

Overcoming Waning Immunity in Pertussis Vaccines: Workshop of the National Institute of Allergy and Infectious Diseases

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Despite high vaccine coverage in many parts of the world, pertussis is resurging in a number of areas in which acellular vaccines are the primary vaccine administered to infants and young children. This is attributed in part to the suboptimal and short-lived immunity elicited by acellular pertussis vaccines and to their inability to prevent nasal colonization and transmission of the etiologic agent *Bordetella pertussis*. In response to this escalating public health concern, the National Institute of Allergy and Infectious Diseases held the workshop “Overcoming Waning Immunity in Pertussis Vaccines” in September 2019 to identify issues and possible solutions for the defects in immunity stimulated by acellular pertussis vaccines. Discussions covered aspects of the current problem, gaps in knowledge and possible paths forward. This review summarizes presentations and discussions of some of the key points that were raised by the workshop. *The Journal of Immunology*, 2020, 205: 877–882.

Pertussis disease is a spasmodic, paroxysmal cough that causes substantial morbidity and mortality in infants and young children and a persistent cough lasting for many weeks in adolescents and adults. The primary agent is the bacterial pathogen *Bordetella pertussis*, with a variable disease burden being due to infections with *Bordetella parapertussis*. In the first half of the 20th century, pertussis was the primary

cause of infant mortality. Devastating disease tolls led to the introduction of highly effective formalin-inactivated whole-cell pertussis (wP) vaccines along with diphtheria and tetanus toxoids in the childhood vaccination series. This vaccine was very successful in reducing cases (1), but concerns about adverse reactions to the wP vaccine led to a switch to acellular pertussis (aP) vaccines formulated into the current pediatric penta- and hexavaccines diphtheria, tetanus, and aP (DTaP) and tetanus, diphtheria, and pertussis booster (Tdap) vaccines. These vaccines have a significantly improved safety profile when compared with wP and are effective in preventing infant mortality and childhood morbidity due to pertussis disease, at least in the short-term.

Over the past decade, there has been an increase in the cases of pertussis seen in adolescents who have been vaccinated solely with the DTaP vaccines. In addition, there have been several pertussis outbreaks in various age groups and increase in pertussis cases in infants too young to have been immunized. This increase in overall pertussis disease even in the face of high levels of vaccine coverage has raised concerns about this emerging problem. Age-incidence data (discussed below) reveal a shift in disease burden toward adolescents and young adults and this has been attributed to waning of Ab titers. Although serum Abs remain the most accessible measure of vaccine-induced protective immunity, they are not the only contributor to protection, and there is a need to identify firm immunological correlates of protection (discussed below). The problem of decreased vaccine-induced protective immunity over time following vaccination is referred to as “waning

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Abbreviations used in this article: aP, acellular pertussis; CDC, Centers for Disease Control; DTaP, diphtheria, tetanus, and aP; FHA, filamentous hemagglutinin; FIM, fimbria; KPNC, Kaiser Permanente Northern California; NIAID, National Institute of Allergy and Infectious Diseases; PRN, pertactin; PT, pertussis toxin; Tdap, tetanus, diphtheria, and pertussis booster; T_{RM}, tissue-resident memory T; wP, whole-cell pertussis.

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Table I. Overcoming waning immunity in pertussis vaccines: workshop of the National Institute of Allergy and Infectious Diseases

<p>Session 1. Vaccine platforms, technology, targets to improve and/or replace aP vaccines—moderated by Kathryn M. Edwards, M.D.</p> <p>Overview of pertussis—Kathryn M. Edwards, M.D.</p> <p>Pertussis epidemiology and vaccination: A United States perspective—Anna M. Acosta, M.D.</p> <p>Pertussis immunity, acellular vaccines, and model-based inference: Modeling effectiveness and durability of vaccination—Pej Rohani, Ph.D.</p> <p>Session 2. Basic research strategies—moderated by Eric T. Harvill, Ph.D.</p> <p>Improving protective immunity by manipulating bacterial immuno-modulators—Eric T. Harvill, Ph.D.</p> <p>Durable vaccine immunity: Challenges and potential solutions—Purnima Dubey, Ph.D.</p> <p>Correlates of immunity and immunological memory—Kingston H.G. Mills, Ph.D.</p> <p>Session 3. Translational research strategies—moderated by Kathryn M. Edwards, M.D. and Eric T. Harvill, Ph.D.</p> <p>Impact of waning immunity on evolving pertussis epidemiology—Nicola Klein, M.D., Ph.D.</p> <p>Landscape of novel vaccine targets—F. Heath Damron, Ph.D.</p> <p>Innovative pertussis vaccine technology platforms—Peter Sebo, Ph.D.</p> <p>Session 4. Clinical research strategies—moderated by Kathryn M. Edwards, M.D. and Eric T. Harvill, Ph.D.</p> <p>Age-specific TLR7/8 adjuvant formulation overcomes hyporesponsiveness to neonatal aP vaccination in a mouse model—Jay Evans, Ph.D.</p> <p>Better memory through TCF1—Ross M. Kedl, Ph.D.</p> <p>Adjuvant effects of monophosphoryl lipid A—Thomas C. Mitchell, Ph.D.</p> <p>Predictive in vitro immunological assays—Marcela F. Pacetti, Ph.D.</p> <p>Baboon model of pertussis: Predictive preclinical animal models—Tod J. Merkel, Ph.D.</p> <p><i>B. pertussis</i> human challenge induces immunizing, asymptomatic colonization—Robert C. Read, M.D.</p> <p>Discussion of knowledge gaps/key recommendations for future research—moderated by Kathryn M. Edwards, M.D. and Eric T. Harvill, Ph.D.</p>
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immunity.” However, the mechanisms of vaccine or infection-induced protective immunity are complex and poorly understood.

As a consequence of nationwide increases in the incidence of pertussis and more frequent local epidemics, in 2015, the National Institute of Allergy and Infectious Diseases (NIAID) added pertussis to the list of Emerging Infectious Diseases/Pathogens. Shortly after, NIAID held the workshop “Waning Immunity and Microbial Vaccines” for six pathogens, including pertussis, with an emphasis on identifying gaps and developing strategies to maintain or promote sustained vaccine efficacy (2). Subsequently, NIAID encouraged more vaccine research and development through dedicated funding opportunities in this area. In 2019, the Division of Microbiology and Infectious Diseases of NIAID convened a workshop titled: “Overcoming Waning Immunity in Pertussis Vaccines” (Table I). The central goal was to “focus on identification of research and knowledge gaps and strategies to overcome waning immunity of acellular pertussis (aP) vaccines.” Participants were asked to identify knowledge gaps and obstacles and to propose novel strategies to combat the growing problem. In this review, we present an overview of the presentations and discussions and highlight some innovative concepts and approaches that have the potential to address the problems associated with waning immunity to aP vaccines.

Charge to the attendees

K. Edwards (Vanderbilt University) provided historical remarks concerning the original development of the wP vaccines that decreased the incidence of pertussis down to a level such that it was rarely observed clinically. Concerns around the safety of wP vaccines lead to the switch to the aP vaccines. Dr. Edwards played a key role in the multicenter aP vaccine trial and described the data demonstrating that DTaP administration provided protection against severe pertussis with minimal side effects.

Epidemiology and modeling

A. Acosta (Centers for Disease Control [CDC]) detailed efforts at the CDC to increase surveillance and closely track pertussis incidence. Whereas disease burden in infants remains low, there is increased pertussis incidence in adolescents and young adults. N. Klein (Kaiser Permanente Northern California [KPNC]) described how maternal immunization with Tdap

has been highly effective (>90%) in preventing pertussis in newborns (3–5), suggesting that maternal Abs provide significant protection during the first months of life. Recent unpublished data from KPNC showed that 7 y after recommendations for the use of maternal Tdap with each pregnancy, pertussis rates in infancy are very low. This suggests that Ab interference following maternal Tdap is not associated with clinically significant blunting of the infant vaccination and increased risk of pertussis during the first year of life. An examination of pertussis risk by DTaP vaccination status and time since the last DTaP in children revealed that the incidence of pertussis was 13 times higher among unvaccinated and 1.9 times higher among undervaccinated when compared with fully vaccinated children. However, over 80% of cases occurred among children vaccinated at the appropriate age but who were several years away from their last DTaP dose, suggesting that waning immunity was contributing to the recent pertussis outbreaks in California (6). Finally, N. Klein reported that KPNC data from a small pertussis outbreak in California in 2018–2019 showed that older teenagers continue to have a high burden of disease (N. Klein, unpublished data). Although surveillance is complicated by reduced pertussis testing of those 18 y of age and older, the incidence of pertussis in adolescents and adults is underappreciated. There is an increasing need for improved detection methods in adolescent and adults, and especially in those who have been immunized with DTaP vaccines as they reach the age of parenthood.

CDC data has provided clear evidence that the incidence of pertussis is increasing in the United States; however, the exact cause is still not clear. P. Rohani (University of Georgia) has undertaken large scale prediction modeling using data from Massachusetts. The model showed that the outbreaks of pertussis in Massachusetts were likely due to incomplete historical coverage with wP vaccines, followed by a new schedule of a DTaP vaccine that is associated with waning immunity (7). Data from the model suggests that immunization with the aP vaccine is reducing overall transmission but is insufficient for elimination of disease. Their data also suggest that schoolchildren represent the core transmission population (8). The need for correlates of protection was discussed during this and numerous subsequent sessions.

Over the past 5 y, N. Klein and her team have published several studies that provide strong evidence of waning immunity

following a complete course of vaccination with DTaP vaccines. Dr. Klein presented data from the most recent study that included 469,982 children aged 3 mo to 11 y with a total of 738 confirmed pertussis cases. This study concluded that unvaccinated and undervaccinated children were at greater risk of pertussis. However, most pertussis cases occurred among children vaccinated at the appropriate age who were further away from their last DTaP dose. Overall, their data suggested that suboptimal vaccine effectiveness and waning immunity played major roles in recent pertussis epidemics in California. Dr. Klein indicated that their data also show evidence that maternal Tdap immunization is protective during the first months of life, and does not appear to cause substantial Ab interference, although more data are needed. Overall, these studies underscore the importance of understanding why aP vaccine efficacy is not durable.

Basic science: bacteriology and immunology

E. Harvill (University of Georgia) opened the session on basic research by discussing the known immunomodulation by *Bordetella* and posing the following question: if we all believe *B. pertussis* modulates immunity in many ways, why do we accept as a given that convalescent immunity is the optimal immunity achievable? Why is it used as the “gold standard” against which vaccine-induced immunity is measured? He described evidence implicating BtrS as a σ factor regulating several known and putative immunomodulatory factors and involved in survival within phagocytes (9, 10). Surprisingly BtrS somehow mediates suppression of T and B cell recruitment to the lungs of infected mice. In its absence, much higher numbers of these resident lymphocytes accumulated in infected lungs and organized into recognizable lymphoid-like structures. Small doses of the *btrS* *Bordetella bronchiseptica* mutant induce protective immunity to all three classical *Bordetella* species: *B. bronchiseptica*, *B. pertussis*, and *B. parapertussis* (10). Dr. Harvill concluded that convalescent immunity is not the best protection possible, and that its use as the gold standard against which vaccines are measured remains questionable. Protection that is superior to that conferred by prior infection is achievable. These ideas will be further discussed later in this review.

Recent studies have demonstrated that tissue-resident memory T (T_{RM}) cells play a critical role in maintaining long term protective immunity to viral and bacterial infections at mucosal surfaces (11). K. Mills (Trinity College Dublin) presented evidence that infection of mice induces *B. pertussis*-specific CD4 T_{RM} cells in the lungs and nasal tissue that have Th1 and Th17 cytokine profile (12) and that these T_{RM} cells play a critical role in maintaining sustained protective immunity against reinfection. Previous infection or immunization of mice with wP vaccines also primes IL-17-producing T_{RM} cells. In contrast, immunization with current aP vaccines failed to do so and failed to protect against nasal colonization with *B. pertussis* (13). To address this failure, alum was substituted with a novel adjuvant comprising TLR2 and STING agonists in an experimental aP vaccine. The change of adjuvant enhanced the vaccine's ability to induce respiratory T_{RM} cells and to confer protection against lung and nasal colonization with *B. pertussis* (14).

P. Dubey (Ohio State University) addressed durable vaccine immunity, focusing on a protein adjuvant, BcfA, that was

first identified in *B. bronchiseptica* by the Deora laboratory (Ohio State University) (15). Addition of BcfA to a current Tdap, Boostrix, accelerated clearance of *B. pertussis* from the lungs of immunized mice. Furthermore, production of Th2 cytokines was reduced (16). Inclusion of BcfA into aP vaccines delivered to mice by i.m. administration and then boosted by the intranasal route “pulled” CD4 T cells to the mucosa, and generated T_{RM} . The addition of BcfA attenuated the Th2 response, skewing the T cell repertoire toward Th1/17 and accelerating clearance of *B. pertussis* from the lungs. Together, these results suggest that booster immunizations with Th1/17 adjuvant-containing vaccines may improve aP vaccine-mediated protection by repolarizing alum-primed immune responses.

Translational research strategies

The selection of pertussis toxin (PT), filamentous hemagglutinin (FHA), pertactin (PRN), and fimbriae (FIM) as Ags for the DTaP and Tdap vaccines was largely based on immunogenicity data. H. Damron (West Virginia University) discussed the landscape of novel pertussis vaccine targets, considering other known Ags as well as the current vaccine components. Suboptimal doses of DTaP vaccine (1/80th human dose) were used to demonstrate that inclusion of the repeats in toxin domain region of the adenylate cyclase toxin could improve clearance of *B. pertussis* from the airways of challenged mice (17). Approximately a dozen *B. pertussis* Ags have been evaluated in similar studies. Comparing *in vitro* and *in vivo* RNA transcriptomes identified genes encoding proteins expressed during infection (18), including several involved in nutrient acquisition, as potentially promising Ags currently under investigation. Most pertussis vaccines have used whole bacterium, protein subunits, or live-attenuated bacteria. Novel platforms are on the horizon such as mRNA or DNA vaccines. mRNA vaccines encode the Ags of interest and instruct host cells to produce Ag that elicit immune responses. An mRNA platform is currently being evaluated in phase I trials for several diseases, including SARS-CoV-2 (19). Dr. Damron also mentioned that mRNA-based platform vaccines can also be used to produce a more diverse response profile to a higher number of Ags than current acellular vaccines composed of proteins that must be isolated and purified individually.

P. Sebo (Czech Academy of Sciences) presented innovative vaccine technology platforms and synthesized anecdotal data, preclinical studies, and clinical trial data. Dr. Sebo argued that part of the pertussis problem is not necessarily waning immunity but rather “misprimed” immunity or linked epitope suppression. He proposed that immunity induced with aP vaccines results in a high number of Abs that recognize nonprotective, potentially decoy epitopes and do not neutralize the native toxins (20–22). Hence, repeated booster immunization by Tdap may lead to overproduction of Abs that interfere with (rather than promote) neutralization of native PT and thus abrogate protective immunity. Dr. Sebo presented datasets and studies that support the idea that intranasal, as opposed to parenteral, immunization can induce mucosal immunity similar to immunity induced by natural infection. Two such studies showed that wP vaccine administered via the intranasal route was effective (23, 24). In a study in Austria, wP was provided to more than 20,000 newborns by

oral route and was safe and effective (25). Intranasal delivery of BPZE1, a live-attenuated pertussis vaccine (26), as well as outer-membrane vesicles (27), have been shown to induce strong mucosal immunity in adults. Dr. Sebo further proposed that the research community should focus efforts on increasing immunity in children whom he believes are the main reservoir of *B. pertussis*. Recent studies in mice showed that nasal immunization with aP vaccines formulated with novel adjuvants induced more protective local immunity than current parenteral alum-adjuvant vaccines. Dr. Sebo was also supportive of returning to highly effective wP vaccines with reduced reactogenicity in place of aP vaccines. However, several participants stated that reversion to the wP vaccine would likely be rejected by regulators because of its reactogenicity.

Adjuvants to elicit persistent immunological memory

The concept of eliciting persistent memory by replacing alum as the adjuvant for aP vaccines was discussed by several workshop participants. J. Evans (University of Montana/Inimmune), R. Kedl (University of Colorado), and T. Mitchell (University of Louisville) presented three different alternatives to alum, the current adjuvant in aP vaccines. Dr. Evans described preliminary studies that suggested that CRX-727, a novel TLR7/8 agonist, could improve protection in mouse models of infection and baboon studies were planned. R. Kedl discussed the role of TCF1 and its ability to induce T cell memory. Using mouse reporter models, the Kedl laboratory has identified IL-27 as a cytokine that predicts induction of memory as early as 12 h post immunization (28). Dr. Kedl advocated the need for early clinical correlate of vaccine safety and effective immunologic priming.

All approved aP vaccines contain alum adjuvant that elicits Th2-polarized immune responses. Dr. T. Mitchell highlighted difference between two HPV vaccines, Gardasil formulated with alum and Cervarix formulated with AS04, a combination adjuvant consisting of alum adsorbed with monophosphoryl lipid A (29). Dr. Mitchell proposed that the switch from wP to aP vaccines resulted in a loss of one critical component, the TLR4 stimulation activity of the endotoxin. He proposed that addition of AS04 into aP vaccines could increase longevity of immunity, decrease Th2 bias, and enhance production of *B. pertussis*-specific Abs. Overall these presentations highlighted the need to further explore new adjuvants and strategies for pertussis vaccines.

Predictive in vitro Ab assays

M. Pasetti (University of Maryland) discussed in vitro serological assays that might help predict protective immune responses. The evaluation of vaccine-induced immunity, relevant serological readouts, end-points, and robust, practical methods to monitor protective responses are critical in the discussion of pertussis waning immunity. Pertussis immune status has traditionally been determined on the basis of circulating Abs specific for vaccine Ags. PT Abs with in vitro-neutralizing capacity can prevent symptoms of experimental pertussis in mice and baboons (30). In addition, PT-IgG avidity has been positively correlated with Ab titer in vaccinated children (31). However, assays for the effects of Ab binding to other Ags suffer for lack of understanding of their true in vivo roles in infection. An opsonophagocytosis assay distinguished symptomatic patients from those who recovered from disease (convalescents)

and controls (32). Analysis of pertussis Ab glycosylation and Fc function revealed distinct structural features of maternal and infant pertussis Abs and a differential capacity to facilitate neutrophil and monocyte phagocytosis and NK-mediated activity (33). Waning immunity can be explained by a combination of low quantity and low quality (reduced avidity) of Abs; these may be enhanced by booster vaccination (31). Defining the Ab function/s and thresholds that can accurately predict vaccine performance could accelerate the evaluation of improved candidates and guide implementation. New assays have been applied for high throughout measurements of *B. pertussis*-specific Abs (34). However, most studies continue to rely primarily on Ab binding, providing limited new knowledge on their actual in vivo effects on various aspects of infection. There are still knowledge gaps in our understanding of the mechanisms of vaccine-induced immunity and the specific roles of different types of Abs to each Ag.

Baboon and human infection studies

The baboon is a highly relevant animal model of pertussis and provides the unique opportunity to study the natural progression of pertussis pathogenesis and transmission as well as the host responses to infection and vaccination. T. Merkel (Food and Drug Administration) presented a brief summary of aerosol transmission and nasal inoculation studies demonstrating they induce comparable infections with similar clinical manifestations. There is good correlation between mouse and baboon infection models, but initial studies with intranasal immunization have not provided protection in baboons. He hypothesized that this may reflect differences in nasal turbinates between these species, but a better understanding of mucosal immune responses in baboons is needed. Dr. Merkel stressed the need for increased capacity to perform baboon studies, partly addressed by the establishment of the baboon model in the laboratory of Roger LeGrand in Paris (35).

R. Read (University of Southampton), funded by the PERtussIS CORrelates of Protection Europe project, presented on the newly established human challenge model of *B. pertussis* infection in Europe with the potential to identify surrogate markers of protection, and to offer a rapid testing platform for new vaccines (36). Volunteers, aged 18–45 who were all immunized with whole cell pertussis vaccines in their primary infant schedule, received an intranasal inoculum of *B. pertussis* and were admitted to a hospital facility for 17 d, with mandatory treatment with azithromycin at 14 d. The challenge isolate used was strain BP1917, which was isolated in 2000 from a Dutch patient with whooping cough and characterized as *ptxP3-ptxA1-prn2-fim3-2, fim2-1* MLVA27, PFGE BpSR11. BP1917 expresses PT, FHA, PRN, and FIM3. In a dose-ranging infection study involving 34 volunteers, an unexpectedly high dose of 10^5 CFUs was needed to induce colonization of at least 80% of the exposed subjects. Very few subjects had any symptoms and only a few experienced mild rhinorrhea and nasal stuffiness toward the end of their hospital admission. Most of those who were detectably colonized exhibited rises in Abs specific for PT, FHA, or PRN, consistent with the expectation that colonization generally results in induction or enhancement of circulating Abs. Nasal washing was the most sensitive diagnostic technique to detect colonization, whereas pernasal swabbing, the conventional diagnostic technique, was relatively insensitive. Reassuringly,

azithromycin cleared carriage in most people by 48 h. These findings added *prima facie* evidence to support epidemiological and serological observations, which suggest that asymptomatic *B. pertussis* colonization is part of the natural life cycle of the organism within a vaccinated population. Further planned studies using this model will seek to identify immune markers of protection against infection and enable more rapid development of novel vaccines.

Discussion of knowledge gaps and recommendations for future research

The overall goal of this workshop was to discuss the issue of waning immunity to DTaP vaccines and its role in re-emergence of pertussis. For improved pertussis vaccines to be brought to market, mechanistic understanding will need to be bridged to preclinical models (murine and baboon) and clinical studies, such as the human challenge studies. However, traditional efficacy trials in humans may be difficult, as the number of human subjects needed to perform a field efficacy trial of a new pertussis vaccine was an important unknown, as was the feasibility and ethics of such a trial. Mathematical models have greatly improved our understanding of pertussis disease in today's population. Can these approaches be combined with preclinical data from animal studies to better define novel correlates of protection? Several presentations noted the need to identify early biomarkers as correlates of protective immunity to bridge preclinical and clinical studies and mentioned the PERISCOPE project as actively generating relevant data that will eventually be made available to the public and the research community (<https://periscope-project.eu/publications/>).

It is possible that additional pertussis Ags might improve vaccine efficacy, especially against nasal colonization, but more studies are necessary to understand the mechanism of protective immunity in the nasal tract. The observation that a growing proportion of recent isolates in the United States of America do not express PRN raises concerns about the use of PRN-expressing reference strains for testing new vaccines (37). Furthermore, the isolation of Prn-negative strains suggests that adding additional Ags to the vaccine, especially those with a putative role in nasal colonization, may improve vaccine responses (38).

The issues surrounding the short-lived and Th2-polarized immune responses elicited by current alum-adjuvanted vaccines was raised regularly during the meeting. Alum does not appear to promote sustained protective immunity, especially in the upper respiratory tract. However, the fact that alum performs well as an adjuvant for diphtheria and tetanus vaccines suggest that pertussis is not simply another toxin-mediated disease. It also supports Dr. Harvill's theory that *B. pertussis* modulates the immune response in ways that other pathogens do not. Substitution of alum for, or in addition to, Th1/Th17-skewing adjuvants and/or mucosal immunization have the potential to improve protective local immunity against *B. pertussis* in the nasal cavity and thereby prevent nasal carriage and transmission.

The NIAID-organized workshop provided a forum for discussion and exchange of ideas regarding the problem of waning immunity to aP vaccines. This topic will be discussed again at the International Symposium on *Bordetella* in Vancouver in 2022 and in subsequent meetings organized by the newly formed International *Bordetella* Society

(bordetella.org). NIAID officials reaffirmed their strong interest in helping advance the understanding of waning immunity and to improve pertussis vaccines. Two generations of pertussis vaccines have been developed and each have encountered their own limitations. The data and ideas presented in this workshop suggest that a successful next generation vaccine that addresses these limitations is possible and will be the result of collaboration between academic institutions, industry partners, and federal agencies.

Disclosures

The authors have no financial conflicts of interest.

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