Meeting report

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The persistence of memory: defining, engineering, and measuring vaccine durability

The US National Institute of Allergy and Infectious Diseases (NIAID) convened a virtual workshop in July 2022 to address the research landscape and identify gaps and opportunities in the understanding of durable vaccine protection.

The durability of vaccines has been an important global public health issue since the rapid development and deployment of successful vaccines for SARS-CoV-2. The continuing evolution of SARS-CoV-2 variants has emphasized the distinction between protection from infection and from severe disease. A variety of vaccine platforms, including mRNA, adenovirus vectors, adjuvanted protein subunit, and whole-inactivated virus, have been used in the SARS-CoV-2 pandemic, with varying efficacy and durability. Protection may be defined by several clinical and immunological endpoints, and vaccination against different viral pathogens requires different levels of protection. For example, a successful preventative vaccine for HIV, with its vast genetic diversity and ability to integrate into the human genome, requires a particularly high bar: durable sterilizing immunity. Immunologically, protection is most often measured by levels of binding or neutralizing antibodies in the serum, which can be convenient surrogates but do not capture the full picture of memory. The virus, vaccine platform, and scientific or clinical endpoint will determine which measures are most relevant. To address this timely topic, the US National Institute of Allergy and Infectious Diseases (NIAID) organized a workshop on 27–28 July 2022, with the objective of identifying knowledge gaps in the research on vaccine durability.

Research landscape

Mark Slifka (Oregon Health and Science University) discussed protective thresholds; durable protection requires maintenance of immune responses over this threshold. A declining immune response may be protective as long as it remains above this threshold. Data presented by Slifka and Matthew Snape (University of Oxford) indicate that peak serum antibody responses tend to correlate in magnitude with plateau levels of antibody[1], but quantity is not always sufficient. Experimental vaccines for HIV elicit high-titer, long-lived serum antibody responses, but do not provide protection from infection[2]. The quality of the antibody response matters; epitope specificity, breadth, and isotype are important determinants of protection.

How to instruct a durable, protective immune response is coming into focus. Deepta Bhatacharya (University of Arizona) and Bali Pulendran (Stanford University) emphasized that early innate and adaptive signals are crucial to establishing long-lived immunity[3]. Long-lived germinal center responses can program durable adaptive immune responses, and have been successfully induced using several strategies discussed below. The tools and technology to engineer durable vaccines are advancing, but we need a clearer understanding of the rules for achieving durable protection (Fig. 1).

In contrast to the unprecedented development and licensure of COVID-19 vaccines, the development of HIV vaccines has been long and challenging. Of the eight vaccine efficacy trials conducted so far, only one, RV144, has shown moderate efficacy, and the follow-up trial, HVTN 702, was unable to replicate this result[4]. The focus in the field has shifted to experimental medicine trials, enabled and expedited by the use of the mRNA platforms licensed for use with COVID-19 vaccines (NCT05217641). There is no doubt that HIV presents a much greater challenge for vaccine developers than does COVID-19. Iterative experimental medicine will determine whether further optimization of Env immunogens, together with advances in vaccine delivery and composition, will overcome this challenge. For viruses such as HIV and SARS-CoV-2, monitoring long-term vaccine-specific durability in real-world settings, in which viral diversity affects efficacy and multiple clinical outcomes are measured, is crucial for developing vaccine policy.

Vaccine platforms

Waning titers of serum antibodies have been observed after vaccination against SARS-CoV-2, although protection against severe disease remains high. David Kaslow (PATH) discussed several factors that affect vaccine efficacy, including the viral incubation period and force of infection (the rate of acquisition of infection)[5]. Longer incubation periods permit an effective recall response, and protection from symptomatic disease. Shorter incubation periods do not allow sufficient time for a protective recall response, and vaccine efficacy decreases[5]. This effect has been widely observed in the SARS-CoV-2 pandemic, in which protection from infection decreased as viral incubation times decreased. Force of infection also affects vaccine efficacy. Vaccine efficacy tends to be higher in areas with lower viral transmission, or force of infection, as observed with a pentavalent rotavirus vaccine with real-world efficacy ranging from 45% to 90%[5]. Protective efficacy and durability are influenced by these factors, as witnessed in the current pandemic[5]. Other factors unique to each virus, such as the tremendous viral diversity of HIV and its ability to integrate into the genome and hide in immune privileged sites, also affect efficacy and durability[5].

Two vaccine platforms dominated the initial SARS-CoV-2 vaccine rollout in the USA: adenovirus-26 (Ad26) and mRNA. These two platforms induced immune responses with different kinetics. Dan Barouch (Ragon Institute) presented data on Janssen's Ad26-based vaccine for SARS-CoV-2. A single dose of this vaccine induced binding and neutralizing antibodies, CD4 and CD8 T cells, which were all detectable up to 8 months, although peak titers were significantly lower than after mRNA vaccines[6]. Protection against hospitalization provided by Ad26 vaccination for adults under age 65 remained consistently above 90% throughout the Delta wave. By contrast, mRNA vaccines induced high peak serum antibody responses that waned by 6 months, although binding antibodies, CD4 and CD8 T cells, and germinal centers persisted as long as 6 months after vaccination, highlighted by Andrea Carfi (Moderna)[6].

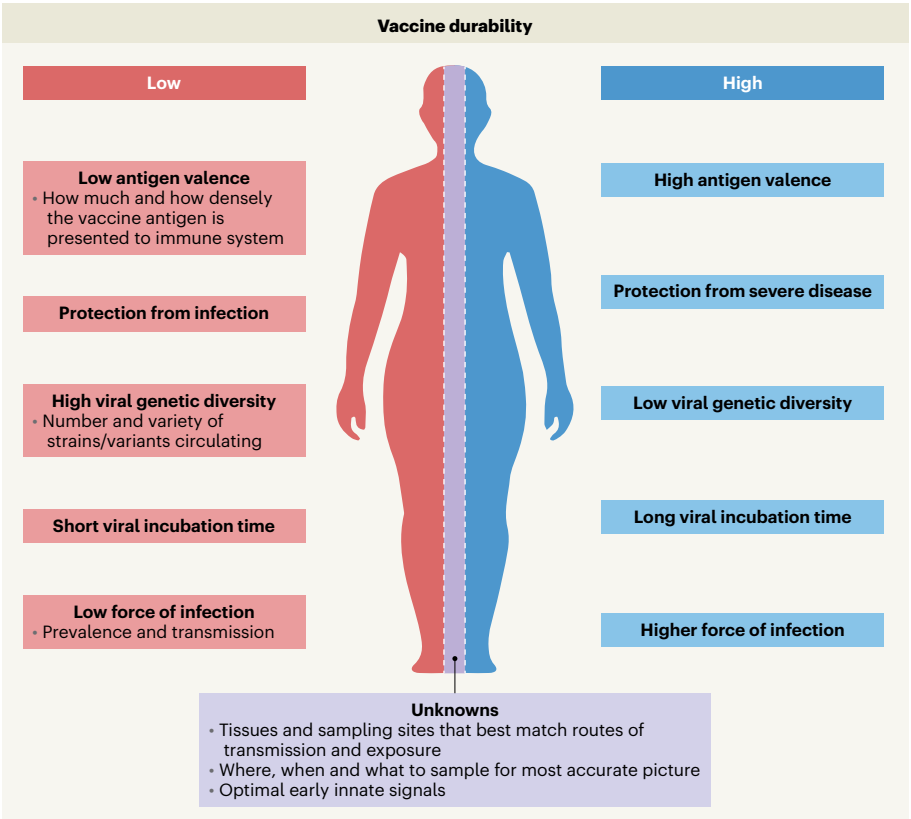

Fig. 1 | Factors that influence vaccine durability. Factors that decrease vaccine durability are listed on the left in red; factors that promote vaccine durability are listed on the right in blue. Current unknowns are shown in the box at the bottom.

Although protection from infection by mRNA vaccines has decreased over time, protection from severe disease remains at around 90%[7,8]. Slifka emphasized that antibody responses to many vaccines plateau by 3 years[9], therefore understanding the true durability of mRNA vaccines will require time.

Boosting with heterologous vectors is commonly thought to enhance immune responses. Snape presented data from the Com-COV2 study, conducted in the UK, demonstrating that heterologous and homologous regimens resulted in varied peak titers, and similar rates of serum antibody decline up to 6 months[1]. In the related COV-BOOST trial and the US MixNMatch trial, a third dose of adenovirus vaccine (AstraZeneca's ChAdOx1 and Janssen's Ad26, respectively) in Pfizer or Moderna mRNA-primed recipients resulted in higher titers of binding antibodies[10]. John Beigle (NIAID) stressed that the MixNMatch study was not designed to assess the superiority of heterologous vaccine regimens. This study found that all combinations of mRNA and Ad26 boosted WA-1 and D614G binding and neutralizing antibody responses up to 6 months after vaccine boost[11], a relevant observation as immunity in the population becomes increasingly heterologous.

The predictive value of commonly used serum antibody and neutralizing antibody titers varies. In the COVE phase 3 efficacy trial, serum neutralizing and binding antibody titers at days 29 and 57 were highly predictive of protection from infection by mRNA-1273 vaccination[12]. By contrast, experimental vaccines for HIV, among others, induced high antibody titers[13], but were unable to protect against infection. Throughout workshop discussions, Carfi, Pulendran, Galit Alter (Ragon Institute), and Shane Crotty (La Jolla Institute for Immunology) remarked that recruiting sometimes rare B cells with the potential for protective specificities and optimal Fc-effector functions is crucial. Serum antibodies are the most accessible measure but may not reflect the full picture of immunity at the site of infection. Although more labor- and time-intensive to measure, T cell responses are an important component of protection, both as direct effectors in the control of viral replication, and as inducers of durable, long-lived antibody responses[9].

The protective antibody threshold has increased with SARS-CoV-2 viral drift, as Barouch observed. Protective serum antibody thresholds are defined for some pathogens, such as hepatitis viruses[9], and even for the VRC01 class of HIV neutralizing antibodies[14]. Adjuvants may enhance plateau levels by increasing peak antibody responses, with mixed effects on durability. Slifka presented data from an AS04-adjuvanted human papilloma virus (HPV) vaccine that elicited higher peak and plateau neutralizing titers than alum-adjuvanted HPV vaccines[9]. By contrast, the administration of various adjuvants had a limited effect on reshaping HIV-specific antibody durability[15]. Thus, engaging specific Toll-like receptors (TLRs) and other innate receptors with adjuvants, singly or in combination, may instruct a durable immune response, but optimizing the effects of adjuvants requires more research.

Engineering durable vaccines

To engineer durable vaccines, we must first define the rules and features of durable protection. These rules may differ for each virus, platform and between prime and boost responses. Knowledge of immune signals that drive the development of long-lived plasma cells (LLPCs) and key factors that affect the persistence of germinal center responses remains insufficient to allow engineering of durable immunity. Crotty discussed data from non-human primate models of HIV, in which an osmotic pump or sequential escalating doses of immunogen for sustained antigen delivery, induced long-lived germinal centers, increased epitope breadth, and enhanced affinity maturation of B cell lineages after a single prime[16]. In humans, whether dose escalation of antigen and adjuvant can prolong germinal center reactions, resulting in increased somatic hypermutation and neutralizing antibody responses, is being tested, based on this non-human primate data (NCT05471076). Rama Amara (Emory University) demonstrated that the route of administration matters as well; intradermal administration of an MVA-vectored vaccine in non-human primates increased antigen retention in the lymph nodes compared with intramuscular administration, resulting in higher and more durable activation of antigen-specific germinal center T and B cells[17]. Intradermal administration resulted in better protection against BG505 SHIV challenge (Amara, unpublished observations), highlighting the effects of

different vaccination regimens and routes of immunization.

Bhattacharya and Eun-Hyung Lee (Emory University) emphasized that further investigation is needed to identify how initial B cell activation imprints key survival programs of LLPCs and which early signals predict a durable immune response[3]. Neil King (University of Washington) observed that infections and replicating viral vectors engage multiple receptors and inflammatory pathways, in contrast to unadjuvanted platforms or those with a single adjuvant. These observations suggest that early innate signals, which vary by pathogen or platform, could be surrogate endpoints to expedite vaccine development, and enable the engineering of more durable vaccines. The format of antigen presentation also affects durability. King further illustrated that higher antigen valence and sustained antigen delivery recruit B cells with a larger breadth of affinities, and more effectively drive B cells into the LLPC pool[3,9,18]. Germinal center interactions ultimately dictate the quality and longevity of the antibody response after vaccination, and each step in the process could be exploited to improve durable humoral immune responses.

Measurement of vaccine durability

Sophisticated tools and sampling methods are being applied to profile immunity in mice, non-human primates, and humans with great precision and detail. Draining lymph nodes can be sampled using ultrasound-guided fine needle aspirates. Ali Ellebedy (Washington University) presented data using this technique that showed high frequencies of SARS-CoV-2 spike-binding germinal center B cells and antibody-producing plasmablasts in draining lymph nodes at least 29 weeks after the second mRNA immunization[19,20]. These studies demonstrated that SARS-CoV-2 mRNA vaccination induces a persistent germinal center B cell response, and robust humoral immunity comparable to seasonal influenza vaccination. Aspirates from lymph nodes and bone marrow can be used to follow B cell maturation by applying single-cell sequencing to track B cell clones and somatic hypermutation[20]. In bone marrow, $CD19^{-}CD38^{hi}CD138^{+}$ LLPCs are the cellular basis of durable antibody responses. Single-cell omics analysis of human bone marrow aspirates uncovered significant heterogeneity in B lineage compartments in the bone marrow[21]. Lee explained that the generation of LLPCs is not fully understood but probably involves the differentiation of antibody-secreting cells in germinal centers, with T cell help, followed by migration and further LLPC adaptation to the hostile, hypoxic bone marrow niche[21]. To explore further, the Lee lab has developed in vitro bone marrow cultures that mimic the unique bone marrow microenvironment needed to sustain LLPCs[21]. The application of advanced culture techniques may uncover environmental and autologous signals that guide the development of LLPCs.

ASCs are a heterogenous effector cell population, due to differences in memory B cell precursors and cytokine milieus. Much of our understanding about memory B cells is derived from studies of responses to systemic antigens in lymphoid organs, whereas initial protective responses occur in mucosal tissues. Troy Randall (University of Alabama) presented research on a new population of mouse influenza-specific, lung-resident memory B cells that rapidly give rise to plasmablasts, reside in bronchus-associated lymphoid tissue, are induced in response to intranasal vaccination, and provide considerable protection from infection[22]. Ignacio Sanz (Emory University) stated that the heterogeneity of the memory B cell compartment in humans remains poorly understood, although antigen-specific B cells can be detected in $CD27^{+}$ and $CD27^{-}$ B cell populations 6 months after three-dose immunization against SARS-CoV-2. It remains unclear which B cell populations are most likely to re-enter the germinal center during a recall response[23]. The requirements for stimulation and maintenance of memory B cell populations have yet to be determined.

Similarly, Susan Kaech (Salk Institute) described distinct memory T cell subsets (memory precursors, effector memory, central memory, tissue-resident memory, and peripheral memory), which vary in their migratory and effector properties. These populations form a layered system of protection, with tissue-resident memory T cells serving as the first line of defense. Indeed, pathogen-specific tissue-resident memory T cells are found in the affected organs and coordinate recall responses: hepatitis-specific T cells tend to be localized to the liver[24]. Antigen-specific memory T cells may be measured using several assays — peptide MHC tetramer staining, activation induced marker assays, and ELISPOT — but the difficulty in detecting and following localized T cell responses remains a notable limitation. In humans, tissue-resident cells may be studied in clinical biopsies and donated organs, although following immune responses over time remains a challenge.

Systems biology has been used to uncover signatures that predict durability and mechanistic correlates. The Pulendran laboratory has shown that live-attenuated yellow fever vaccine induced extremely long-lived protection, engaging several TLRs and inflammasome activators[25]. Notably, the Pfizer BioNTech mRNA vaccine did not engage TLRs or inflammasome activators. It did, however, engage MDA-5, an RNA sensor associated with T cell responses[26]. Knowledge of these pathways has been applied in combining TLR-activating adjuvants with subunit or nanoparticle vaccines to induce strong, durable responses in non-human primates and in clinical trials[27,28]. Mechanistic knowledge of the immune system, sampling of difficult-to-access lymphoid tissues, and powerful computational methods are being combined to predict and engineer durable immune responses. To understand durability, it will be necessary to uncover more signals required to move an antibody-secreting cell to the bone marrow, to survive in the hostile environment of the bone marrow, and to become an LLPC.

Heterogeneity in memory T and B cell subsets is a feature of the immune system, providing several layers of defense and adaptability. Protective immune responses require layered, tissue-specific defenses; it is necessary to induce both serological memory and tissue-specific memory. Mucosal responses differ in their potential to generate immunity and memory and are distinct from systemic responses. Kanta Subbarao (University of Melbourne) and Chris Chiu (Imperial College London) posited that the short half-life of IgA in the nasal mucosa may be a major factor in the lack of protection from re-infection by respiratory viruses. By contrast, long-lived serum IgG is accessible to the lower respiratory tract and protects against severe disease from respiratory infections[29]. Combining different platforms and routes of delivery has the potential to induce superior protection. In animal models, intranasal prime with live-attenuated influenza virus followed by intramuscular boost with a subunit vaccine and intramuscular mRNA–lipid nanoparticle prime followed by intranasal protein boost induced robust immune responses to influenza and SARS-CoV-2, respectively[30]. Understanding the adaptive immune cells in their changing microenvironments is crucial to enhancing vaccine durability. Pulendran and Lee echoed that understanding which lymph node plasmablasts acquire the lifespans of LLPCs upon residence in the bone marrow is essential, as they likely acquire distinct intrinsic signals and molecular programs in the microniche[21].

Ellebedy raised the limitation that spatial information is not captured by techniques such as lymph node aspirates; developing a method of visualizing T and B cells in situ would enable the interrogation of the microenvironment and of cellular interactions. Subbarao and Chiu also noted that studying mucosal immunity in humans is challenging, in part owing to variability in sampling, particularly for the respiratory tract. As serum responses are not a reliable correlate of mucosal immunity, understanding and measuring mucosal immune memory remains a gap.

Conclusions

Investigators participating in the workshop, particularly Pulendran and Julie McElrath (Fred Hutchinson Cancer Center), championed a need for standardized human studies that directly compare vaccine platforms, including traditional recombinant protein with or without adjuvants, replicating and nonreplicating viral vectors, and nucleic acid DNA and mRNA approaches. There is precedent in the harmonized COVID-19 vaccine efficacy trials conducted by the NIAID-funded Coronavirus Prevention Network (CoVPN), and in human clinical vaccine trials comparing multiple adjuvants with a common stabilized HIV Env trimer immunogen (NCT04177355). Valid comparisons require similar or identical qualified or validated assays to profile the innate and the adaptive response, with in-depth sampling of not just blood, but also mucosa, lymph nodes, and bone marrow. Standardized clinical trials and assays will enhance the ability to define vaccine durability and correlates of protection, speeding licensure for vaccines and uncovering the requirements for durable protection.

Although there is abundant information about serum antibody responses to vaccination, many mechanistic protective responses remain unclear. There is a need to define biomarkers of a durable protective response, and the rules for induction of a durable protective response at the site of infection, as Randall and Subburao emphasized. Advances in bioengineering have provided the tools necessary to engineer durable vaccines once the parameters are known. With nanoparticle and virus-like particle vaccines, antigen valence, density, and pathogen variants can be tightly controlled. Advances in biomaterials can allow controlled, sustained delivery of antigen. An ever-expanding panel of new adjuvants can be used to activate specific innate immune pathways. The tools exist. What remains is to define the rules of durability for a particular pathogen, platform, and population. Uncovering these rules will require rigorous, standardized human experimental medicine.

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Competing interests

G.A. is a founder and/or equity holder in Systems Seromyx and Leyden Labs, and since October 2022 is an employee of Moderna Therapeutics. S.C. has consulted for GSK, JP Morgan, Citi, Morgan Stanley, Avalia NZ, Nutcracker Therapeutics, University of California, California State Universities, United Airlines, Adagio, and Roche. B.P. serves on the External Immunology Network of GSK, and the scientific advisory board of Medicago. M.P.D., M.C.L., M.L., C.M., M.J.M., A.C.P. and A.S. declare no competing interests.