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EDITORIAL COMMENTARY

One Step Forward in the Road Toward a Universal Influenza Vaccine

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(See the major article by de Vries, on pages 3–11.)

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Despite the major health and societal benefits associated with influenza immunization, current influenza vaccine programs continue to face significant skepticism in the general public and even from some healthcare providers. Although many of those concerns have limited scientific basis and are mostly related to misinformation, one of the major limitations of the current strategy is the need to formulate the vaccine composition every year because of influenza virus antigenic drift and the difficulties in predicting the circulating strains for the upcoming season. This is a significant shortcoming, as studies have demonstrated reduced vaccine effectiveness that led to increased morbidity in years when there was a poor match between the viral strains included in the vaccine and those circulating in the community [1].

These obvious limitations have inspired major research efforts to develop improved influenza vaccines that can provide broad cross-protective and durable immunity against the diverse influenza virus strains and, as such, will avoid the yearly inexact process of selecting the strains composing the vaccine. Many strategies are actively being investigated to develop such universal vaccines, focusing on a variety of antigens designed to elicit both cross-protective antibodies and broad T-cell responses [1–3]. One unique target for developing a universal vaccine that has attracted major research is a structurally conserved region of the hemagglutinin (HA) glycoprotein, the HA stalk [4, 5]. Influenza virus HA is a glycoprotein on the surface of the virus that plays a major role in the pathogenesis of infection. HA is responsible for binding of the virus to the sialic acid present on the membrane of the respiratory cells; in a second step, HA facilitates the fusion of the virus envelope and cell membranes to allow the entry of the viral genome into the target cells. HA has 2 major components, the head and the stalk. The head (HA1) is variable and immunodominant; it is a major component of the current influenza vaccines and induces neutralizing antibodies that protect against the same virus strain. The stalk is structurally conserved but less capable of inducing neutralizing antibodies. The HA stalk induces cross-protective antibodies, but these antibodies are poor inhibitors of hemagglutination. This represents an additional challenge to measuring their activity by using standard assays, as regulatory agencies recognize hemagglutination-inhibiting antibody titers as the primary correlate of protection and vaccine efficacy.

In this issue of the *Journal,* de Vries et al [6] provide important new evidence of the ability the HA stalk to induce cross-protective antibodies in humans. In addition, they show that novel antibody-dependent cellular cytotoxicity (ADCC) assays specifically designed to measure cross-reactive antibodies directed to the HA stalk can be implemented using a simplified approach that should facilitate their standardization across different laboratories. In this report, the investigators evaluated a cohort of nonimmunized children 1–7 years of age with serologic evidence of primary influenza B virus (IBV) infection. This cohort was well characterized and selected from a larger population by measuring hemagglutination-inhibiting antibodies against multiple representative influenza A virus (IAV) and IBV strains. The cohort included 41 children; of those, 18 had B/Victoria (B/Vic) lineage infection and 23 had B/Yamanashi (B/Yam) lineage infection. First, de Vries et al [6] measured ADCC antibodies by means of a solid-phase assay, using representative full-length HAs for both B/Vic and B/Yam lineages and the NK92.05-CD16 cell line. They demonstrated significant HA lineage–specific ADCC titers. Most significantly, there was also evidence of ADCC activity against the heterologous lineage, although at lower titers. These HA-specific cross-reactive ADCC antibodies were detected despite the lack of HI heterologous antibodies. Next, to determine whether the IBV infection induced HA head and/or HA stalk antibodies, they used an enzyme-linked immunosorbent assay (ELISA) with a variety of targets,

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including representative full-length lineage HAs, HA1 subunits (HA heads) for both lineages, and a chimeric HA with an irrelevant IAV H8 subtype head and a B/Yam stalk. Children with B/Vic and B/Yam infections demonstrated antibodies against full-length HAs of both lineages but only lineage-specific antibodies against the HA1 subunits, whereas the HA stalk–specific antibodies were detected in children infected with both the B/Vic and B/Yam strains. These data indicate that the cross-reactive antibodies measured by ELISA are directed to the HA stalk. Using the same antigen targets, they measured ADCC antibodies. Those experiments showed ADCC cross-reactive antibodies against the full length HAs, but, in contrast to the ELISA results, no ADCC antibodies against the HA1 subunits were detected. Finally, using the chimeric HA, they demonstrated that HA stalk–specific ADCC antibodies were present in children infected with both the B/Vic and B/Yam lineages. Taken together, these studies provide convincing evidence that IBV infection results in antibodies directed against the HA head and the HA stalk, but only the HA stalk–specific antibodies, not those against the HA head, mediate ADCC activity. The authors reasoned that this provides additional evidence, as previously reported [7, 8], that effective ADCC activity requires 2 points of contact between the target and the effector cells: the contact between Fc and FCyRIII and the interaction between HA and sialic acid.

Overall, this study confirms and provides new understanding of the value of the structurally conserved HA stalk as a target for developing universal influenza vaccines. The investigators also demonstrated that solid-phase assays to measure ADCC activity can be implemented with a simplified approach using recombinant HA antigens and a continuous natural killer cell line [9]. The study has significant strengths that may have implications for future research. First, de Vries et al took advantage of a unique and well-characterized clinical cohort of children that allowed detailed and precise analyses of cross-reactive antibodies. Second, it underscores the need to move beyond the traditional hemagglutination inhibition or neutralizing assays and to incorporate immunologic assays that can provide a more comprehensive assessment of functional protective antibodies. It is urgent that these nontraditional assays become standardized and incorporated into ongoing clinical studies and regulatory pathways. It should be mentioned, as the authors indicate, that despite all these advances in defining the role of ADCC antibodies, the best correlate of protection still needs to be defined. Nevertheless, these studies confirm the importance of the structurally conserved HA stalk as a target antigen and represent an important step forward in the road toward the development of novel universal influenza vaccines.

Notes

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