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MEETING HIGHLIGHTS

Waning Immunity and Microbial Vaccines—Workshop of the National Institute of Allergy and Infectious Diseases

Xin-Xing Gu,[a] Stanley A. Plotkin,[b] Kathryn M. Edwards,[c] Alessandro Sette,[d] Kingston H. G. Mills,[e] Ofer Levy,[f,g] Andrea J. Sant,[h] Annie Mo,[a] William Alexander,[a] Kristina T. Lu,[a] Christopher E. Taylor[a]

Division of Microbiology and Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, USA[a]; University of Pennsylvania, Philadelphia, Pennsylvania, USA[b]; Vanderbilt University, Nashville, Tennessee, USA[c]; La Jolla Institute of Allergy and Immunology, La Jolla, California, USA[d]; School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland[e]; Precision Vaccines Program, Boston Children's Hospital,[f] and Harvard Medical School,[g] Boston, Massachusetts, USA; University of Rochester Medical Center, Rochester, New York, USA[h]

ABSTRACT Since the middle of the 20th century, vaccines have made a significant public health impact by controlling infectious diseases globally. Although long-term protection has been achieved with some vaccines, immunity wanes over time with others, resulting in outbreaks or epidemics of infectious diseases. Long-term protection against infectious agents that have a complex life cycle and antigenic variation remains a key challenge. Novel strategies to characterize the short- and long-term immune responses to vaccines and to induce immune responses that mimic natural infection have recently emerged. New technologies and approaches in vaccinology, such as adjuvants, delivery systems, and antigen formulations, have the potential to elicit more durable protection and fewer adverse reactions; together with *in vitro* systems, these technologies have the capacity to model and accelerate vaccine development. The National Institute of Allergy and Infectious Diseases (NIAID) held a workshop on 19 September 2016 that focused on waning immunity to selected vaccines (for *Bordetella pertussis*, *Salmonella enterica* serovar Typhi, *Neisseria meningitidis*, influenza, mumps, and malaria), with an emphasis on identifying knowledge gaps, future research needs, and how this information can inform development of more effective vaccines for infectious diseases.

KEYWORDS *Bordetella pertussis*, *Neisseria meningitidis*, *Salmonella*, adjuvants, immunity, influenza, malaria, mumps, vaccines

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Address correspondence to Xin-Xing Gu, guxx@niaid.nih.gov.

Public health has benefitted greatly from the development of over 70 licensed vaccines that prevent infections or limit the infectivity of approximately 30 common pathogens that have historically been significant causes of morbidity and mortality (1–3). Although the goal of vaccination is to establish durable, lifelong immunity, it has become clear that for a number of infectious diseases, vaccine-induced host protective immunity wanes over time (4–10). Participants in an NIAID-sponsored workshop, "Waning Immunity and Microbial Vaccines," reviewed research on six representative vaccines and assessed the issue of waning immunity and possible approaches to generate long-term protection. The workshop discussion focused on novel strategies to elicit, detect, and enhance the persistence of protective immunity elicited by vaccination (11). Here, we provide summaries of the presentations and discussions for each group of vaccines (against pertussis, influenza, malaria, *Salmonella enterica* serovar Typhi, meningococcus, and mumps), along with a description of novel research strat-

egies to provide insights into waning immunity, a summary of the gaps in knowledge for the specific infectious agents, and general recommendations for future vaccine development.

PERTUSSIS VACCINES

Kathryn Edwards (Nashville, TN, USA) noted that in the 1920s and 1930s there were about 200,000 pertussis cases reported annually in the United States (12, 13). Following the introduction of the diphtheria, tetanus toxoid, and pertussis vaccine (DTP) in the 1940s, the number of cases declined (14). However, significant local and systemic reactions after receiving the DTP whole-cell vaccine (WCV) promoted work to develop a less reactogenic component vaccine consisting of purified pertussis antigens (Ags). The first of these acellular vaccines (ACVs) for pertussis combined with diphtheria and tetanus toxoids (DTaP) was introduced in children in the 1990s, after which the Tdap vaccine was introduced for administration to adolescents and adults. As the percentage of individuals who received the ACVs increased, the number of cases of pertussis also increased (15).

During the development and clinical testing of current ACVs, the only immune responses that were assessed were antibodies (Abs), quantified by enzyme-linked immunosorbent assays (ELISAs). While the Ab responses induced by ACVs were higher than those induced with WCVs, by 2 years postvaccination the Ab titers had waned substantially in children immunized with ACVs (5, 6). Importantly, repeated booster doses of ACVs in children primed with an ACV resulted in protection that waned rapidly after immunization. Immunization with an ACV elicited mainly Th2 responses, IgG1, and higher-affinity Abs, and during boosting IgG4 was elicited. In contrast, immunization with WCV elicited mainly Th1 and Th17 responses, moderate-affinity Abs, and IgG1 and provided protection through recruitment and activation of phagocytes by T cells and induction of complement-mediated opsonophagocytosis (16, 17). The immune responses induced with WCV were directed against additional Ags not contained in the current ACV. The progressive loss of protection induced by ACV compared with that by WCV might reflect a failure to induce complement-mediated killing, the induction of Th1 cells, the recruitment and activation of phagocytic cells, antimicrobial defenses, and opsonizing Abs.

During the group's discussion, ongoing studies in The Netherlands were cited, including some focused on a cohort of children who had been primed with ACV or WCV and were followed for 10 to 12 years. The findings to date suggest that B cell memory responses can be boosted more effectively in children primed with WCV than those who receive ACV. Findings from studies in Australia and data extrapolated from a Kaiser Health Plan study suggest that the first immunization to a pertussis vaccine might set the type of the subsequent immune response enhanced by future booster vaccinations (18).

Using a murine model, Kingston Mills (Dublin, Ireland) compared immunity induced with WCV to that induced with ACV and found that the bacterial burden in the lungs was significantly lower after *Bordetella pertussis* aerosol challenge of mice immunized with WCV than in those immunized with ACV. *B. pertussis*-specific Ab titers, quantified by using an ELISA, were moderate in mice immunized with WCV and high for those immunized with ACV. Similar to studies in humans, the T cell responses induced with ACV in mice were skewed to Th2, whereas the responses induced by WCV were skewed to Th1 and Th17 cells. Furthermore, the Ab subclasses induced with WCV in mice were IgG2a/c, which have an opsonizing function, whereas ACV induces IgG1, which has a toxin-neutralizing role.

In studies of lung tissue-resident memory (T_{RM}) cells, K. Mills used FTY720, a selective S1P antagonist that inhibits lymphocyte egress from the lymph nodes (LNs) of *B. pertussis*-infected animals. He reported that CD4 and $\gamma\delta$ T_{RM} cells expanded in the lungs despite a block of T cell migration from LNs and that these cells expanded rapidly in the lungs following reinfection and mediated immediate bacterial clearance (19, 89). Further experiments showed that transfer of CD4 T_{RM} cells from the lungs of conva-

lescent mice conferred protection to naive mice. Importantly, CD4 T_{RM} cells expanded in the lungs following *B. pertussis* challenge of mice immunized with WCV, but not in those immunized with ACVs. Additionally, the lung CD4 T_{RM} cells were *B. pertussis* specific and secreted more gamma interferon (IFN-γ) and interleukin-17 (IL-17) than circulating CD4 T cells.

To determine whether the immune response generated by ACV vaccine could be enhanced, Mills and colleagues used a novel adjuvant combination that included LP1569, a synthetic lipopeptide derivative of BP1569, a lipoprotein Toll-like receptor 2 (TLR-2) agonist that is also an antigenic component of *B. pertussis* (20). In mice, the combination was superior to alum as an adjuvant for an ACV and equal to a WCV with respect to induction of Th1 and IgG2c Abs and protection against *B. pertussis* challenge (A. C. Allen and K. H. G. Mills, unpublished data).

Alessandro Sette (La Jolla, CA, USA) explored the mechanism of waning immunity by comparing human T cell responses to ACV versus WCV at least 17 years after the original priming and following repeated ACV boosts (21–23). By studying *ex vivo* responses and a "pertussis megapool" of defined human T cell epitopes, 601 epitopes were defined. The results indicated that the Th1 versus Th2 polarization induced following immunization in childhood with WCV and ACV, respectively, persisted after boosting of these subjects as adolescents and adults with ACV. Priming with WCV, but not with ACV, was associated with higher $CD4^+$ T cell counts after booster immunization with ACV. The studies suggested that there was no difference in memory T cell subsets induced by ACV (with or without an ACV boost) or WCV (with or without an ACV boost).

Studies of the evolution of proliferative responses to *B. pertussis* Ags in WCV-primed versus ACV-primed individuals as a function of age showed that cells from ACV-primed 4-year-old children proliferated better than those from WCV-primed 4-year-old children following an ACV boost. However, there was no difference in proliferation in 10-year-old children. Sette hypothesized that there is a teenage-related shift in relative proliferative capacity and tested this hypothesis with the carboxyfluorescein succinimidyl ester (CFSE) assay. Unpublished results showed that the original WCV priming is associated with a higher proliferative capacity, suggesting that in 18- to19-year-old teens, the proliferative response decreases in comparison to that in WCV-primed adults and that original WCV priming generates a T cell response associated with a long-lasting proliferative capacity.

Tod Merkel (Silver Spring, MD, USA) described a baboon model of pertussis that has been used to test new vaccine development strategies. The model recapitulates many aspects of the clinical manifestations of the human diseases and the induction of protective immunity by infection or vaccination (24, 25). Infected baboons exhibited classical signs of pertussis, including paroxysmal coughing, leukocytosis, and a high number of bacteria in the airway. After *B. pertussis* infection, the animals became colonized and the bacteria initially persisted in the airway. Over time their numbers declined slowly, and the animals cleared the infection after approximately 4 weeks. Th17-associated cytokines, but not Th1- or Th2-associated cytokines, were found in nasopharyngeal washes. Challenge studies showed that although ACVs protected the animals against disease, as determined by evaluation of coughing and leukocytosis, ACVs failed to protect against *B. pertussis* carriage, and animals remained colonized for a longer period of time than naive animals. Additional studies found that the ACVs failed to protect against subsequent infection by natural transmission or prevent transmission from colonized asymptomatic vaccinated animals. In comparison with ACV and convalescent animals, animals receiving WCV were initially infected at the same level as animals receiving ACV, but they were able to clear bacteria more rapidly. Both ACV and WCV reduced CFU of *B. pertussis* in the lower airway. Studies assessing cytokine induction (IFN-γ, IL-5, and IL-17) found parallels with humans and mice, with ACV inducing a strong Th2 response (IL-5). However, the host immune response to WCV was more like the response to natural infection, but with lower concentrations of IL-17 and IFN-γ.

INFLUENZA VACCINES

Influenza virus infections and pandemics in humans have been documented for over 500 years, with high morbidity and mortality globally (26, 27). After the successful culturing of the virus in the 1930s, inactivated influenza vaccines (IIV) were developed and led to successful human protection from the infection in the 1940s. Since then, many improvements have been made, with rapid production of influenza virions and increased purity and safety. Most influenza vaccines contain proteins from two influenza A virus strains and one to two influenza B virus strains (28, 29). The vaccines are designed to promote neutralizing Ab responses to the hemmaglutinin (HA) protein, which controls influenza virus attachment and entry. Sterilizing immunity can be generated in humans and animal models, with correlates of protection generally associated with the titer of serum anti-HA Ab. Current licensed IIV are produced from virions grown within infected embryonated eggs or cultured mammalian cells. A live-attenuated influenza vaccine administered by the intranasal route has also been used, with the aim of eliciting both mucosal and systemic immunity against viral infection. Recently, vaccines composed of purified HA proteins produced in baculovirus have been licensed. Most recently, non-HA-specific Abs, such as Abs for neuraminidase, have been shown to provide protection from influenza virus infection, with most of the alternative correlates of protection induced by vaccines with nonneutralizing Abs and cellular immunity, diminished virus replication and virus-induced disease, rather than providing complete sterilizing immunity (29, 30).

Despite these advances in production and vaccination, complete vaccine efficacy in prevention of disease is lacking. One of the key factors is the ability of influenza virus to evade vaccine-induced immunity via mutation. Genetic drift, i.e., mutations in the HA protein, which are enabled by an "error protein" DNA polymerase, allows for continual generation of variants that can escape neutralization. Every 1 to 2 years, there are sufficient genetic and serological alterations in the circulating influenza viruses to require reformulation of the vaccine using the influenza virus strains anticipated to be the most prevalent in the upcoming season, followed by readministration of the vaccines. Nevertheless, even in the absence of genetic drift, there has been a noted persistent problem over time of waning immunity induced by the influenza vaccines. Depending on the vaccine administered, the study population, and the method of quantifying immunity, protective immunity to influenza virus wanes between 6 and 18 months after vaccine administration (4, 31, 32). The events involved in diminished protection induced by influenza vaccines were the focus of two presentations at the workshop, each of which generated much discussion.

Rafi Ahmed (Atlanta, GA, USA) focused on the relationship between influenza virus-specific Abs that were detectable in the serum and in bone marrow (BM) plasma cells in humans. He noted that the long-lived plasma cells that reside in the BM could secrete Abs without any antigenic stimulation and that the plasmablasts in the blood were not the cells that provided long-term Ab responses. His studies found a strong correlation between the levels of influenza virus-specific IgG in the blood and the number of influenza virus-specific plasma cells in human BM. Interestingly, the correlation between influenza virus-specific memory B cells and serum Ab levels was not as strong. Studies are in progress to examine how BM plasma cell numbers change after immunization with the seasonal influenza vaccine. A subsequent discussion focused on how infection and vaccination differ in generating long-lived immunity (33, 34). It is known that humans infected with influenza virus decades previously possess circulating Abs reactive to the original infecting strain. This raised the issue of whether purified inactivated protein vaccines administered in the absence of adjuvant might elicit plasma cells that have less persistence in the BM or whether such niches of the human host are preferentially occupied by B cells elicited by pathogen infection early in the life of the host.

Andrea J. Sant (Rochester, NY, USA) addressed the role of CD4 T cells in the early

response to influenza virus infection and vaccination based on her work with a murine model in which the host is prepopulated with CD4 T cell memory cells specific for either HA or a control nucleoprotein (NP). The results have shown that populating a host with CD4 T cell memory cells enhances the germinal center (GC) and Ab responses to influenza virus infection, suggesting that CD4 T cell help is a limiting factor in the B cell response to infection. Analysis of serum Ab specific for HA and NP by ELISA showed that a match between CD4 T cell help and Ab specificity, as well as HA-specific CD4 T cell memory, are needed for accelerated Ab responses (35). These results suggested that efforts to promote CD4 T cell immunity specific for HA would likely enhance long-lived neutralizing Ab responses, as CD4 T cells help promote B cell affinity maturation to plasma cells and B cell memory cells.

To explore the potential response to pandemic strains of influenza virus, she described a study done with John Treanor at the University of Rochester to examine the vaccine implications for involved individuals who had received one vaccine (A/Vietnam/1203/04 2005), two vaccines (A/Hong Kong/156/97 1998 and A/Vietnam/1203/04 2005), or no vaccine 5 years earlier. These studies revealed that prior H5 HA vaccination, years earlier, with a serologically distinct H5 protein led to persistent H5-specific CD4 T memory cells that were recalled into the subsequent response and that the CD4 T cell response to HA, but not NP, correlated with higher microneutralization titers (36). Sant noted that because many conventional influenza vaccines were produced from virions, they contained internal virion proteins. Thus, CD4 T cells of many specificities, including those specific for proteins such as NP and M1, will be recruited into the response (36–38). She speculated that repeated vaccination with the IIV annually might lead to expansion and accumulation of CD4 T cells specific for those conserved virion proteins, and they might compete with or antagonize HA-specific CD4 T or B cell responses (39). Collectively, this competition may lead to diminished long-lived neutralizing Ab responses. She discussed ongoing experiments evaluating the response of healthy volunteers to egg-derived IIV (Fluzone), MDCK cell-derived IIV (Flucelvax), and pure HA (FluBlok) to determine if diminished levels of certain proteins might elicit a more robust Ab response. Additional discussion focused on issues of CD4 T cell immunodominance and the nature of the Ag taken up by the HA-specific B cells after infection and vaccination. Also considered was that the contribution of CD4 T cells to the neutralizing Ab response may be either positive or negative, depending on the viral Ag specificity and the effector phenotype of the CD4 T cells (40). The discussion group suggested that the specific composition of administered influenza vaccines might have an important role in dictating long-term immunity. Future efforts might consider the modes of vaccination needed to generate persistent memory B cells, memory T cells, and long-lived plasma cells. Inclusion of adjuvants may successfully recruit those necessary functional cells and initiate robust and persistent immunity in the host. These strategies, together with new candidates for universal influenza vaccines (41), offer the potential for long-term immunity with better coverage against influenza virus infection.

MALARIA VACCINES

Malaria is one of the most important tropical diseases globally, with 212 million clinical cases and 429,000 deaths reported in 2015 (42). Recent advances in the field of malaria vaccine development have led to a promising vaccine, RTS,S, which is being considered for widespread deployment. The RTS,S vaccine is a self-assembling virus-like particle (VLP) thast consists of a fragment of the *Plasmodium falciparum* circumsporozoite protein (CSP) fused to the hepatitis B virus surface Ag (HBVsAg), and it is formulated with the AS01 adjuvant. The vaccine, however, only produces a moderate level of efficacy against clinical malaria, with 33 to 50% protection in older infants, and this protection wanes over time without additional boosters (43). Other promising vaccine approaches include whole sporozoite-based vaccines that are either attenuated by irradiation (e.g., PfSPZ) or **c**hemo**p**rophylaxis and **s**porozoite (CPS) vaccination strategies, in which volunteers are given live sporozoite immunizations together with

chloroquine (CQ) chemoprophylaxis (CPS-CQ) to block development of parasitemia and diseases (parasite "attenuation" *in vivo*). Clinical experience with the PfSPZ vaccine in the Controlled Human Malaria Infection (CHMI) Trials in malaria-naive individuals also indicated a decrease in vaccine efficacy over time, despite the initial relatively high level of protective efficacy (44). The underlying mechanisms of the losses in vaccine efficacies over time are unclear.

Eleanor Riley (London, UK) summarized immunological observations from clinical trials of RTS,S and from studies of natural immunity. A mathematical model of sporozoite infection fitted to data from early RTS,S clinical trials with the CHMI in a malaria-naive population indicated that an extremely high anti-CSP Ab titer was required to achieve more than 90% vaccine efficacy (45). In the large multicenter phase III trial (43) in areas where malaria is endemic, the study involved two groups of children, 6 to 12 weeks of age and 5 to 17 months of age; low vaccine efficacy as well as short durability (after the initial vaccination without a booster) were demonstrated. Immunologically, peak anti-CSP Ab titers of 1,000 μg/ml, the requisite for 80 to 90% efficacy, were only achieved in less than 50% of vaccinees. In addition, the overall IgG titers declined rapidly following a biphasic model (short-lived and long-lived components) after primary vaccination. The last booster vaccination at 18 months did extend the vaccine efficacy and anti-CSP Ab durability in the group of older children and increased the long-lived Ab-secreting cells (ASCs) from ~10% to ~30%. Overall, the anti-CSP Ab titers seemed to correlate with the level of vaccine efficacy over time.

On the other hand, evidence accumulated from studies of natural infections seems to suggest that chronic Ag exposure might also prolong Ab responses. For example, anti-malaria Abs showed a sharp peak pattern in children but a rather stable level in adults in Gambia (46). In Ghana, IgG titers decayed more slowly in persistently infected children and in older children (47). Comparison of a cohort of children in Ghana from birth to 2 years of age with a cohort in Gambia aged 1 to 6 years also suggested that the proportion of long-lived ASCs was higher in the older group (48). In Kenya, where there are many malaria infections, serum IgG titers rapidly declined in children with acute malaria, in contrast to sustained Ab and memory B cell responses in adults in Thailand, where malaria infections are low and less acute malaria disease is found (49). Interestingly, animal data have suggested that severe malaria infections lead to inflammatory cytokine production and hence impair the GC response by inhibiting Tfh cell differentiation (50), which may explain the failure to boost the peak response, the slow affinity maturation, and the accumulation of "atypical" memory B cells in population studies.

Robert Sauerwein (Nijmegen, Netherlands) explored the topic by comparing CPS-CQ immunization in which live sporozoite immunizations were carried out by mosquito bites versus transmission of the disease naturally in the field. In this case, the CPS-CQ induced 100% homologous protection in naive individuals in the CHMI trial and long-lived cellular responses, including IFN-γ production by $\alpha\beta$ T cells and $\gamma\delta$T cells (51, 52). Clearly, sustained antimalaria immunity, which cannot be easily achieved in field situations, can be induced in naive individuals. Comparison of the CPS-CQ Ab responses with those from semiimmune Kenyans showed different antigenic profiles. CPS-CQ-immunized individuals reacted preferentially to 84 preerythrocytic Ags, while the Kenyan participants reacted preferentially to 238 blood-stage Ags. Both groups reacted to about 90 cross-stage Ags (53, 54). It is possible that different antigenic profiles contribute to the differences in immune sustainability or/and the CQ drug may modify the T and B cell memory responses.

In summary, long-lived antimalaria immunity seems possible via live sporozoite CPS-CQ immunization strategies for malaria-naive individuals. However, vaccination in the field did not seem to easily induce long-lived cellular or humoral immunity. Exposure to malaria infection and Ag expression characteristics seem to be associated with the duration of natural immunity. Yet, frequent malaria and other infections may also impair BM homing and persistence of ASCs, as inflammation

may draw the ASCs to tissues where they die, rather than to the BM where the response is sustained in long-lived plasma cells. There remains a great challenge to determine if longevity of vaccine-induced immunity is affected by the intrinsic biological complexity of the parasite infection or the suboptimal performance of vaccination.

SALMONELLA ENTERICA SEROVAR TYPHI VACCINES

Marcelo Sztein (Baltimore, MD, USA) described work with *S.* Typhi, which infects over 20 million individuals and causes over 200,000 deaths annually (55). Moreover, antibiotic resistance is appearing in Southeast Asian populations (56, 57). Currently, Typhim Vi and Vivotif are the two licensed typhoid vaccines in the United States. Typhim Vi contains the cell surface Vi polysaccharide extracted from the *S.* Typhi Ty2 strain and protects persons 2 years of age and older for 2 years after one injection. Vivotif contains the live attenuated strain *S.* Typhi Ty21a and protects persons older than 6 years of age for at least 5 years following four doses administered orally. The waning of protection from these vaccines creates the need to revaccinate and, therefore, increases the cost and feasibility to roll out these vaccines for areas where typhoid is endemic. An updated Vi conjugate vaccine is expected to potentially improve the nature of the antigenic response, with better durability (58).

Though the immunological correlates of protection are unclear, Abs to *S.* Typhi Ags (e.g., Vi, lipopolysaccharide) might play an important role in defense against typhoid bacilli when they are extracellular. Since *S.* Typhi persists intracellularly, cell-mediated immunity (CMI) is expected to be essential in eliminating *S.* Typhi from the infected cells. In those orally immunized with attenuated *S.* Typhi vaccines, the key effector CMI components to *S.* Typhi-infected targets included cytotoxic T lymphocytes and IFN-γ production mediated by both $CD8^+$ (dominant) and $CD4^+$ cells (59–62). These responses also involve proliferative responses and dominant production of type 1 cytokine responses to soluble *S.* Typhi Ags (e.g., IFN-γ). Long-term multifunctional HLA-E-restricted $CD8^+$ cells coexpressing IFN-γ, tumor necrosis factor alpha (TNF-α), and CD107 were also found (63). Interestingly, expression of homing molecules that direct these *S.* Typhi-specific central and effector memory T (T_{EM}) cell subsets to migrate to mucosal and nonmucosal tissues have also been reported (59, 64).

To assess if defined effectors are associated with protection from typhoid disease (TD), Sztein worked with Andy Pollard at Oxford University to develop a human typhoid challenge model. The first challenge study involved 41 healthy participants who received either a high or a low dose of wild-type *S.* Typhi. Of the participants, 61% were diagnosed with TD between 6 and 14 days after challenge, received antibiotics, and were followed for up to a year (65). Higher baseline *S.* Typhi-specific responses were associated with protection from TD, and subjects with TD had a delayed time to diagnosis, with multifunctional T cells likely playing an important role in protection (66). The postchallenge kinetics of *S.* Typhi-specific $CD8^+$ T_{EM} cell responses differed, depending on the clinical outcome. While participants who developed TD exhibited marked decreases in circulating *S.* Typhi-specific T_{EM} cells expressing CD107a and producing cytokines following challenge and before TD, the levels of *S.* Typhi-specific cells remained unchanged in volunteers who did not develop TD. These observations, together with results of studies on the expression of homing molecules in *S.* Typhi-specific $CD8^+$ T cells in these volunteers, were suggestive of the homing of these effector cells to the gut (the primary infection site) and nonmucosal sites. Studies of T regulatory (T_{reg}) cells in these volunteers showed that expression of integrin $\alpha4\beta7$ is upregulated before challenge in circulating *S.* Typhi-specific T_{reg} cells in TD volunteers (67). Increased expression of activation molecules in *S.* Typhi-specific T_{reg} cells was seen in TD volunteers and suggested that a balance between T_{EM} and T_{reg} responses is needed for protection. Testing of improved vaccine candidates in the human typhoid challenge model would allow for measurement of the balance between T_{EM} and Treg responses and their link to the duration of protection.

MENINGOCOCCUS VACCINES

Lee Harrison (Pittsburgh, PA, USA) noted that *Neisseria meningitidis* is a major cause of invasive bacterial disease globally and resulted in an estimated annual 500,000 cases, before the recent widespread use of vaccines, which are transforming the meningococcal disease landscape (68). Polysaccharide-protein conjugate vaccines, with their superior immunogenicity in infants and their ability to reduce pharyngeal carriage of *N. meningitidis* and induce immunologic memory, are replacing polysaccharide vaccines. Current challenges of nonspecific symptoms, the sudden onset, and the rapid progression of the infection have resulted in a limited time frame for both diagnosis and treatment of patients (69). Therefore, a long-term immunity after vaccination is desired. Data suggested that children receiving the meningococcal C conjugate vaccine at 2, 3, and 4 months of age had good immunological memory at 12 months of age (70). However, there was a rapid waning of serum bactericidal Abs, with only 46% and 12% of children having protective levels at 9 to 12 months of age and 4 years of age, respectively (71). In contrast, Abs were more persistent in children who were immunized during adolescence, with 95% retaining protective levels after 1 year and 92% retaining protective levels of Ab 3 years after immunization.

Despite the induction of immunologic memory, there is no evidence for vaccine effectiveness in infants more than 1 year after immunization (72). In addition, it was demonstrated that substantial memory Ab responses in adolescents require 6 to 7 days to be generated (73), which is longer than the meningococcal disease incubation period (74). Taken together, these data indicate that long-term protection from meningococcal disease requires persistence of serum bactericidal Abs such that immunologic memory in the absence of protective (bactericidal) Ab levels is insufficient.

During the discussion, it was noted that the 2-, 3-, and 4-month meningococcal C conjugate vaccine regimen used in the United Kingdom has been modified to administration at 3, 4, and 12 months, which provides a more durable (persistent) Ab response. The schedule has been revised since then.

MUMPS VACCINES

Steve Rubin (Silver Spring, MD, USA) noted that in the prevaccine era, mumps was an acute infection of childhood and the leading cause of viral aseptic meningitis and encephalitis in developed countries, and it was the most common cause of unilateral acquired deafness in children. Studies indicate that protection is mediated by Abs, that seronegative persons are highly susceptible, with attack rates correlating inversely with the levels of neutralizing Abs, and that maternally acquired Abs and passive administration of mumps virus-specific Ig confer protection. Although virus-specific CMI persists for more than 20 years postinfection and T cell help is clearly needed for Ab responses, there is no correlation between the nature or magnitude of the CMI and protection.

The number of mumps cases has declined from over 200,000 to a few hundred cases since the introduction of the monovalent mumps vaccine in 1967 and subsequent licensure and use of MMR (measles, mumps, and rubella) vaccines in the United States. Similar success was achieved globally. However, between 2004 and 2006, large outbreaks began appearing in countries where mumps was previously under control, including the United States. Uncharacteristically large outbreaks have occurred globally. Most cases occurred on college and university campuses among young adults, nearly all of whom had been vaccinated during early childhood (9). Data from outbreak studies showed that the odds of developing mumps increased by 10 to 27% with each year postvaccination, suggesting waning immunity as the basis for these outbreaks (10, 75). This observation was consistent with data from other studies that showed that mumps virus-neutralizing Ab titers declined significantly with time postvaccination (76, 77), although the level of Ab that confers protection was not known.

MMR was initially given at 12 to15 months of age and then at 4 to 6 years of age. The CDC and FDA investigated use of a third dose during adolescence as a means of addressing the apparent waning immunity. In that study, virus-neutralizing Ab titers

increased significantly in response to the additional dose but then retreated to baseline within 1 year (78). The results were not supportive of inclusion of the routine administration of a third dose of vaccine. Research is now focused on other approaches to improving the longevity of the immune response to vaccination, such as use of a heterologous prime-boost regimen or rational design of vaccine Ags to stimulate greater numbers of virus-specific memory B cells and greater Ab avidity. The latter is based on data demonstrating that the numbers of virus-specific memory B cells and the avidity of Abs are significantly lower for the mumps virus component of the vaccine, compared to the measles and rubella virus components (79, 80).

NOVEL TRANSLATIONAL AND CLINICAL RESEARCH STRATEGIES TO PROVIDE INSIGHTS INTO WANING IMMUNITY

New technologies and strategies have emerged with capacities not only to measure and predict potential waning immunity but also to elicit and moderate long-term immunity for some vaccines.

Galit Alter (Boston, MA, USA) addressed systems serology as a technology that could capture information on structure-related Ab functions, which could then provide insights into how Abs themselves may contribute to durability of an immune response. Abs such as IgG can tune their functionality through two modifications in the Fc domain: (i) the selection of one of the four subclasses and (ii) changes in the glycan that is located between the two Fc arms. Deconvoluting the mechanisms of Abs that individuals generate to an Ag can provide insight into how the host controls the immune response after infection or vaccination. We can measure (i) the Abs themselves and (ii) Ab functionality, e.g., complement fixation. Such signature analyses can provide correlates of immunity and insights into Ab function.

Galit Alter then provided an example of this approach for determining the factors that predict the evolution of broadly neutralizing Ab responses to HIV. Abs from neutralizers exhibit enhanced functional activity. When purified Abs are then used to immunize mice, the neutralizer Abs elicit quality Abs and higher frequencies of B cells, including in the GC of the draining LNs, compared to vaccination with nonneutralizer immune complexes. The superior immunogenicity of immune complexes from neutralizers is linked to changes in Ab glycosylation, as previously shown by Dilillo and Ravetch (81). Galit Alter noted that sialylated Abs might have other functions, beyond their role in regulating B cell activation, as shown by Wang et al. (82), functions specifically related to GC formation. Thus, during the discussion, she stated that the carbohydrates could be both biomarkers and predictors and that the carbohydrate selection was controlled at the level of B cell priming.

Ofer Levy (Boston, MA, USA) discussed the Precision Vaccines Program, a program for characterizing vaccine-induced protection that can inform development of vaccine formulations for distinct and vulnerable populations. The program employs cutting edge technologies, such as human *in vitro* platforms and systems biology. Many factors, including age, sex, genetics, demographics, and the environment, affect the human immune response. Infectious causes of death are most common in the very young and the elderly, with the former being especially vulnerable, including the ~11% of newborns who are delivered preterm and have demonstrated impaired Th1 immune responses and heightened susceptibility to infection through the teenage years (83). Levy's team has developed microphysiological systems to model immune ontogeny, considering the humoral and cellular differences related to age, and they anticipate that these systems provide distinct information from animal models, which are costly and reflect species-specific biology. Given the large number of candidate adjuvant systems, Ags, and possible formulations, large-scale human clinical trials of each potential vaccine formulation would be prohibitive with respect to time and resources. In this context, human *in vitro* systems may allow selection of lead formulations tailored to distinct populations for *in vivo* studies, thereby increasing predictability/yield and reducing the risk of failure.

Key to the *in vitro* modeling approach are the use of human systems that are age

specific and include autologous human plasma, which is a rich source of age-specific immunomodulatory factors (84), the use of primary leukocytes and epigenetic programs, and avoiding the use of exogenous cytokines or heat-treated conditions. The use of *in vitro* systems that model soluble (plasma) and cellular age-specific human immune responses and the systems biology ("omics") approaches to biomarker and pathway discovery have enabled (i) the identification of age-specific adjuvants/adjuvant combinations to inform targeted adjuvanted vaccine development (85) and (ii) the benchmarking of new versus licensed vaccines to accelerate age-specific vaccine development. *In vitro* human newborn and adult monocyte studies have shown the following: (i) adjuvant, age, and kinetic concordance of monocyte secretome proteins *in silico* with gene expression levels in adults immunized with an monophosphoryl lipid A-adjuvanted malaria vaccine; (ii) TLR7/8-activating imidazoquinolines are more potent and efficacious than alum in inducing IL-1β and TNF from human neonatal and adult monocytes (86); and (iii) TLR7/8 adjuvantation dramatically accelerates and enhances neonatal immune responses to pneumococcal conjugate vaccine (PCV-13) in newborn nonhuman primates (87).

Jay Evans (Missoula, MT, USA) discussed adjuvant systems as an approach to address some of the challenges in vaccine development, and he defined the role of adjuvants as being able to enhance, modulate, and direct the appropriate immune response to a vaccine Ag, promote CMI or humoral immune responses, enhance immunogenicity to weak Ags, reduce the dose of Ag required, improve efficacy in hard-to-reach populations, improve immunologic memory, and expand T and B cell repertoires in order to gain vaccine-induced long-term immunity.

Historically, adjuvant selection and development have been largely based on trial and error. The next generation of adjuvants are being developed with a focus on directing an appropriate/desired immune response and improved therapeutic index that is based on the pathogen/disease and target population. With challenging diseases or target populations, a single adjuvant might not be sufficient, and adjuvant systems, i.e., specific combinations of adjuvants and/or formulations tailored to enhance the most appropriate specific immune response, may be needed. The first generation of adjuvant systems are included in licensed vaccines for human use or are in late-stage clinical testing, e.g., AS04, AS01 (Liposome, MPL, QS21 [for malaria and varicella zoster]), AS03, and MF59 (squalene, Tween 80, and Span 85 in an oil-water emulsion [for pandemic influenza]), with the next generation of adjuvant systems in development. There are several Th1-, Th2-, and mixed Th1/2-based adjuvant systems, but additional work is necessary to identify and develop new Th17 and T_{reg} cell-modifying adjuvants either to suppress or direct immune cells. Long-term humoral and cellular immunity could benefit from the use of adjuvant systems targeting CMI and proper GC development.

Evans presented two studies related to adjuvants and waning immunity. The first study, by Martins et al., was related to an Ebola-VLP vaccine with which mice had great short-term protection against Ebola challenge but lost protection over time (88). Testing a series of different adjuvants to increase long-term durable immunity showed that only poly(IC) was effective at inducing long-term immunity. Mechanistic investigations showed that the correlates of long-term immunity were a Th1-skewed Ab response, the IFN-γ CD4 T cell frequency, and draining LN T_{fh} cell frequency at 7 days postvaccination. The second study (unpublished data) used an influenza virus challenge model in mice, in which two different adjuvants (adjuvant 1 [Th1/2] and adjuvant 2 [Th1]) were used with intramuscular split-flu vaccinations. Animals were challenged early and late after immunization. Both adjuvants promoted strong serum anti-influenza virus Ab titers that protected animals from challenge at 28 days postboost. When mice were challenged at 248 days postvaccination, animals vaccinated with adjuvant 1 lost more weight than control animals or those who had received adjuvant 2, and this increased weight loss seemed to represent disease exacerbation. It was noted that all mice vaccinated with either adjuvant recovered from the challenge, leading to 100% survival. Subsequent histopathological studies found differences in the

levels of eosinophilia in the lungs, with mice that received Ag alone showing moderate to severe levels, mice that received Ag plus adjuvant 2 showing mild levels, and mice that received Ag plus adjuvant 1 showing moderately severe to severe levels. Thus, both the short-term and long-term effects of adjuvants on humoral and CMI responses and on protection need to be assessed.

SUMMARY OF THE GAPS IN KNOWLEDGE FOR SPECIFIC AGENTS

Pertussis. For pertussis, the knowledge gaps include the following: the mechanism of accelerated waning protective immunity despite repeated immunization with ACVs; the effect(s) of adding new pertussis Ags to the existing DTaP vaccines to enhance immunity with ACVs; the impact of skewing of the Ab response to IgG4 and IgE on waning immunity with ACVs; and the potential for a third-generation pediatric ACV with a Th1/Th17/T_{RM} cell-inducing adjuvant(s) to induce long-term immunity.

Influenza. Regarding influenza vaccines, there remain the following knowledge gaps: the mechanism through which some antagonistic CD4 T cell specificities develop and mediate suppression of Ab responses; the effect of selective CD4 priming on establishment of B cell memory; the role of adjuvants or repeated priming/boosting in potentiating or antagonizing subsequent responses; and the mechanisms/factors that lead to reduced life span/persistence of influenza vaccine-induced new Ag-specific BM plasma cells.

Malaria. Gaps in knowledge about malaria include the following: how confounders (e.g., pathogen exposure, Ag persistency, coinfection, etc.) affect vaccine-induced immunogenicity and efficacy, and/or how a diverse array of vaccine products (e.g., different Ags, formulations, delivery systems, etc.) influence the durability of immune responses; the effects of drug treatment and the contribution of trained innate immune cells on the vaccine response; identification of target Ags and phenotypical and molecular characterization of Ag-specific T cells and the balance of T_{eff}/T_{reg} cell functionality.

***Salmonella*.** In order to obtain more complete knowledge of *S.* Typhi, the following gaps require attention: the need for additional volunteer challenge studies with wild-type *S.* Typhi to investigate the innate and adaptive responses to candidate vaccines; a focus on the identification of correlates of protection between protected versus unprotected individuals; integration of study findings with systems biology and gut microbiome assessments; and exploration of the effects of age, gender, and preexisting immunity on vaccine responses.

Meningococcal disease. New technologies are needed to induce faster immune memory responses to meningococci, given the rapid course of this disease, and to achieve more vigorous and persistent bactericidal Ab responses, especially in infants.

Mumps. As boosting with the current vaccine is unlikely to address waning immunity, immune informatics and novel adjuvant systems hold promise for the design of improved vaccines.

GENERAL RECOMMENDATIONS

The NIAID Workshop produced the following general recommendations.

- Apply modern immunologic tools to delineate the correlates of immunity for vaccines that induce durable immunity, compared with vaccines associated with waning immunity in human trials, and identify components of sustained and effective long-term T and B cell memory kinetically
- Establish validated assays to facilitate a comparison of immunity induced after natural infection versus immunity elicited by vaccination
- Study the effects of various adjuvants, delivery systems, formulations, and immunization routes to enhance long-term protective immunity induced by existing vaccines
- Develop novel technologies (adjuvants, delivery systems, formulations, and im-

munization routes) to induce long-term immunity, especially in the very young, elderly, and pregnant populations

- Determine if the low efficacy of a vaccine is inherent in the product or a reflection of the pathogenic properties of the infectious agent
- Use and develop appropriate animal models to evaluate and confirm long-term immunity with comparable vaccines/Ags
- Evaluate the impact of demographic factors, existing immune status, Ag/pathogen persistence, special populations, and/or genetic determinants on the quality, magnitude, and persistence of effective immunity from vaccination and natural infection
- Assess innate and adaptive effector and regulatory immune responses, including local humoral (IgA) and cellular (T_{RM} cells) responses, to understand how multiple host defense mechanisms operate in concert to induce long-term immunity
- Develop human *in vitro* systems to model age-specific innate and long-term adaptive responses to accelerate and derisk development of vaccines that induce persistent immunity
- Build capacity to integrate and analyze systems biology (e.g., "omics" measurements, biomarkers, microbiome) with functional immunological assays to better understand protective host immunity and to predict long-term immunity

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