Journal of the Pediatric Infectious Diseases Society

ORIGINAL ARTICLE

Mucosal Immunity: The Forgotten Arm of the Immune System

Synopsis of the Pediatric Infectious Disease Society's 2017 Stanley A. Plotkin Lecture in Vaccinology

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The 2017 Stanley A. Plotkin Lecture in Vaccinology was delivered by Professor Peter F. Wright at the Pediatric Academic Societies Annual Meeting in San Francisco, California, in May 2017. The presentation provided an overview of the mucosal immune system as it applies to vaccinology. Specifically, Professor Wright's lecture highlighted the remarkable opportunities for mucosal immunity research afforded by having both topically administered live vaccines and systemically administered inactivated vaccines available for the same pathogen. Using influenza and poliovirus case studies, Professor Wright described the use of live attenuated vaccines for human challenges and discussed how recent technological advancements in immunological assays have ushered in a new era for investigating the correlates of immune protection against wild-type infections at mucosal sites.

Keywords. human challenge; inactivated vaccine; live oral vaccine; mucosal immunity.

The mucosal immune system is highly complex and heterogeneous in its structure and function across the body's mucous membranes. Variability in mucosal environments can be observed in terms of protective defense mechanisms (eg, diarrhea, sneezing, and coughing), microbial colonization patterns (eg, vaginal mucosae are populated predominately by *Lactobacillus* species, whereas the uterine mucosae remain relatively sterile), and relative concentrations of immunoglobulin (Ig) isotypes (eg, the ratio of IgA to IgG is greater than 400:1 in the parotid saliva and less than 1:1 in cervical secretions). However, common to all mucosal tissue substrates is the key role of the mucosal immune system in protecting the body from microbial pathogens. Indeed, in many cases, the immune mediators at mucosal sites serve as the body's first line of defense against infection. In addition to its function in protecting individuals from disease, mucosal immunity is also essential for preserving population health. For pathogens that replicate in mucosal tissues (eg, influenza [1], respiratory syncytial virus [2], and poliovirus [3]), a robust mucosal response is capable of rapidly controlling microbial shedding and thereby interrupting the onward transmission of the infectious agent to susceptible individuals.

Received 6 September 2017; editorial decision 21 October 2017; accepted 30 October 2017; published online December 22, 2017.

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Journal of the Pediatric Infectious Diseases Society 2019;8(1):53–4

DOI: 10.1093/jpids/pix102

Despite its clinical significance, mucosal immunity in conventional studies has been limited by a combination of technological and biological constraints. Sample collection can be invasive (eg, mucosal biopsy during endoscopy), specimen storage can be challenging (eg, proteolytic enzymes can degrade antibodies), and immunoassays can be labor-intensive to perform and might provide limited sensitivity for detect clinically meaningful differences. Vaccine challenge studies have enabled us to overcome some of these limitations. For these clinical trials, individuals (who might be immunonaïve or randomly assigned to receive a primary vaccine on a specific schedule) are administered a challenge dose of a live vaccine as a proxy for natural exposure to the pathogen. During subsequent follow-up visits, a longitudinal series of mucosal samples are collected, and the amount of vaccine-derived virus recovered from the samples is quantified. The presence or absence of a given virus and the titer of viral shedding provide surrogate indicators of mucosal immune protection. In our own practice, this research has revealed that virus is less likely to be recovered after challenge with a live attenuated influenza vaccine (LAIV) in nasal washes from children primed with LAIV than in children primed with inactivated influenza vaccine [4]. Similarly, this approach has revealed that after challenge with a type 2 oral polio vaccine (OPV), the titers of poliovirus type 2 recovered in stool collected from children immunized with trivalent OPV are lower than those recovered from children immunized with bivalent (types 1 and 3) OPV, with or without a supplementary dose of trivalent inactivated polio vaccine [5].

Today, recent advances in biotechnology have enabled us, in a convincing way, to identify immune parameters that are associated with the induction of mucosal immunity and that provide

protection from viral replication at mucosal sites on challenge with a live vaccine. Specifically, the development of luciferase-expressing pseudoviruses (eg, polio pseudoviruses [6]) has made it possible to quantify antibody function in its ability to neutralize virus present in mucosal samples. Because this pseudovirus assay measures a single cycle of virus growth, it facilitates rapid turnaround, reduces contamination risks, and is associated with fewer cellular toxicity issues. Equally important have been Luminex (Austin, Texas) multiplex bead–based assays that can quantify the concentrations of several serotype-specific binding antibodies in a single assay [7]. A prime example of these technologies in action has come from our recent investigations into vaccine-induced mucosal immunity to poliovirus. Using these assays in the study of infant stool samples, we have observed (1) a brisk mucosal response to live OPV challenge that closely tracks the temporal kinetics of viral shedding, (2) significant pairwise correlations between serotype-specific IgA concentration, pseudovirus-neutralizing activity, and diminution of viral shedding, and (3) a notable lack of correlation between mucosal responses and a child's prechallenge serum immunity [5, 7]. In our ongoing investigations, we are comparing the induction of mucosal immunity between various infant polio vaccine schedules by using fecal samples collected from a series of clinical trials conducted under the auspices of the Bill and Melinda Gates Foundation. If robust correlations continue to be established between poliovirus shedding and pseudovirus-neutralizing activity and polio type-specific Ig concentrations in stool samples, then it is plausible that we can examine the primary mucosal immunogenicity of vaccines in the absence of a live vaccine challenge. This examination could be of exceptional value for evaluating individual mucosal responses, in real time, to emerging vaccines such as the "new" OPVs, which are a class of highly attenuated and stable live vaccines designed to confer reduced risks of reversion to neurovirulence and capacity for transmission relative to those of conventional OPVs [8].

As we look to the future, much remains to be learned about the mucosal immune system and its significance for vaccines. Some specific questions that warrant further investigation include the following:

- What is the duration of vaccine-induced mucosal immune protection?
- Can mucosal antibodies be primed via natural exposure or immunization with live vaccine such that mucosal responses can be generated after subsequent exposure to an inactivated vaccine?
- How broadly does mucosal protection reach beyond the epithelial cell layer? Can mucosal immunity influence the replication of viruses (eg, human immunodeficiency virus [HIV] and poliovirus) in perimucosal sites?
- Which biological features underlie the observed variability in the mucosal immune responses to specific vaccines?
- Why does viral replication seem to be necessary for the induction of mucosal immunity against certain pathogens?
- How do we explain the effectiveness of selected inactivated vaccines, such as those against human papillomavirus and cholera toxins, in conferring mucosal protection?
- To what degree do neutralizing antibodies in the serum transudate to mucosal surfaces? Does boosting the neutralizing response in the serum confer ancillary benefits for mucosal immunity?
- Are there adjuvants capable of enhancing the mucosal immunogenicity of vaccines?
- Can the aforementioned pseudovirus-neutralization approaches and highly targeted bead-based immunoassays be adapted for investigations of other mucosally replicating pathogens? Can the platforms be used with other biological matrices (eg, cervical secretions, breast milk, parotid saliva)?

In conclusion, for too long mucosal immunity has been the forgotten arm of the immune system. A combination of highly effective mucosally delivered vaccines, vaccine challenge studies for determining mucosal immunity, and new assays with high specificity and the capacity to measure functional immune responses are now unraveling the long-suspected role of mucosal immunity in our immune armamentarium.

Note

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

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