during human infection. mBio **2019**; 10:e00760–19.

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A Multicenter, Longitudinal Cohort Study of Cryptococcosis in Human Immunodeficiency Virus–Negative People in the United States

To the Editor—Marr and colleagues [1] report the results of a longitudinal follow-up of human immunodeficiency virus (HIV)–negative patients with cryptococcosis, and found substantial long-term neurological sequelae and morbidity in this multicenter cohort. This is a striking finding, because in patients living with HIV (PLHIV) presenting with cryptoccal meningitis, long-term neurological sequelae do not seem to be as prevalent if patients are treated promptly.

We would like to make 2 further comments. First, we do not know what the prevalence of stroke was in this study, which may account for the long-term neurological disabilities. Stroke as a consequence of cryptococcal meningitis in PLHIV is relatively rare—timely restoration of the immune system with antiretroviral therapy seems to prevent excessive brain swelling [2]. However, this was not an option for most patients in this study, who had to remain permanently immunosuppressed after diagnosis of cryptococcosis. Antifungal treatment also has poor penetration across the blood–brain barrier [3]. In patients on long-term immunosuppression, these factors may contribute to persistence of the organism within the subarachnoid space, prolonged brain inflammation and swelling, and consequently a higher burden of brain infarction or hemorrhage.

Second, the authors mention that they were unable to examine the significance of delayed diagnosis on mortality using Cox regression due to collinearity. A prolonged delay to diagnosis, however, is especially significant in the context of neurological disability, and may be central to its reduction if related to brain ischemia. It may be interesting to plot a simple graph of time to diagnosis and time to death, or severity of neurological deficits after diagnosis and examine for this relation descriptively.

Following on from this, future studies on cryptococcosis in patients without HIV should focus on the prevalence and prognostic significance of any brain imaging or cerebrospinal fluid results (opening pressure, India ink stain, glucose, and protein). This may also further help to characterize this particular cohort of patients and facilitate an earlier diagnosis.

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