Neuraminidase: Another Piece of the Influenza Vaccine Puzzle

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(See the major article by Monto et al on pages 1191–9.)

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The hunt for immune correlates of vaccine-mediated protection from influenza is by no means a recent endeavor. The most recognized unit for such a correlate, a hemagglutination inhibition (HAI) assay titer of ≥40, is derived from studies published in 1972 [1]. This unit has proven useful for licensure of inactivated human seasonal influenza vaccines, but the increase in zoonotic infections and an influx of vaccine effectiveness data from test-negative case-control studies in recent times have reinforced the desire to improve and broaden the specificity of influenza vaccines and rekindled the drive to understand other immune correlates of protection.

Although the cellular immune response to influenza virus infection is known to target a diverse array of viral proteins, studies of the humoral response have concentrated on those that target the major viral surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA). HA-specific antibodies dominate the response to influenza virus infection and vaccination, and a subset are known to be neutralizing and protective. It is widely considered that the induction of high titers of HA-specific antibodies is a hallmark of a successful influenza vaccine, although it has been long recognized that antibodies targeting the viral NA can also be generated. Antibodies directed to NA do not neutralize virus infection, but rather limit subsequent spread and have been shown able to reduce severe disease and mortality in a variety of animal challenge studies [2]. Epidemiologically, there has also been circumstantial support for a role of NA immunity in reducing the severity of the 1968 influenza pandemic. In this instance, the NA was conserved between the previously circulating influenza A(H2N2) virus and the 1968 influenza A(H3N2) pandemic virus. NA-based immunity developed because of exposure to the influenza A(H2N2) virus and was postulated to reduce severity of subsequent influenza A(H3N2) infection [3]. Despite these observations, solid data to support a protective role of NA immunity in humans have been lacking. In this issue of The Journal of Infectious Diseases, Monto et al [4] now provide the most convincing evidence to date that NA-directed humoral immunity is protective and is a correlate of protection in its own right.

Armed with a recently optimized assay more conducive to better measurement of antibodies that inhibit the enzymatic activity of NA (ie, the NA inhibition [NAI] assay) [5] and stored serum specimens from a prior randomized, placebo-controlled trial of trivalent live-attenuated influenza vaccine (LAIV) and trivalent inactivated influenza vaccine (IIV) [6], Monto et al determined the contribution of NA-specific antibodies to protection against laboratory-confirmed influenza. Consistent with the trends seen with HA-reactive antibody titers, vaccine-induced responses to NA were higher in recipients of the inactivated form of the vaccine than in those who received LAIV, with 37% and 6% of individuals responding, respectively. Although, because of assay differences, it is not entirely correct to directly compare HA- and NA-specific titers, the responses to NA appeared to be less rigorous than those seen to HA. This could be a result of intrinsic differences in the immunogenicity of HA and NA, poor sensitivities of NAI assays, and/or different amounts of the corresponding antigens in the vaccine formulations. IIVs are standardized on HA content only, whereas LAIV is standardized on infectious virus titers. Regardless, the biggest take-home message from this work lies in the authors’ result that “as NAI assay titers rose, the frequency of vaccine failure fell” [4]. Although it is difficult to separate the effects...
of NA-specific immunity from other immune responses, this suggests that vaccines can induce NA-directed antibody responses that are independently predictive of protection. The study has provided convincing data that support prior circumstantial observations that NA-specific antibodies are protective in humans.

The biggest question now is how to move forward. Is it time to propose that a minimum level of NA be present in current IIV formulations (by default it is present in LAIV)? After all, there are now solid data to suggest a protective role for NA immunity. The authors have stopped short of promoting this, but rather suggest that “[a]s new influenza vaccines are developed, NA content should be considered” [4]. And perhaps this is a more appropriate path to take. The current seasonal influenza vaccine pipeline, or, more accurately, time line, is fragile, and mandating an additional requirement may lead to an inability to deliver vaccine. Vaccine manufacturers, regulatory agencies, and public health entities work collaboratively to select appropriate antigens, produce vaccine, develop and release reagents, and fill, distribute, and ultimately deliver vaccine in a very tight time frame. Studies have shown that some IIV contains enzymatically active NA [7], but this is likely batch and formulation dependent. Failure at any of the above steps, including any future recommendation of minimum NA content, is crippling. Even if mandating an element of NA content is unlikely in the current manufacturing process, the data suggest that it is prudent to optimize production processes to maximize vaccine NA content, as suggested by guidance provided by the European Pharmacopeia [8]. The study also adds measurement of NA-specific antibodies as an important component in the evaluation of emerging vaccine platforms. It should also be noted that the assay used in this study only measures antibodies that neutralize the enzymatic activity of NA; little is known about the protective effect of antibodies targeting other regions of the protein.

While Monto et al have shown convincing evidence for NA-directed antibodies as an additional correlate of protection, data from the study also show ample room for improvements in influenza vaccines. If high titers of HAI and NAIs assays are independent correlates of protection, then it was a bit disheartening to learn that the authors found that “cases were still identified even among those with high log₂ HAI and NA assay titers” [4]. In other words, even vaccines that produce robust antibody responses may not be protective. Does this tell us that some individuals are just more susceptible to influenza virus because of genetic or environmental influences, regardless of immunity, or does it suggest that other independent or dependent correlates of immunity are yet to be discovered? Although it is unrealistic to expect vaccines to be 100% effective, there is a general consensus that time and resources spent improving current or developing new influenza vaccine platforms are well spent. To achieve either of these goals effectively, defining strong correlates of protection is critical. As such, Monto et al are to be congratulated for their contribution to the field. But there is, as usual, much to do. While there has been an increase in the number of clinical studies aimed at determining the efficacy and/or effectiveness of current vaccines, there has not been a corresponding increase in the number of laboratory-based studies to determine what underpins these measures. There is no doubt that adding immunologic components to efficacy studies is resource intensive, but it is possible, and the data generated are remarkably synergistic. The study highlighted by this editorial is a perfect example.

Note
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