Advancing Treatment Options for Influenza: Challenges With the Human Influenza Challenge

Alicia M. Fry, Weimin Zhong, and Larisa V. Gubareva
Influenza Division, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia

(See the major article by Ramos et al on pages 1038–44.)

Current therapeutic options for the treatment of influenza virus infections are limited. Only 2 classes of agents have licensed products, neuraminidase (NA) inhibitors and M2 inhibitors, and only NA inhibitors are active against currently circulating seasonal viruses. While observational studies show a benefit of NA inhibitor treatment for hospitalized patients with influenza [1], optimal treatment for severely ill patients and those who are immunocompromised will likely require additional treatment options with a different mechanism of action. Since the 1918 pandemic, convalescent serum or plasma from persons previously infected with influenza virus has been investigated as treatment for individuals with severe influenza virus infection, including, more recently, individuals with human avian influenza and 2009 pandemic A(H1N1) infections [2, 3]. However, receipt of convalescent serum can be associated with severe adverse effects, and the resources necessary to produce such serum in large quantities are prohibitive [2]. Compared with convalescent serum, human monoclonal antibodies (mAbs) against influenza viruses may be more feasible for widespread use, without the adverse effects associated with human blood products. However, identifying a human mAb that can reproduce the clinical anti–influenza virus effects of polyclonal convalescent serum is a challenge. A handful of mAbs against influenza virus proteins are currently in early phases of evaluation for human use [4]. In this issue of The Journal of Infectious Diseases, Ramos et al report results from a human influenza challenge study evaluating the effect on clinical illness of a human mAb targeting the external portion (ie, ectodomain) of the M2 protein (M2e) [5].

M2 protein is an attractive target for broadly cross-reactive influenza vaccines and therapeutic Abs because of the highly conserved nature of the amino acid sequences of its ectodomain among isolates from different antigenic subtypes of influenza A viruses [6]. A number of mAbs targeting either linear or conformational epitopes within M2e have been described [7–11]. Studies in mouse models have shown that passive transfer of anti-M2 mAbs into recipient mice can offer survival advantages against lethal viral challenges and reduce virus replication in the lungs; however, the degree of the therapeutic effect varied depending on the particular mAb, challenge virus, and other factors [8, 12–14].

The mechanisms of anti-M2e Ab–mediated protection are not fully understood. Anti-M2 Abs do not possess hemagglutination inhibition ability or in vitro virus neutralization activity [15]. It is believed that the main target for the anti-M2e antibody is virus-infected cells, which abundantly express M2 protein on their surface [16]. Conceivably, by binding to M2e, the nonneutralizing anti-M2 Abs may facilitate elimination of virus-infected cells in vivo by triggering complement-dependent cytotoxicity and/or antibody-dependent cell-mediated cytotoxicity [12, 15]. The involvement of alveolar macrophages in humoral M2e-specific protection by phagocytosis has been reported [17]. Moreover, the possibility of an alternative mechanism of protection (eg, interference with M2 ion channel activity) was suggested for an anti-M2e mAb that targeted dimeric or multimeric M2 peptides [10]. Elimination of infected cells results in reduction of virus replication and spread. However, concerns have been raised with regard to a potential disease exacerbating effect of nonneutralizing Abs, presumably through the similar immune mechanisms, as observed in the swine model of influenza [18, 19].

Evaluation of any new anti–influenza therapeutics, such as anti-M2e mAbs, in humans is a daunting task. Human challenge models were developed to aid in such evaluations. Challenging healthy adult volunteers with a well-characterized influenza virus provides several advantages: synchronization of infection and illness course, prescreening for susceptible participants by testing for Abs against the hemagglutinin of the challenge virus, and knowledge of baseline symptoms and
characteristics of participants. In theory, human challenge studies can be instrumental in providing a proof of principle that a new therapeutic agent can reduce disease symptoms and virus shedding, as well as provide some information on safety and the development of resistance (eg, the ease by which resistant viruses emerge and markers of resistance) [20]. These data can be informative in determining whether a new therapeutic agent should proceed to more-resource-intensive human clinical trials dependent upon natural infections, and they can also help to structure collection of information from those trials. As one might expect, human challenge studies are carefully monitored, and extensive testing is required before a challenge virus is approved for human studies [20].

The study by Ramos et al highlights some of the challenges and pitfalls associated with human challenge models. The authors infected participants with an intranasal A(H3N2) influenza virus (A/Wisconsin/67/2005) and randomly assigned them to receive TCN-032, the anti-M2e mAb, or placebo 1 day later. The authors compared symptoms, symptom severity, and virus shedding between study groups. Finding susceptible participants was difficult and was cited as a reason for only enrolling half of the target sample size. This is not surprising since A/Wisconsin/67/2005 virus was included in seasonal influenza vaccines as the antigenically representative strain of the influenza A(H3N2) subtype circulating during 2006–2008. The authors reported a small and statistically nonsignificant difference in the main study outcome, presence of fever or a higher grade of symptoms, and no serious adverse events in recipients of TCN-032. One secondary outcome, total symptom score for 7 days, was statistically less by a third in TCN-032 recipients, compared with the placebo group. However, the other secondary outcomes, including median virus shedding, duration of symptoms, and weight of nasal secretions in tissues, were lower, but not statistically significantly so, in the TCN-032 recipients, compared with placebo recipients.

The choice of challenge virus and challenge dose is one of the critical steps in human challenge studies. Although virus strains causing severe infections cannot be used, the use of an overly attenuated virus strain can limit the information collected from a human challenge study. In the study by Ramos et al, there are some suggestions that either the challenge A(H3N2) strain was overly attenuated and/or the infective dose too low. Among the placebo-treated patients, only 2 of 24 participants (8%) reported fever, and the median tissue weight from nasal secretions was low (6 g), suggesting that the challenge virus caused very mild illness. A similar finding was reported in another study among untreated participants challenged with the same virus: only 3 of 14 subjects (21%) developed fever [21]. It would be helpful to know if all of the infected participants shed virus or whether only those with infection confirmed by seroconversion shed virus. By comparison, in a human challenge study evaluating the efficacy of oseltamivir, 12 of 33 patients (36%) receiving placebo had fever after challenge with A/Texas/36/91(H1N1) virus, the weight of tissues collected from nasal secretions was 12 g, and 70% of inoculated participants shed virus [22].

Clues regarding potential virus attenuation could be sought in details from characterization of the challenge A(H3N2) virus, including the sequence of the virus genome; posting of the challenge virus sequence would be helpful for future studies using this virus. Finally, determination of infectious titers can be complicated in some contemporary A(H3N2) viruses because of a loss of ability to agglutinate erythrocytes, an important step in determining virus titer (expressed as median tissue culture infective doses per milliliter). Details about viral titer determination may provide additional clues to explain the attenuated clinical response by infected participants. When virus shedding is low in placebo recipients as a result of an attenuated virus infection, it is not possible to assess for the emergence of drug resistance, a potential concern raised after therapeutic treatment of severely immunocompromised mice with an anti-M2 mAb [9]. One additional piece of information that would have been helpful is the composition of the placebo. A control antibody is commonly used in animal mAb challenge studies, which in some instances showed a small protective effect [11].

In summary, the authors report a trend toward a very modest protective effect from TCN-032, the anti-M2e mAb, in this human challenge study. This is the first published evaluation of a mAb as a therapeutic agent for influenza virus infection, a novel approach and potentially important adjunct to current treatment options for a common virus infection that can be associated with severe outcomes. We will not know whether the anti-M2e mAb TCN-032 has clinical benefit until it is evaluated in a clinical trial with a larger number of participants with natural acquired influenza. Human challenge studies are not always predictive of the magnitude of clinical effect determined by randomized clinical trials [20]. A larger number of participants will be needed to evaluate for adverse effects of a new immunomodulatory agent, and more research is needed to determine whether clinically significant resistance will emerge. In addition, the effect of combined use of TCN-032 and a NA inhibitor needs to be assessed. Finally, whether a nonneutralizing antibody, such as TCN-032, will be useful in patients who are severely ill or immunocompromised remains an important question.

**Notes**

**Disclaimer.** The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

**Potential conflicts of interest.** All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.
References


