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# Mucosal Immunity: The Forgotten Arm of the Immune System

*A 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, USA, in May of 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 the case studies of influenza and poliovirus, 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 wildtype infections at mucosal sites.*

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 (e.g., diarrhea, sneezing, and coughing), microbial colonization patterns (e.g., vaginal mucosae are predominately populated by *Lactobacilli* species while the uterine mucosae remain relatively sterile), and relative concentrations of immunoglobulin (Ig) isotypes (e.g., 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 (e.g., 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 agents to susceptible individuals.

Despite its clinical significance, conventional study of mucosal immunity has been limited due to a combination of technological and biological constraints. Sample collection can be invasive (e.g., mucosal biopsies during endoscopies), specimen storage can be challenging (e.g., proteolytic enzymes may degrade antibodies), and immunoassays can be labor-intensive to perform and may have limited sensitivity to detect clinically meaningful differences. Vaccine "challenge" studies have allowed us to overcome some of these limitations. For these clinical trials, individuals (who may be immune-naïve or randomized to receive a specific primary vaccine 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/absence of a given virus or the titer of viral shedding provide surrogate indicators of mucosal immune protection. In our own practice, this research has demonstrated that vaccine-derived live 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 shown that lower titers of poliovirus type 2, after challenge with a type 2 oral polio vaccine (OPV), are recovered in stool collected from children immunized with trivalent OPV than bivalent OPV (types 1 and 3) 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 (e.g., polio pseudoviruses [6]) have made it possible to quantify antibody function in its ability to neutralize virus present in mucosal samples. As 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 multiplex bead-based assays utilizing Luminex® (Austin, Texas, USA) 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 been our recent investigations into vaccine-induced mucosal immunity to poliovirus. Utilizing these assays in the study of infant stool samples, we have observed: (i) a brisk mucosal response to live OPV challenge that closely tracks the temporal kinetics of viral shedding, (ii) significant pairwise correlations between serotype-specific IgA concentration, pseudovirus neutralizing activity, and diminution of viral shedding, and (iii) a notable lack of correlation between mucosal responses and a child's pre-challenge serum immunity [5, 7]. In our on-going investigations, we are comparing the induction of mucosal immunity between varying infant polio vaccine schedules, 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 immunoglobulin concentrations in stool samples, then it is plausible that we could examine the primary mucosal immunogenicity of vaccines in the absence of a live vaccine challenge. This could be of exceptional value for evaluating individuals' mucosal responses, in real time, to emerging vaccines like the 'new' oral polio vaccines (nOPVs), which are a class of highly attenuated and stable live vaccines designed to have reduced risks of reversion to neurovirulence and capacity for transmission relative to conventional OPVs [8].

Looking 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:

- What is the duration of vaccine-induced mucosal immune protection?
- Can mucosal antibodies be primed via natural exposure or immunization with live vaccines, such that mucosal responses can be generated upon subsequent exposure to inactivated vaccines?
- How broadly does mucosal protection reach beyond the epithelial cell layer? Can mucosal immunity influence replication of viruses (e.g., 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 appear 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 have 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 utilized with other biological matrices (e.g., cervical secretions, breast milk, and parotid saliva)?

In conclusion, mucosal immunity has, for too long, been the forgotten arm of the immune system. A combination of highly effective mucosally delivered vaccines, vaccine challenge studies to define 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.

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