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Peter's Paradigm and Pandemic Preparedness

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Peter Doherty and Rolf Zinkernagel's discovery of how the body's immune system distinguishes virus-infected cells from normal cells is an essential element to curbing influenza infection and preventing severe disease. The recognition that CD8+ T cells kill virus-infected cells by engaging with an ''altered self'' (20), a complex of self major histocompatibility complex (MHC) molecule and the foreign antigen, enabled others to identify peptides of conserved influenza proteins as the primary targets of these killer cells (12, 17), providing a mechanism to explain protection against influenza A/H1N1 (or other subtypes) after influenza A/H3N2 infection. Vaccines that target the induction of CD8+ T cells and heterosubtypic immunity have, therefore, become an important strategy in the development of universal vaccines capable of broad protection and preventing severe disease during a pandemic (9, 3).

During my postdoctoral training in the Doherty laboratory at St. Jude Children's Research Hospital, we investigated several aspects of CD8+ T cell immunity. ''Tea time'' each morning in the old St Jude cafeteria is where ideas were mulled and experimental plans developed. The ideas were big, leaving lots of room for alternatives and testable hypotheses. We discussed how the immune response was initiated and argued about whether antigen persisted. Conversations continued throughout the day, with Peter always available to answer questions or discuss a problem. Peter's love for learning, openness to sharing ideas, and generosity in spending time with his staff in discussions not only of work but also life, provided a foundation for my career period. His example has been a model that I still try to follow and encourage others to aspire to.

We worked as a team in the Doherty laboratory; it was common for us to contribute to one another's experiments even if only to infect mice or harvest lymph nodes. In Peter's laboratory there were many opportunities to collaborate with molecular immunologists who were creating knockout or transgenic mice; an incredible time to show the *in vivo* function of single genes and to demonstrate the contribution of CD8+ T cells to influenza immunity. These opportunities to collaborate and Peter's amazing ability to communicate clearly provided me with a good number of quality publications (2, 4–8, 18) that advanced my career.

Although most questions during my time working with Peter were targeted at understanding specific immune mechanisms, some of his work was translational, including several projects to identify and understand the human T cell response to viruses. From this came my interest to apply paradigms established in the laboratory to the human immune response, and for that reason, I joined the Center for Immunization Research at Johns Hopkins School of Public Health.

The first DNA vaccine trial, a naked plasmid expressing hemagglutinin (HA), was conducted soon after I arrived at Hopkins. My group was responsible for establishing tests to measure antibody and T cell responses. Unfortunately, the vaccine was not immunogenic at any of the doses tested; the antibody responses were undetectable, and T cell responses were negligible (unpublished). Since HA does not have well-characterized class I human leukocyte antigen (HLA)-restricted epitopes, this study missed an opportunity to examine the ability of this novel vaccine to induce CD8+ T cell responses. DNA vaccines can indeed activate CD8+ T cells; a later DNA vaccine expressing a known target of human CD8+ T cells nucleoprotein (NP) increased the number of γ-interferon-producing T cells (16).

Unfortunately, many prelicensure clinical studies have shortcomings due to designs that do not consider findings from basic research. This may include discoveries related to vaccine immunogenicity or improvements that have been made to measure the immune response. Although research in mice should not be used as a substitute for human studies, incorporation of lessons learnt from mouse studies will improve the chance of success of a universal vaccine. For example, vaccines that target the induction of cytotoxic T cells should be formulated or designed to express a known T cell target antigen in dendritic cells or to allow cross-presentation. This is easily achieved by live viruses, recombinant vectors that express the targeted antigen, or messenger RNA vaccines, whereas inactivated or peptide vaccines require the use of delivery vehicles such as liposomes, or adjuvants to deposit the antigen appropriately.

The long history of studies by the Doherty laboratory and others demonstrating protection against influenza by T cell responses is finally being followed by clinical trials addressing that form of protection (9, 11). When human vaccine studies are planned, they should also consider findings from other clinical or epidemiologic studies. Despite strong evidence from studies conducted during the 1968 influenza

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pandemic that antibodies to neuraminidase (NA) contribute to immunity against influenza (14), responses to NA have only recently been considered more routinely as a secondary immunogenicity end point.

NA inhibiting antibodies do not prevent infection but limit virus release from infected cells, resulting in ''infection-permissive'' immunity (13, 1). It is highly likely that NA-specific antibodies also provide a frontline defense against influenza infection by preventing virus release from mucins, thereby reducing the number of infectious particles that are available to infect mucosal epithelial cells. Although we have a good understanding of the mechanism of NA-specific antibody action, clinical studies of most vaccines containing both HA and NA are designed in such a way that they cannot evaluate the contribution of NA immunity. For example, vaccine efficacy studies routinely use PCR-confirmed influenza as an end point. Considering NA immunity does not prevent infection but rather reduces disease by limiting. Virus spread, the contribution of NA immunity would require clinical measures of illness severity or duration of virus replication.

Test negative postlicensure observational studies are typically used to evaluate influenza vaccine effectiveness. In these studies, all subjects have symptoms of acute respiratory illness (i.e., influenza-like illness); the vaccine status of subjects positive for influenza by PCR testing is compared with the status of subjects who had a negative PCR test result. This type of study is very different from earlier observational studies of vaccine effectiveness in which cases were patients with influenza-like illness and controls were individuals without symptoms. With an understanding that NA immunity does not prevent infection but reduces clinical signs of disease, there is a good chance that the apparent poor effectiveness reported from studies using a test negative design may be the result of not counting individuals with subclinical or mild disease as benefiting from vaccination. The same issue applies to T cell vaccines.

In my opinion, a universal vaccine that targets CD8+ T cells may be somewhat effective when CD8+ T cell memory is established in lymph nodes; however, there is a delay when the T cells are recalled to the site of infection (10). Therefore, the most effective vaccine may be one that induces local T cell immunity and results in memory T cells in the lungs. The benefit of having such CD8+ T cell memory located in the lungs in reducing virus load and recovery from infection is evident in a mouse model (19). Vaccines that target the induction of local immunity would need to be administered intranasally. This idea is verified by the rapid and robust protection observed in mice that were immunized with universal vaccine candidates intranasally (15).

Although animal studies can demonstrate that influenza-specific CD8+ T cells have been induced and are present in the nasal or bronchial-associated lymphoid tissue or lungs, this would be difficult to evaluate during a human vaccine study. Evaluation of the benefit of vaccination is also difficult; as for NA, influenza-specific cytolytic T cells do not protect from infection and, therefore, clinical benefit such as shortened duration of infection or reduced signs of disease would need to be demonstrated by daily monitoring of clinical signs and samples collected at several time points to determine virus titer or duration of infection. This is not possible to achieve in a typical observational study of vaccine efficacy.

Clinical challenge studies may be essential to establish overall benefit NA or CD8+ T cell-inducing vaccines in reducing symptoms and/or duration of influenza-like illness, and some such studies have been carried out. Although CD8+ T cell immunity may have minimal impact on seasonal influenza in a background of robust antibody responses to vaccines well matched to the virus, it is likely to be critical during a pandemic or an outbreak of an unexpected strain.

Given the current emphasis and need for development of a universal influenza vaccine, it would serve funding bodies and regulators well to make sure Peter's discoveries are considered in the development of universal influenza vaccines that target induction of CD8+ T cell responses. This type of vaccine could save millions of lives during a pandemic.

Author Disclaimer

My comments are an informal communication and represent my own best judgment. These comments do not bind or obligate the Food and Drug Administration.

Author Disclosure Statement

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