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CONCISE REPORT

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES

Progress in vaccine development for infectious diseases—a Keystone Symposia report

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Abstract

The COVID-19 pandemic has taught us many things, among the most important of which is that vaccines are one of the cornerstones of public health that help make modern longevity possible. While several different vaccines have been successful at stemming the morbidity and mortality associated with various infectious diseases, many pathogens/diseases remain recalcitrant to the development of effective vaccination. Recent advances in vaccine technology, immunology, structural biology, and other fields may yet yield insight that will address these diseases; they may also help improve societies' preparedness for future pandemics. On June 1–4, 2022, experts in vaccinology from academia, industry, and government convened for the Keystone symposium "Progress in Vaccine Development for Infectious Diseases" to discuss state-of-the-art technologies, recent advancements in understanding vaccine-mediated immunity, and new aspects of antigen design to aid vaccine effectiveness.

KEYWORDS
antigen design, mRNA vaccines, structural vaccinology, vaccines, vaccine delivery, vaccine hesitancy

INTRODUCTION

The advent of vaccines and antibiotics has dramatically reduced infectious disease–related mortality.[1] Prior to COVID-19, vaccination was attributed to saving approximately 3 million lives each year worldwide.[2] Since COVID-19, it has become clear how important vaccines are to not only global public health but also for keeping societies open during pandemics. Recent technologic advances in vaccines and immunology, partially spearheaded by the concerted efforts to create COVID-19 vaccines, have ushered in a new age of vaccinology. There is considerable optimism that these advances will facilitate interdisciplinary cooperation between experts in fields such as immunology, structural biology, and biomedical engineering to develop effective vaccines against hard-to-target pathogens while ensuring the technologies used are effective for the areas that need them most.

On June 1–4, 2022, experts in vaccines from academia, industry, and government convened for the Keystone symposium "Progress in Vaccine Development for Infectious Diseases" to discuss current

state-of-the-art vaccine technologies, such as mRNA vaccines, viral vectors, and protein nanoparticles; recent advancements in understanding vaccine-mediated immunity; and key aspects of antigen design. A session on vaccine diplomacy explored the concepts of vaccine hesitancy and antiscience rhetoric that has cast a shadow over the acceptance of COVID-19 vaccines.

PANDEMIC VACCINES—WHERE WE ARE AND WHERE WE NEED TO BE

Barney S. Graham, former deputy director of the Vaccine Research Center at the National Institutes of Health (NIH), and **Katalin Karikó** from BioNTech RNA Pharmaceuticals provided their perspectives on how decades of research on pathogens, vaccine platforms, and RNA delivery mechanisms led to the approval of highly efficacious COVID-19 mRNA vaccines in record time and what this means for pandemic preparedness moving forward. While the unprecedented speed at which COVID-19 vaccines were developed and approved resulted in an unfortunate backlash of vaccine hesitancy and antiscience rhetoric, Graham and Karikó discussed how the vaccines represent the culmination of decades of research on other viruses, technologies, and vaccine platforms; lessons from prior pandemics; established partnerships; and investments in clinical and research infrastructure.

Graham stressed the importance of proactively developing tools and candidate clinical products for pathogens with pandemic potential. Recent experiences with Ebola outbreaks in 2014–2015[3] and Zika outbreaks in 2016 demonstrate all too well the importance of having vaccine candidates available before an outbreak occurs. In both cases, despite the prior availability of the product or rapid development, vaccine efficacy trials were not initiated until incidence rates had already begun declining and were not able to provide definitive field data. Without the potential for conclusive phase 3 efficacy data and ensuing regulatory approval, pharmaceutical companies have little incentive to develop vaccines during an outbreak. To address this concern, Graham espouses the prototype pathogen approach in which representative viruses are chosen among the 27 virus families known to infect humans to be the focus of basic research and development of reagents and clinical products through phase 1 trials. When an outbreak occurs, the research and products can be leveraged and adapted to the pathogen of interest.[4,5] This approach was instrumental in the timely development of the COVID-19 vaccines. Previous work on the respiratory syncytial virus (RSV) established the concept that stabilizing class I fusion proteins made them better vaccine antigens. Applying that concept to other coronaviruses[6–10] had already demonstrated a generalizable antigen design approach that enabled researchers to quickly identify the SARS-CoV-2 spike protein as a key immunogenic protein and to incorporate key stabilizing mutations to make it a suitable vaccine antigen. Graham also stressed the importance of preexisting public, private, and academic partnerships and infrastructure, largely established by previous effort to develop HIV vaccines, that enabled a relatively smooth transition from basic research to clinical trials and commercial vaccine development.

One of the legacies of COVID-19 is the establishment of mRNA as a viable modality for safe, efficacious vaccines. mRNA vaccines provide several advantages for pandemic preparedness, both mechanistically—antigens are presented in a native-like form and induce both antibody and T cell responses—and practically—they can be synthesized rapidly without the need for bioreactors, and changes to the RNA sequence can be made without changing manufacturing protocols.[11] Karikó gave an overview of some of the key developments in RNA research that were necessary for mRNA therapies, both as vaccines and as protein replacement therapeutics. In the late 1970s, researchers first demonstrated that mRNA could be delivered into mammalian cells via liposomes to drive the translation of functional protein.[12,13] Over the following two decades, several labs demonstrated the ability to deliver *in vitro* transcribed mRNA into living cells and animals.[14–19] However, several challenges precluded mRNA from being a viable therapeutic modality. RNA is inherently unstable, and methods to use mRNA to deliver proteins resulted in low translation and immunogenicity. Over time, an increased understanding of both mRNA and lipid nanoparticles paved the way for advancing therapeutic mRNA.[20] Karikó's work has shed light on which RNA modifications are important for immunogenicity and inflammation and how those modifications impact protein translation.[21–23] In 2013, Karikó joined BioNTech, a pharmaceutical company focused on mRNA-based therapeutics. There, she focused on improving mRNA therapies via codon optimization, untranslated region (UTR) and cap optimizations, and nucleoside modifications to increase protein expression and develop scalable therapeutics.[24] mRNA-based therapies are now being investigated in multiple clinical and preclinical settings, both as protein replacement therapies and as vaccines.[25–27]

As the world looks forward and prepares for future pandemic threats, Graham stressed that a new era in vaccinology, ushered in by technological advances in structure-based antigen design and rapid platform manufacturing, has made it feasible to achieve generalizable vaccine solutions. However, as was demonstrated too often during the COVID-19 pandemic, there is still a need to improve access to research, manufacturing, and vaccine delivery to achieve greater equity among low- to middle-income countries as well as to build trust and establish transparent means of engagement between science and the public to improve vaccine uptake.

GENETIC VACCINES: FROM MECHANISMS OF ACTION TO CLINICAL APPROVAL

Immune responses to adenovirus-based and mRNA COVID-19 vaccines

Dan H. Barouch from Harvard Medical School described the immune responses to an adenovirus-based COVID-19 vaccine, Ad26.COV2.S, which was developed by Johnson & Johnson's Janssen and is currently approved worldwide for adults.[28] Barouch's lab initially developed a replication-incompetent adenovirus 26 (Ad26) in the mid-2000s as part of an HIV vaccine program.[29] Since then, Ad26-based

vaccines have been developed for multiple pathogens, including Zika virus, Ebola virus, and RSV. This work demonstrated the potential for Ad26-based vaccines to elicit durable immune responses and the feasibility of their mass production and global distribution.[30,31] In January 2020, Barouch's lab shifted from HIV vaccine efforts toward developing an Ad26-based COVID-19 vaccine, which would ultimately become Ad26.COV2.S and demonstrate 85% efficacy against severe COVID-19 in global phase 3 trials.[32] Barouch's lab evaluated the magnitude, durability, and kinetics of immune responses following vaccination with Ad26.COV2.S, as well as for two mRNA COVID-19 vaccines, Moderna's mRNA-1273 and Pfizer's BNT162b2. They found that while the mRNA vaccines generally elicit high antibody titers in the weeks following vaccination, antibody levels fall off rapidly. In contrast, Ad26.COV.2 generates lower peak antibody titers than the mRNA vaccines, but antibody levels are more durable. By 8 months after vaccination, antibody titers for all three vaccines were comparable. All three vaccines also elicited durable T cell responses, with Ad26.COV2.S eliciting higher $CD8^+$ T cell responses than the mRNA vaccines.[33] These data are consistent with real-world efficacy data demonstrating that while mRNA vaccines are more effective than AD26.COV2.S initially, their efficacy wanes over time, while the efficacy of the Ad26-based vaccine is more constant.[34,35]

Barouch also discussed how the vaccines held up in the face of the Omicron variant. A consistent picture has emerged in which vaccine effectiveness against viral variants has decreased for preventing infection but remains high for preventing severe disease. For example, neutralizing antibody titers against Omicron are significantly lower with the first vaccines, yet recipients are still largely protected against hospitalization and death.[36,37] It is clear that immune correlates of protection other than neutralizing antibodies play a role in vaccine efficacy. Recent data have shown that the T cell response is more robust to viral variants, including Omicron. Most of the $CD8^+$ T cell epitopes are unaffected by the mutations in Omicron.[38] Barouch's group showed that vaccines elicit a durable, cross-reactive T cell response that protects against Omicron.[39] In a challenge study in non-human primates (NHPs), Barouch's group found that vaccine failure was associated with moderate neutralizing antibody titers and low $CD8^+$ T cell responses. Consistent with human data, the Ad26-based vaccine elicited a higher $CD8^+$ T cell response than an mRNA-based vaccine.[40] Taken together, these data indicate that protection against asymptomatic infection is largely mediated by antibodies, while protection against more severe disease is largely mediated by T cell responses, which are more durable and cross-reactive than antibody responses.

Determining COVID-19 vaccine-induced correlates of protection

Richard A. Koup from the Vaccine Research Center at the NIAID/NIH expanded on the notion of vaccine-induced correlates of protection. As part of what was originally called Operation Warp Speed, the US government partnered with vaccine developers to run and validate immune assays on clinical trial samples to identify immune signatures that correlate with vaccine efficacy. The goal was to identify correlates of protection that predict vaccine efficacy, with the aims of accelerating the clinical development of future vaccines, supporting approval in special populations, and guiding modifications to vaccine regimens, such as boosters, dosing, and vaccines developed against new strains. The partnership included six vaccines representing mRNA, adenovirus vector, and recombinant protein platforms.[41] A critical aspect of the project was to harmonize the assays and analyses across multiple clinical trials conducted by different companies. Toward that goal, the US government partnered with academic and government labs to develop, qualify, and validate assays to test trial samples as well as to develop a common statistical plan so that results could be compared across trials and vaccines. The assays developed to identify correlates of protection included binding antibody titers for the spike protein and receptor-binding domain (RBD), as well as neutralizing antibody assays. Unfortunately, the lack of collection of peripheral blood samples during the trials precluded the analysis of T cell responses.[41]

Koup described the correlates of protection identified for the Moderna and Janssen vaccines. For both vaccines, peak binding and neutralizing antibody levels were lower in vaccine breakthrough cases. However, there was no clear threshold that defined protection.[42,43] For the Janssen vaccine, the correlation between binding antibody titers and protection was not statistically significant. This trial was unique in that it was a global trial and conducted when SARS-CoV-2 variants had started to emerge.[43] Correlates of protection are now being used to assess the impact of different boosting strategies,[44] protection against variants of concern,[45] and to expand regulatory approvals to other populations, such as children. Research is ongoing to identify correlates of protection at later time points and to determine whether correlates of protection differ based on disease severity.

Analysis of variant vaccine boosting on B cell, neutralizing responses, and protection in NHPs

Robert A. Seder from the Vaccine Research Center at the NIAID/NIH discussed SARS-CoV-2 variants including BA-1, BA-2, BA-4, and BA-5, all highly transmissible and have substantial resistance to antibody neutralization following immunization with ancestral spike vaccines. A key question is whether boosting with variant-specific mRNA vaccines would enhance immunity and protection compared to boosting with the homologous ancestral WA1 vaccine. In a series of studies in NHPs, Seder's group assessed how animals that received mRNA-1273 at weeks 0 and 4 and boosted 6–9 months later with either mRNA-1273 or mRNA encoding Beta or Omicron variants influenced immunity and protection against the variants. The data show that boosting by the ancestral mRNA-1273, Beta, or Omicron variant mRNA significantly enhanced neutralizing antibody titers against all variants tested. Variant mRNA boosting did not significantly enhance neutralizing responses compared to mRNA-1273. Following boosting with mRNA 1273, Beta, or Omicron, 70–80% of spike-specific B cells were cross-reactive against WA1,

Beta, or Omicron. All boosts led to significant and equivalent control of virus replication in lower airways and more limited protection in the upper airways. These data suggest that initial mRNA-1273 vaccination induces B cells that are cross-reactive against multiple variants and such responses are potently boosted by ancestral and variant vaccines. While changing vaccines to match variants will enhance immunity and protection against the severe disease, it is likely that mucosal responses required to prevent airway infection and transmission may require intranasal boosting.

Arenavirus-based viral vectors elicit SIV-specific T cell responses

Bhawna Sharma from Gilead Sciences presented preclinical data on arenavirus-based vector vaccines in NHP models of SIV infection. Arenavirus-based vectors proprietary to HOOKIPA Pharma Inc. have been shown to generate T and B cell responses,[46] and $CD8^+$ T cell immunity is known to be important for controlling HIV infection in humans and SIV infection in NHPs.[47–49] Sharma described the results of an SIV challenge study in NHPs to investigate whether arenavirus-based vectors induce SIV-specific T and B cell immune responses. The study included four vector constructs: nonreplicating and replicating arenavirus vectors based on either lymphocytic choriomeningitis virus (LCMV) or Pichinde virus (PICV). Animals were immunized four times by either a single-vector approach (LCMV- or PICV-based) or a two-vector approach with alternating administration of LCMV and PICV vectors. The study also assessed the differences between intravenous and intramuscular administration. Sharma showed that both alternating two-vector therapies induced robust SIV antigen-specific T cell and antibody responses, with the alternating two-vector therapy inducing greater T cell responses than single-vector therapy. This translated to a significant reduction of viral load in NHPs with replicating vectors (alternating two-vector therapy).[50] Further, intramuscular administration showed a modest benefit over intravenous administration in both the magnitude of T cell response and the consistency and durability of antibody response. This study supports the clinical evaluation of arenavirus-based vectors for therapeutic HIV vaccine development.[50]

INFECTIOUS DISEASE mRNA VACCINES

Optimizations to mRNA vaccines to increase immunogenicity

Susanne Rauch from CureVac described the company's COVID-19 mRNA vaccine program. CureVac's first-generation COVID-19 vaccine, CVnCoV, demonstrated 48% efficacy against symptomatic disease in a phase 2b/3 clinical trial,[51] markedly lower than that of other COVID-19 mRNA vaccines.[52] CureVac is developing a second-generation COVID-19 vaccine, CV2CoV, that consists of the same lipid nanoparticle and protein coding sequence as CVnCoV but contains modifications in the 5′ and 3′ UTRs as well as the 3′ tail to both stabilize the mRNA and increase protein expression. Preclinical immunogenicity studies in rodents and NHPs showed that CV2CoV elicited higher neutralizing antibody titers at earlier time points than CVnCoV while also producing a robust T cell response.[53,54] Antibody levels remained stable over 3 months and could be boosted with subsequent vaccination.[55] In NHPs, CV2CoV showed higher potential for cross-reactivity than did CVnCoV, and CV2CoV elicited neutralizing antibodies against multiple SARS-CoV-2 variants, including Alpha, Beta, Lambda, and Delta. In a challenge study in NHPs, CV2CoV protected the animals against infection. Vaccination with CV2CoV reduced viral load in both the lower and upper respiratory tracts and protected against pathological changes in the lung. In contrast, vaccination with CVnCoV only reduced viral load in the lower respiratory tract.[54] CV2CoV is currently being investigated in a phase 1 dose escalation study in the United States in collaboration with GSK.[56] CureVac is also developing second-generation bivalent COVID-19 vaccines that contain the same modifications as CV2CoV but code for SARS-CoV-2 Beta and Delta spike proteins. Rauch showed promising preclinical data demonstrating that a bivalent vaccination strategy that contains antigens for both the Delta and Beta strains may provide cross-reactive protection.

Development of broadly protective, multivalent mRNA-based influenza vaccines

Norbert Pardi from the University of Pennsylvania described work in collaboration with several labs at Mount Sinai to develop a multivalent mRNA-based universal influenza vaccine. The mRNA platform Pardi is using is very similar to that used to develop the COVID-19 vaccines—nucleoside-modified purified mRNA coding for a protein antigen is encapsulated in a lipid nanoparticle. Pardi's group is taking advantage of the flexible nature of this platform to coformulate several antigens within a single vaccine with the aim of generating broadly protective responses against the range of influenza viruses. The project is divided into four stages. Stages one through three, which have been completed, were to develop mRNA vaccines against influenza A Group 1 viruses, influenza A Group 2 viruses, and influenza B viruses. The final stage, which is in development, is to generate a multivalent universal influenza virus vaccine. Pardi focused on the effort to develop vaccines against influenza A Group 1 and influenza B viruses. The influenza A Group 1 virus vaccine includes four antigens: a headless hemagglutinin (HA) construct, neuraminidase (NA), matrix-2 (M2) protein, and nucleoprotein (NP). In mice, this combination vaccine elicited strong antibody responses to all four antigens and protected mice from a lethal challenge of the H1N1 influenza virus as well as against diverse influenza A strains. In contrast, monovalent vaccines produced variable levels of protection based on the infecting strain. Serum transfer studies showed that protection was primarily mediated by antibodies.[57] Pardi also showed similar data for a pentavalent mRNA-based vaccine that targets multiple influenza B virus antigens.[a]

[a] *Nat Commun*. 2022 Aug 9;13(1):4677. doi: 10.1038/s41467-022-32149-8.

Development of mRNA-based seasonal vaccines

Raffael Nachbagauer from Moderna described the company's efforts to develop mRNA-based seasonal influenza vaccines. Moderna has a multipronged influenza vaccine portfolio. The most advanced candidate, mRNA-1010, is a quadrivalent seasonal influenza vaccine that encodes for four HA molecules from seasonal influenza strains based on WHO recommendations. In a phase 1 dose-ranging trial in which the vaccine was based on 2021 southern hemisphere influenza strains, mRNA-1010 elicited high antibody titers across age groups. In a subsequent phase 2 trial, in which the vaccine used antigens from northern hemisphere influenza strains, mRNA-1010 elicited high antibody titers, exceeding the threshold associated with a 50% reduction in risk of infection and a consistent immunogenicity profile across age groups. Antibody levels against influenza A strains, which are responsible for the majority of influenza-related disease, were higher in those vaccinated with mRNA-1010 than in those vaccinated with a commercial influenza vaccine. Both studies demonstrated a consistent safety profile, with no Grade 4 adverse events reported. mRNA-1010 is now being investigated in a phase 3 safety and immunogenicity trial that enrolled approximately 6000 participants across the southern hemisphere, as well as a phase 3 efficacy trial that aims to enroll approximately 23,000 participants in northern hemisphere countries.[58]

In addition to mRNA-1010, Moderna is also developing influenza vaccines that contain NA. While HA has been the primary target of current vaccines, it is prone to antigenic drift and is highly variable across subtypes, making it difficult to create a broadly reactive vaccine based on HA alone. In contrast, NA has lower antigenic drift and is more conserved across strains.[59] In addition, anti-NA antibodies can inhibit multiple stages of the influenza lifecycle, which could reduce the opportunity for antigenic escape.[60] Manufacturing issues have limited the use of NA as a vaccine antigen; however, these are not a concern for mRNA-based vaccines. Moderna has two mRNA-based vaccines that contain NA antigens, mRNA-1020 and mRNA-1030, which are being investigated in early clinical trials. Finally, Moderna is expanding on mRNA-1010 with mRNA-1011 and mRNA-1012, which include additional HA antigens to expand strain matching.

Longitudinal assessment of post-vaccination immune responses among special care home residents in Sweden

Mattias N. E. Forsell from Umea University described a longitudinal study to assess post-vaccination immune responses among special care home residents across Sweden. The study consisted of approximately 2600 individuals across 93 special care homes with a median age of 86 years. In addition to age, residents of special care homes often have several other factors that put them at high risk of infection-related complications. Forsell showed that elderly individuals can mount a robust immune response following vaccination with the COVID-19 mRNA vaccines. Among residents who received a full two-dose course of the Pfizer or Moderna mRNA vaccine, a third dose boosted antibody levels by 96-fold, while the increase in antibodies following a fourth dose was more modest (three- to fourfold). Individuals who had previously had COVID-19 had higher antibody titers after the third dose than those who had not been infected. The study demonstrates the feasibility of longitudinally monitoring the immune and general status of elderly residents at special care homes. Forsell hopes that studies such as this one can inform future decision-making regarding vaccination policies for the elderly.

Identifying correlates of protection against SARS-CoV-2 variants

Michael Schotsaert from Mount Sinai presented unpublished data on the immune response to suboptimal SARS-CoV-2 vaccination in a hamster model to identify immune correlates of protection and cross-reactivity outside of neutralizing antibodies.

OPTIMIZING VACCINE DELIVERY AND ADJUVANT STIMULATION

Mechanisms of B cell maturation following vaccination

Ali H. Ellebedy from Washington University School of Medicine in St. Louis presented work on monitoring the maturation of B cell responses following mRNA vaccination. Ellebedy's group samples cells from the draining lymph nodes following vaccination to understand vaccine-induced germinal center responses. Prior to the COVID-19 pandemic, they were tracking germinal center responses induced by influenza virus vaccines. Ellebedy showed that they can detect and track vaccine-induced germinal center B cells over 2 months after influenza vaccination. By tracing the origins of these B cells, they found that both memory and naive B cells contribute to germinal center reactions.[61] They have also investigated the B cell response to the Pfizer mRNA-based COVID-19 vaccine. Germinal center reactions following COVID-19 vaccination were much more robust than those observed following influenza virus vaccination and persisted for at least 6 months after the last immunization. The group analyzed samples from blood, draining lymph nodes, and bone marrow to track the evolution of responding B cell clones following Pfizer's mRNA-based COVID-19 vaccine. Shortly following vaccination, antibody levels are very high; these antibodies are produced primarily by short-lived plasmablasts and have relatively low affinity. Over time, antibody levels decrease; most of the antibodies detected late after vaccination are produced by lymph node plasma cells and bone marrow plasma cells that have matured within the germinal centers and show higher affinity. Therefore, vaccination results in affinity-matured long-term antibody responses that can potently neutralize the virus.[62] Ellebedy hopes that data like these will inform ways to improve the durability of responses to other vaccines.

Initial innate immune responses following mRNA vaccination

Karin Loré from the Karolinska Institutet discussed the initial innate immune responses that occur following mRNA vaccination. A study carried out with Moderna in NHPs of an mRNA-based vaccine that encodes a fluorescent protein showed that following vaccination immune cells, particularly neutrophils, monocytes, and dendritic cells, are rapidly recruited to the site of injection.[63] This is not unique to mRNA vaccines, having been observed with several other vaccine platforms.[64,65] While neutrophils were the most efficient cell type at taking up the lipid nanoparticles, monocytes and myeloid dendritic cells were most efficient at translating the mRNA-encoded protein. The group also looked at markers of innate immune activation and found upregulation of genes involved in monocyte activation and type I IFN responses.[63] Additional work comparing the innate immune response to CureVac's mRNA-based influenza vaccine, a nonadjuvanted split virion vaccine, and an adjuvanted split virion vaccine showed that mRNA vaccination induced similar or higher antibody titers as well as stronger innate immune responses than the other vaccine platform. Similar to the previous study, mRNA vaccination induced upregulation of genes associated with monocyte and dendritic cell activation and the type I IFN response. These two studies indicate that the innate immune activation gene profile is generally similar between the two mRNA vaccines. Similar results were seen after vaccination in humans. Bulk RNA-seq analyses following vaccination with Pfizer's COVID-19 mRNA vaccine showed upregulation of genes involved in monocyte and dendritic cell activation as well as the type I IFN response. A rapid, transient induction of type I IFN cytokines was also observed. Innate immune activation was more pronounced after the priming dose in SARS-CoV-2–experienced individuals and after the second, boosting dose in SARS-CoV-2–naive individuals, indicating that prior immunity can enhance immune activation.

Tracking vaccine antigens *in vivo*

Darrell J. Irvine from MIT presented work on tracking the fate of protein antigens *in vivo* after vaccination, with the goal of developing more effective vaccines. Irvine's group has monitored the fate of antigen-bearing nanoparticles,[66,67] assessed the effects of vaccine timing on humoral immunity,[68,69] and analyzed the effects of adjuvants on antigen trafficking.[70–72] These studies consistently demonstrated that following primary immunization with protein vaccines, an antigen can be detected in the draining lymph nodes within hours but is virtually gone within days. Irvine showed that antigen proteolysis within the lymph nodes was at least partially responsible for this disappearance. This could negatively impact the humoral immune response as it would activate B cells that recognize nonnative proteolytic products, thus leading to nonrelevant antibodies unable to provide protection. Single-cell RNA-seq and immunostaining showed that transmembrane and secreted proteases are expressed by several cell types in the draining lymph nodes, including stromal cells, macrophages, and dendritic cells. However, there was little protease expression within the follicles.[73] Using a technique known as imaging zymography,[74] Irvine's group confirmed that protease activity was high within the subcapsular sinus and low within the follicles.[73] Using a FRET-based assay to monitor the degradation of an HIV antigen consisting of a gp120 nanoparticle, they found that the antigen was quickly degraded outside of the follicles but remained intact within the follicles through 14 days.[73] These data indicate that targeting vaccine antigens to the follicles can maintain their structural integrity and improve the B cell response. Irvine showed two strategies by which antigens can be targeted to the follicles—immunizing with heavily glycosylated protein nanoparticles[66,67,75,76] and repeated vaccine administration.[68,69] In the first case, the binding of mannose-binding lectin to glycosylated nanoparticles triggers an innate immune response that results in the trafficking of the immunogen to the follicles by macrophages. This has been observed in both rodents and NHPs.[66,67,75,76] In the case of extended antigen exposure, it is believed that initial doses prime the B cell response and affinity maturation. Subsequent doses are met with more mature antibodies, which can form immune complexes and traffic the antigen to the follicles.[68,69] Irvine hopes that studies like these will lead to more effective vaccines and vaccination strategies.

Effect of lipid composition on lipid nanoparticle trafficking

Emily Pilkington from Stephen Kent's lab at the University of Melbourne presented work on how the lipid composition of lipid nanoparticles affects their trafficking to different immune compartments. Lipid nanoparticles have become a common vehicle to deliver nucleic acids both for therapeutics and vaccines.[77] They typically consist of ionizable lipids, which are responsible for the capture and release of their nucleic acid cargo, as well as several types of stabilizing lipids. Work in Kent's lab has shown that the lipid formulation can impact where the lipid nanoparticles end up in the body. For example, replacing the stabilizing lipid PEG with Tween within a standard lipid nanoparticle redirected the particle from the site of injection to the draining lymph node in mice, which can have implications for vaccine delivery.[78] A separate study investigated different compositions of ionizable lipids and identified lipids that result in more efficient delivery to the spleen.[79] Pilkington also presented unpublished data on how the charge of the ionizable lipid impacts its biological behavior. Together, these data show the importance of lipid composition on lipid nanoparticle trafficking, which could ultimately impact the efficacy of lipid nanoparticle–based vaccines and therapeutics.

IL-1 as a regulator of the inflammatory response to mRNA vaccines

Siri Tahtinen from Genentech presented work on better understanding the inflammatory response to mRNA vaccines and why the inflammatory response is more pronounced in humans than in animals used in

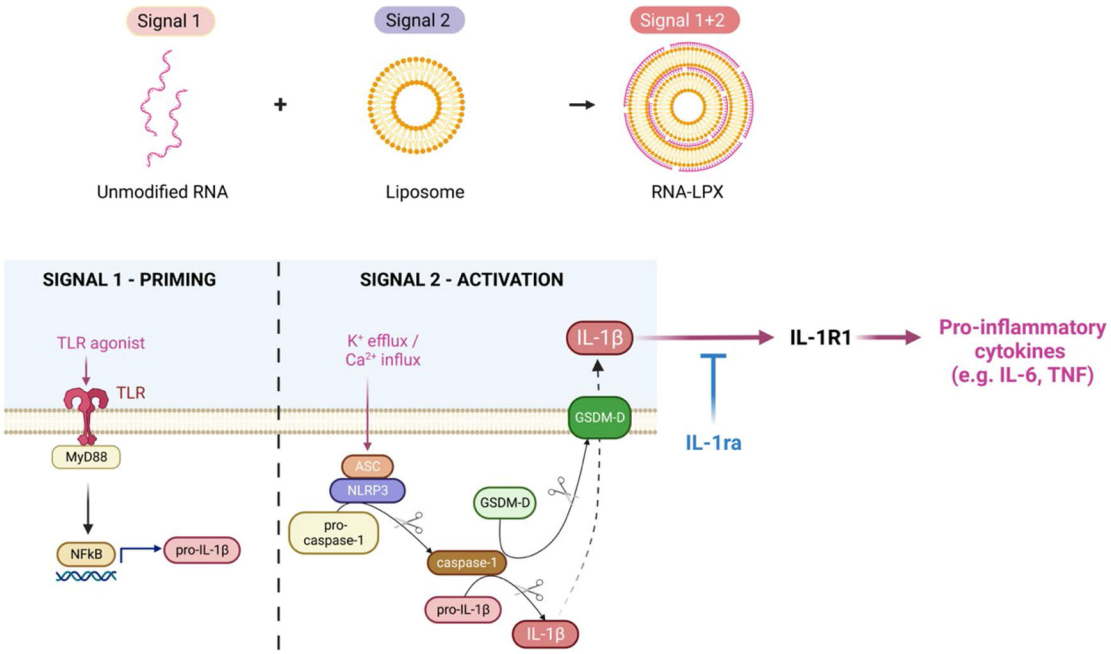

FIGURE 1 RNA-LPX vaccine activates the NLRP3 inflammasome pathway, leading to the robust IL-1β release from human monocytes. Subsequent binding of IL-1β to IL-1 receptor 1 (IL-1R1) on the surface of other immune and nonimmune cells triggers a downstream cytokine cascade. This pathway is substantially inhibited by high IL-1ra levels in preclinical mouse models, protecting them from IL-1–induced inflammatory toxicities.

preclinical studies, such as mice. Genentech's RNA vaccine platform, RNA-LPX, is a cancer vaccine platform in which unmodified RNA is encapsulated in a liposome; the liposome particles are targeted to the spleen and taken up by antigen-presenting cells.[80] Tahtinen focused on IL-1, a proinflammatory cytokine secreted by innate immune cells, including dendritic cells, monocytes, macrophages, and neutrophils. IL-1 activation requires two steps. In the first signal, Toll-like receptor (TLR) signaling induces the expression of an IL-1 proprotein, which is subsequently cleaved into an active form by the NLRP3 inflammasome upon a second signal (Figure 1). IL-1 activity can be inhibited by IL-1 receptor antagonist (IL-1ra), an endogenously expressed anti-inflammatory cytokine that competes with IL-1 for its receptor. Tahtinen showed that RNA-LPX vaccines induce the secretion of IL-1β both *in vitro* and *in vivo*, and that in both cases IL-1 mediates vaccine-induced release of other proinflammatory cytokines (such as IL-6 and TNF). Mechanistically, it appears that the unmodified RNA portion of the vaccine provides signal one via TLR7/8 sensing, while the liposome provides signal two (Figure 1). In humans, IL-1β levels are roughly equivalent to those of IL-1ra at tolerated vaccine dose levels, while in mice levels of IL-1ra are much higher, suggesting that IL-1ra may protect mice from the inflammatory effect of IL-1β. Consistent with this, knocking out the IL-1ra gene in mice sensitized them to toxicities related to high-dose RNA-LPX and resulted in increased inflammatory cytokines in the systemic circulation. Tahtinen also showed how the modified RNA lipid nanoparticle vaccines used in the COVID-19 vaccines impact IL-1 expression. Modified RNA does not activate the innate immune response like unmodified RNA does. However, COVID-19 vaccines are still clearly effective and elicit vaccine-mediated inflammation. Tahtinen showed that in modified RNA lipid nanoparticle vaccines, it is the lipid nanoparticle formulation that induces a cytokine response, while mRNA is the main driver of the innate immune response with RNA-LPX vaccines.[81]

B cell responses to COVID-19 vaccination among immunocompromised individuals

Elizabeth A. Thompson from Andrea Cox's lab at Johns Hopkins University presented unpublished work on the B cell response to COVID-19 vaccination among immunocompromised individuals, specifically solid organ transplant recipients. Immunocompromised individuals experience reduced responses to COVID-19 vaccination.[82] Thompson described an observational study of solid organ transplant recipients to evaluate the effect of a third dose of the COVID-19 vaccine.[83] She showed how B cell phenotypes differ between those who respond to the vaccine and those who do not and how understanding this basic B cell biology can inform future vaccine design and strategies for immunosuppressed cohorts.

Innate memory to a vector-based vaccine

Yanis Feraoun from the French Alternative Energies and Atomic Energy Commission discussed innate immune memory in the context of modified vaccinia Ankara (MVA), a viral vector vaccine. While the innate immune response is often considered nonspecific, recent

data indicate that it can be endowed with memory, which can impact innate responses to subsequent stimuli. Feraoun described studies in NHPs immunized with MVA with different dosing regimens and routes of administration. In a prime–boost dosing strategy, the interval between the two doses impacted the innate immune response to the second immunization. For shorter intervals (2 weeks), the innate immune response was similar for the prime and boost doses. However, a longer interval (2 months) boost resulted in an abundance of ready-to-respond innate immune cells, which correlated with a higher quality secondary antibody response.[84] Similarly, the quality of the response to the priming dose, which differs depending on the route of vaccine injection, was important for inducing innate memory and impacted subsequent innate responses to boosting doses.[85] Feraoun stressed the importance of considering innate immune memory in vaccine design.

PROTEIN NANOPARTICLE VACCINES

Computational design of self-assembling protein nanoparticles

Neil P. King from the University of Washington presented work using computational methods to design self-assembling protein nanoparticles for vaccines.[86] King's group has developed a generalizable computational method to design self-assembling proteins *de novo* with atomic-level accuracy.[87–91] These particles can be leveraged for multiple applications. King described efforts to design protein nanoparticles as a platform for multivalent antigen presentation. Self-assembling proteins are a promising platform for vaccines—they create uniform structures that can multivalently display complex antigens without the need for conjugation. King's group has designed self-assembling nanoparticles that display the SARS-CoV-2 spike protein (or RBD). The RBD-based vaccine induced potent and protective neutralizing antibodies in both mice and humans.[92,93] The vaccine, GBP510, was developed by GSK and SK Bioscience and was approved in 2022 in the Republic of Korea,[94] marking the first computationally designed protein medicine.

King primarily focused on how they are using computational protein nanoparticle design to systematically vary key features to define structural determinants of vaccine-elicited immunity. In short, they design and create a series of self-assembling protein nanoparticles expressing a given antigen(s) in which one key characteristic is altered. For example, King showed that modulating the valency of the antigen impacts antibody titers: higher antigen density was associated with higher antibody titers.[95] In another study, King's group created a series of immunogens with varying glycan density and composition to elucidate how these features impact antigen trafficking. As Darrell Irvine showed in his talk (see pg. 7 above), heavily glycosylated protein nanoparticles are more likely to be trafficked to the B cell follicles, which protects the antigen from proteolysis.[66,67,96] Protein nanoparticles are also ideal to codisplay multiple antigens. King's group has designed a mosaic nanoparticle that displays four influenza HA proteins. The hope is that this strategy will lead to more broadly protective vaccines by more strongly activating B cells that recognize multiple antigens.[97–99]

Nanoparticle vaccines to elicit broadly reactive anti-HIV responses

Kevin Saunders from Duke University discussed the development of nanoparticle vaccines to elicit broadly neutralizing HIV-1 antibody responses. The ability to elicit broadly neutralizing antibodies is generally considered required for a protective HIV vaccine. Individuals with chronic HIV infection have had years of affinity maturation to yield broadly reactive antibodies. By isolating these antibodies and using computational methods to reconstruct their maturation history, Saunders and colleagues hope to design immunogens that target different stages of antibody development, ultimately resulting in a broadly neutralizing response.[100] Because these broadly reactive antibodies have had years to mature within infected individuals, it is necessary to tease out which mutations are critical for reactivity. The program ARMADiLLO simulates antibody somatic mutations, assigning a probability score to each mutation. This process has been used to identify four improbable mutations that occur early in antibody maturation and confer neutralization breadth to a broadly reactive antibody that targets the V3-glycan binding site on ENV[101] Saunders described HIV ENV-based nanoparticle vaccines, including protein-based and mRNA-LNP platforms, developed to engage early, unmutated common ancestors of broadly reactive antibody lineages. Two protein-based gene fusion vaccines are moving into clinical trials in 2023; these vaccines are designed to induce and mature broadly reactive antibodies that target the CD4 and V3-glycan binding site on ENV, respectively.[102] Saunders also described work to improve upon gene fusion protein–based nanoparticles with conjugated nanoparticles. Conjugating ENV to the nanoparticles provides more control over antigen quality. In mice, a conjugated protein nanoparticle vaccine elicited a similar broadly reactive antibody response while eliciting fewer off-target, non-neutralizing antibodies.[102] Saunders also showed that an mRNA-LNP vaccine, in which the mRNA encodes HIV ENV, can successfully lead to assembled protein nanoparticles *in vitro* and elicit neutralizing antibodies in mice.[103]

Systems biology approach to identify correlates of immunity

Galit Alter from the Ragon Institute presented work using systems serology to define correlates of immunity to SARS-CoV-2. While neutralizing antibodies are commonly used as a correlate of vaccine protection, one of the key observations as SARS-CoV-2 variants emerge is that vaccines continue to protect against severe disease and death despite the loss of neutralization. Alter is looking into what other antibody functions are important for protection. Antibodies do a lot more than block infection via neutralization. Binding

antibodies can recognize pathogens and target them for destruction via innate immune cells. Alter's group has developed high-throughput, systems biology tools, dubbed *systems serology*, to correlate antibody functions and biophysical features with disease outcomes. Using these methods, they have identified patterns of antibodies associated with the natural resolution of severe COVID-19, with vaccine efficacy, and with hybrid immunity. Among patients with severe COVID-19, spike-specific antibodies involved in opsonophagocytic functions, such as monocyte and neutrophil phagocytosis and complement activation, were higher among patients who survive severe infection than those who do not. These data indicate that non-neutralizing Fc-effector functions, specifically opsinophagocytosis, are likely key to the natural resolution of infection.[104] Regarding vaccine-mediated immunity, Alter showed that different vaccine platforms induce unique functional humoral fingerprints that can be linked to vaccine efficacy.[105] For example, higher rates of breakthrough infection were observed with the Pfizer vaccine than with the Moderna vaccine when the Alpha and Delta variants emerged.[106] Alter's group profiled antibody responses among people who received either vaccine and found that the Moderna vaccine elicits higher IgA across multiple viral epitopes as well as high levels of NTD-specific antibodies that interact with various Fc receptors. Therefore, the Moderna vaccine appears to elicit an immune response that spreads across the spike protein, which may contribute to its ability to protect against viral variants.[107] Finally, Alter discussed why hybrid immunity (resulting from infection and vaccination) is more robust than vaccine-induced immunity.[108] They found unique antibody profiles among those with hybrid immunity. In particular, hybrid immunity was associated with high Fc-effector quality against the S2 region of the spike protein, which is highly conserved.[109]

Protein nanoparticles targeting the HA stem

Syed M. Moin from the Vaccine Research Center at the NIAID/NIH presented work on inducing cross-group neutralizing antibodies against influenza A viruses by targeting the HA stem region. Targeting the HA stem has become a strategy for a universal influenza vaccine to focus the immune response toward the conserved stem region and generate a broad antibody response.[110,111] Moin focused on two vaccines in which the HA stem is genetically fused and displayed on self-assembling ferritin nanoparticles: H1ssF and H10ssF, which are derived from the HA stem of group 1 (H1N1) and group 2 (H10N8) influenza viruses, respectively. Administering either of these vaccines alone provided heterosubtypic protection from lethal challenges in mice but did not result in cross-group protective responses.[110,112] However, coimmunizing with H1ssF and H10ssF elicited cross-group neutralizing antibody responses across several animal models, including NHPs.[113] Coimmunization induced cross-group reactive B cell population in NHPs and a bnAb isolated from an immunized NHP broadly neutralized and conferred protection against diverse group 1 and group 2 viruses.[113] Genetic, structural, and immunological analyses showed homology between NHP and human bnAb and revealed a common mode of HA recognition through a DH gene–encoded motif among the two.[113] H1ssF and H10ssF are being evaluated in humans in phase 1 trials.

An HCV E2E1 nanoparticle vaccine

Kwinten Sliepen from the University of Amsterdam presented work on inducing cross-neutralizing antibodies against the hepatitis C virus (HCV) using nanoparticles that display a novel recombinant form of the HCV E1E2 glycoprotein. E1E2 is the major target for neutralizing antibodies; however, sequence diversity, glycan shielding, and lack of structural knowledge have made it difficult to develop E1E2-based immunogens that elicit a broad antibody response.[114,115] Sliepen presented unpublished data on the design of a permutated E1E2 (E2E1) nanoparticle vaccine. Combining recombinant E2E1 from different strains on the same "mosaic" nanoparticles elicited significantly broader antibody responses. He also showed how a high-resolution cryoEM structure of E1E2 was obtained by stabilizing the glycoprotein with antibody Fabs. The structure revealed key interactions that stabilize the E1/E2 interface as well as novel epitopes. Sliepen hopes that these structural insights will inform the design of new HCV immunogens and ultimately lead to an HCV vaccine.[116]

DIVERSITY AND EVOLUTION OF IMMUNITY TO VACCINATION

Elucidating the diversity of V, D, and J genes

Gunilla B. Karlsson Hedestam from Karolinska Institutet presented work on understanding how diversity in V, D, and J genes affects the adaptive immune response. V, D, and J genes are among the least well-characterized portions of the genome; they are typically not covered by traditional whole genome sequencing methods, and little is known about the diversity of these sequences across populations. Karlsson Hedestam's group has developed a germline inference tool, IgDiscover, that allows the identification of germline immunoglobulin alleles with single nucleotide precision from expressed BCR and TCR repertoires.[117] In a population study covering 80 individuals from Europe, South Asia, East Asia, and sub-Saharan Africa, Karlsson Hedestam's group identified over 100 previously undescribed immunoglobulin heavy chain V (IGHV) alleles, most of which were present in the African population group. The ability to genotype individuals for their V, D, and J allele content and to couple this to functional studies offers new possibilities to study the role of germline gene variations on elicited adaptive immune responses. Karlsson Hedestam further showed how the group has isolated mAbs from persons infected with SARS-CoV-2 and, similar to previous studies, identified IGHV genes that were overrepresented in spike-specific memory B cells. Further characterization of some of the strongest neutralizing mAbs representing a public antibody class demonstrated that affinity maturation played a major role, leading

to antibodies that displayed both potent and broad neutralization activity, even against Omicron variants. Somatic hypermutation of key amino acids in a representative mAb was responsible for the increase in neutralization breadth, results supported by structural analyses.[118] Karlsson Hedestam also showed how they characterized another class of SARS-CoV-2 neutralizing antibodies where the specific IGHV allele present played an important role in antibody recognition of the target epitope. She concluded that because some IGHV genes used in SARS-CoV-2 antibodies are characterized by considerable allelic variation in the population, some classes of antibodies may be more personal and depend on specific IGHV allele usage.

Immunity across the circumsporozoite protein

Hedda Wardemann from the German Cancer Research Center discussed work on assessing the human antigen receptor repertoire to guide vaccine design. Wardemann focused on the immune response to circumsporozoite protein (PfCSP) from *Plasmodium falciparum*, the parasite that causes malaria. Circumsporozoite protein has been a primary target for vaccine design, including the only commercially available malaria vaccine, RTS,S/AS01. RTS,S/AS01 is composed of key immunogenic regions of the circumsporozoite protein: the NANP motifs within the central repeat domain, which elicit a B cell response, and the C-terminus, which activates the T cell response. However, RTS,S/AS01 suffers from limited and short-term efficacy. Several studies on anti-PfCSP responses in humans have demonstrated that protective, high-affinity anti-PfCSP antibodies target the central repeat domain and the N-terminal junction.[119–121] Wardemann's group has shown that antibodies against the C-terminus fail to protect against infection.[122] Wardemann presented, unpublished data that further support the fact that anti-C-terminus antibodies are nonprotective. With regard to vaccine design, preventing this nonprotective B cell response, while maintaining the C-terminus–mediated T cell response, may provide better efficacy. Wardemann's group has identified a short epitope within the C-terminus that is recognized by 94% of TCRs. However, the T cell response was highly specific, with cross-reactivity to common PfCSP variants limited by HLA types.[123]

Leveraging systems biology to decipher vaccine-mediated immune responses

Bali Pulendran from Stanford University presented several examples of how systems biology can provide key insights into vaccine-mediated immunity and inform vaccine design.[124] Pulendran incorporates transcriptomics, epigenetics, metabolomics, and other "omics" analyses to derive insights about the molecular networks impacted by vaccines. For example, in individuals who received a shingles vaccine, this multiomics approach revealed that expression of SREBP was highly correlated with both antibody and T cell responses.[125] SREBP is involved in cholesterol metabolism and had not previously been implicated in vaccine-mediated immunity. Pulendran presented unpublished data elucidating the role of SREBP in the antibody response and B cell function. In a second example, Pulendran showed how systems biology has shed light on the impact of the microbiome on vaccine-mediated immunity. Multiomics analyses of individuals receiving influenza vaccines revealed a high correlation between the expression of TLR5, which plays a role in sensing bacterial flagella, and antibody responses.[126,127] Follow-up studies in mice[128] and humans[129] showed that impairing the microbiome altered vaccine-mediated immunity. In humans, vaccinees given broad-spectrum antibiotics had an altered metabolome characterized by a decrease in secondary bile salts and a corresponding increase in inflammation and dendritic cell activation. Somewhat paradoxically, these effects correlated with impaired vaccine immunity and influenza-specific antibody response.[129] Finally, Pulendran showed how his group is using systems vaccinology to understand the durability of the response to COVID-19 vaccines.[130] Work with yellow fever vaccine identified innate receptors critical to durability.[131,132] However, modified mRNA vaccines are designed to prevent the activation of immune receptors.[22] Pulendran's group has identified which innate immune pathways are triggered by the Pfizer COVID-19 mRNA vaccine. He showed that both antibody and T cell responses are independent of several innate pathways, including TLR signaling and inflammasome activity, while T cell responses are dependent on the innate receptor MDA-5.[133] His group is continuing to work on understanding which innate responses lead to durable antibody responses.

The importance of COVID-19 vaccine boosting to protect against variants

Nicole A. Doria-Rose from the NIH presented data on the importance of boosting to improve the durability and breadth of COVID-19 vaccines. At the time of the meeting, two doses of either the Pfizer or Moderna mRNA vaccines were considered fully vaccinated in the United States. A third booster dose was recommended for all adults, with discussions on a second booster for at-risk populations. Data from individuals who received two doses of the Moderna mRNA COVID-19 vaccine indicated that neutralizing antibody titers persist for at least 6 months but that neutralization is lower for more recent variants. Several assays, including pseudovirus neutralization, live virus neutralization, ACE2 blocking, and spike binding, demonstrated similar kinetics and variant susceptibility.[134] The necessity of a third dose became critical as new, more contagious variants emerged that are able to escape neutralization. By mid-2021, it was clear that waning antibody titers, new variants, and the emerging highly contagious Delta strain overwhelmed the protection offered by the first round of immunizations. A study done in Israel showed that a third dose of the Pfizer mRNA vaccine improved binding, neutralization, and avidity, which correlated with a lower risk of breakthrough infection.[135] Toward the end of 2021, the main variant of concern was Omicron. Doria-Rose

presented data[b] showing that while neutralization titers against Omicron were significantly lower after two vaccine doses, they could be boosted to levels comparable to those that provide protection against the original strain with a third dose. The boosting dose likely provides breadth because it selectively expands antibodies that have had several rounds of affinity maturation.[136] Doria-Rose ended by noting that a strategy involving continually vaccinating the entire population every 6 months is not feasible. Despite the importance of boosting, rates of boosting in the United States are low—approximately one-third of Americans had received a third dose by mid-2022—and vaccine access and inequity preclude booster doses throughout much of the developing world.[137] Improving the durability of vaccine response and developing vaccines with broader activity will be key to ending the revolving door of variant-related surges.

Eliciting cross-neutralizing responses against SARS-CoV-2 in an NHP model

Wan-Ting He from Raiees Andrabi's lab at The Scripps Research Institute presented a study on eliciting broadly neutralizing antibodies to SARS-related viruses in NHPs. He's unpublished work focuses how different immunization strategies impact antibody responses and the ability to elicit cross-neutralizing responses. Characterizing these broadly neutralizing antibodies may inform new rational vaccine design efforts.

Understanding T cell responses and memory to SARS-CoV-2

Jennifer A. Juno from the University of Melbourne presented work on understanding the T cell response and T cell memory to SARS-CoV-2 infection and COVID-19 vaccination. Both infection and vaccination induce a robust $CD4^+$ T cell response. Juno's group had previously identified a spike-specific immunogenic T cell epitope in COVID-19 convalescent patients and confirmed its HLA restriction. They are using pHLA tetramers to study spike-specific T cells and T cell memory under varying conditions, including following mild SARS-CoV-2 infection, vaccination, hybrid immunity, and breakthrough infections. Juno showed that both mild COVID-19 disease and primary vaccination established long-lasting $CD4^+$ T cell memory, including a persistent spike-specific cTfh pool. Infection and vaccination established similar T cell repertoires, with similar α/β pairings. They also identified motifs and clones that were shared across vaccinees and convalescent individuals, indicating that the T cell response is fairly well conserved. In individuals with hybrid immunity (convalescent patients who received a vaccine), the spike-specific T cell memory was rapidly and efficiently recalled upon vaccination.[138] Juno showed additional unpublished work suggesting that T cell recall may be less robust among those with breakthrough infection.

[b] *N Engl J Med*. 2022 Mar 17;386(11):1088-1091. doi: 10.1056/NEJMc2119912.

Eliciting broader influenza immunity in humans

Sila Ataca from the Kanekiyo laboratory at the Vaccine Research Center, NIAID/NIH, presented unpublished study on the effects of an immunization regimen on the breadth of influenza HA stem–directed B cells in a human immunoglobulin VH1-69 gene knock-in mouse model. Because of its uniquely hydrophobic CDR H2, human VH1-69 binds group 1 HA but not group 2 HA. Ataca found that even in the highly group 1–biased B cell repertoire in the VH1-69 knock-in mice, priming them with a group 2–based HA stem immunogen altered the HA stem–specific B cell repertoire and led to the elicitation of cross-group reactive B cell responses upon subsequent group 1 HA stem immunizations. The cross-group HA stem–directed B cells isolated from the mice share a common CDR H3 motif, instead of the CDR H2, to engage HA. The study offers insights on vaccine strategies for eliciting broader influenza immunity in humans.

STRUCTURAL IMMUNOLOGY–GUIDED RATIONAL VACCINE ANTIGEN DESIGN

Balancing B and T cell responses in antigen design

Adam K. Wheatley from the University of Melbourne presented work on the importance of considering both the B and T cell responses in vaccine design. Wheatley showed two examples in which B and T cell epitopes are found in different regions of an immunogen. For both the influenza HA and the SARS-CoV-2 spike, B cells tend to recognize conformational epitopes that cluster in a domain. In contrast, T cells recognize linear peptide sequences found throughout an antigen.[139] In the case of HA, the exposed head domain is the immunodominant domain and the focus of seasonal vaccines. However, there is interest in redirecting immunity toward the more conserved stem region to elicit a broader response. Wheatley's group showed that immunizing mice with a truncated HA stem construct did not elicit a high antibody response. They mapped the T cell epitopes in HA and found that Tfh repertoires were heavily skewed toward a small number of epitopes in the HA head domain. Other immunogens, such as ovalbumin, also elicited a restricted Tfh repertoire. Wheatley concluded that the HA stem does not contain effective Tfh epitopes in mice, which limits the immunogenicity of HA stem monomeric vaccines.[139] He showed similar, unpublished, work on the localization of T cell epitopes within the spike protein of SARS-CoV-2. Wheatley stressed that it is important to balance B and T cell epitopes when designing an immunogen. His work suggests that there may be inherent tension in selecting the optimal B cell immunogen, which would ideally be small to focus on neutralizing epitopes and limit the number of distracting epitopes, and the optimal T cell epitope, which may require a larger immunogen. He suggested two strategies to boost the effective T helper response to smaller immunogens. Conjugating the HA stem to a carrier protein as well as making multimeric antigens have been shown to increase the immunogenicity of the HA stem.[139]

Stabilizing prefusion F protein for an hMPV vaccine

Jason S. McLellan from the University of Texas at Austin described work on an RSV F vaccine antigen and an analogous antigen for hMPV. The RSV F protein has become a classic story of how structural biology can guide antigen design. The F protein is a glycoprotein found in the membrane of RSV. In the absence of a host cell, the F protein exists in a metastable, prefusion state. When it engages with a host cell, it undergoes a significant conformational change that enables it to attach to the host cell membrane and bring the membranes together, eventually adopting a highly stable, postfusion state. McLellan has been instrumental in determining the structures of RSV F protein in the postfusion[140,141] and prefusion[7] states, as well as in elucidating the conformational changes that occur as the protein switches between the two states.[142] Prefusion F is too unstable to use as an antigen. With the structure solved, McLellan incorporated stabilizing mutations that lock the protein in the prefusion state, rendering it suitable to be used as an antigen. In both mice and NHPs, a stabilized prefusion F construct was shown to be more immunogenic than a postfusion construct.[6] This was corroborated by findings that the majority of high potent neutralizing antibodies target prefusion-specific conformational epitopes that do not exist in the postfusion state.[143] RSV vaccines based on stabilized prefusion F protein are currently being investigated in phase 3 clinical trials.[144–146] McLellan showed how his group is expanding on their work with RSV F protein to create a vaccine for hMPV, which contains a similar F protein. They created a stabilized prefusion hMPV F construct and showed that it adopts a similar fold as prefusion RSV F. However, unlike RSV prefusion F, hMPV prefusion F was not more immunogenic than the postfusion form in mice in their initial studies; this may be due to the glycosylation pattern in hMPV F, which is likely to shield prefusion antibody binding sites.[147] McLellan's group has incorporated additional mutations into hMPV F to improve the yield and further stabilize the prefusion form. He showed that this construct elicits robust neutralizing antibody titers compared to postfusion F in mice.[148] Clinical trials in humans began in 2022.

Memory B cell responses to Omicron breakthrough infection

Laura M. Walker from Adagio Therapeutics presented work on the memory B cell response elicited by Omicron breakthrough infection. Omicron was the first variant with major antigenic shifts. It contains mutations in key antigenic sites within the RBD recognized by all three classes of neutralizing antibodies, allowing it to nearly universally escape the neutralizing response.[149] Walker's group evaluated the acute B cell response among vaccinated individuals infected by Omicron. During her talk, Walker focused on the subvariant BA.1, though other Omicron subvariants emerged. Walker showed that BA.1 breakthrough infection drives a broader serum-neutralizing antibody response that is cross-reactive against other SARS-CoV-2 variants, but not against other sarbecoviruses. Somewhat paradoxically, breakthrough infection shifted the B cell immunodominance hierarchy from the S2 subunit to the RBD. The S2 subunit is highly conserved between variants, and it is expected that antibodies that target S2 are more likely to be cross-reactive. Walker proposed that the shift in immunodominance may be caused by circulating S2-specific antibodies, which bind to S2 and mask its ability to activate B cells. In contrast, many of the pre-existing circulating antibodies would not recognize the BA.1 RBD. Walker showed that most of the BA.1-activated B cells are clonally diverse and exhibit high levels of somatic hypermutation. This indicates that the acute B cell response following BA.1 breakthrough infection is primarily mediated by the recall of vaccine-induced WT/BA.1 cross-reactive memory B cells that have undergone affinity maturation. Walker's group also identified two antibodies with that broad activity against SARS-CoV-2 variants and SARS-CoV-1, which may be instrumental as therapeutics. The fact that the two antibodies cross-neutralize SARS-CoV-1 indicates that they target an immunorecessive site under less selective pressure.[150]

Influenza virus NAs: Targeting the conserved catalytic site

Julia Lederhofer from the Kanekiyo laboratory at the Vaccine Research Center, NIAID/NIH, presented unpublished work on the discovery of mAbs against influenza NA from a convalescent individual of a recent H3N2 infection. Lederhofer identified immunoglobulins that cross-react with NAs across various influenza A and B viruses. The expressed antibodies derived from isolated B cells not only bound tightly to N1 subtype NAs, but also to influenza B NA along with avian influenza NAs. This work illustrates the ability of humans to generate broadly protective NA-directed antibodies through infections and/or vaccinations by targeting the conserved catalytic site on the otherwise hypervariable NA protein.

Toward a broadly reactive norovirus vaccine

Lisa C. Lindesmith from the University of North Carolina discussed the development of a human norovirus vaccine candidate, with a focus on broad activity across the different norovirus variants. Lindesmith's group has incorporated a genetically diverse mouse model system to recapitulate the range of responses to mRNA vaccination seen in humans. She described unpublished work on a chimera virus-like particle vaccine that incorporates immunogens from the common GII.4 variant into another epidemiologically important genotype.

Structure-based vaccines for prion diseases

Holger Wille from the University of Alberta described work using structure-based approaches to design a vaccine against prion diseases. Prion diseases are caused by pathological misfolded proteins. Prion protein-based vaccines have had limited success due to a lack of control

over the antigen structure, which is critical. Structures of the healthy and infectious prion protein[151,152] reveal a conformational epitope that is present on the misfolded prion protein but not on the properly folded protein. Wille's group has incorporated this epitope into a scaffold protein and is investigating the potential for it to act as a prion vaccine for various animal and human prion diseases.

VACCINE DIPLOMACY AND DISTRIBUTION

The development of highly effective, safe COVID-19 vaccines within a year of the beginning of the pandemic was an unprecedented scientific achievement that leveraged decades of research and preexisting partnerships. Since then, one of the key challenges has been getting these vaccines into people's arms. The barriers are two-fold. Vaccine hesitancy and, in some cases, flat-out antiscience sentiment have fomented the antivaccine movement, particularly in developed countries where vaccines are accessible to virtually anyone who wants one. At the same time, vaccine inequities related to logistical hurdles, limited resources, and stockpiling persist such that vaccination rates in low- to middle-income countries remain low.[153,154] During the "Vaccine Diplomacy and Distribution" session, **Peter J. Hotez** from Texas Children's Hospital and Baylor College of Medicine and **Linda-Gail Bekker** from the University of Cape Town discussed ways to improve vaccine uptake by addressing both logistical and psychological barriers.

Before COVID-19, Hotez, together with his colleague Maria Elena Bottazzi, led a group at the Texas Medical Center focused on developing vaccines for multiple parasites that afflict the developing world. Hoetz calls these "antipoverty vaccines" because while the diseases they target are often not lethal, they can be very debilitating in terms of reproductive health, child development, and productivity. Preventing these diseases, therefore, has significant potential to improve the quality of life across rural and impoverished urban areas.[155] These diseases do not garner the interest of Big Pharma as they have limited commercial value. The group does much of the early development and pilot manufacturing themselves and transfers the technology to members of the Developing Country Vaccine Manufacturers Network, which consists of over 40 manufacturers in 14 countries and territories.[156] When COVID-19 emerged, much of the focus from US and European pharma companies was on developing vaccines using new technologies, including mRNA, adenovirus vectors, and nanoparticles. Hotez noted that there was not enough consideration on leveraging pre-existing infrastructure in developing countries that had been producing billions of doses of recombinant hepatitis B vaccines. Hotez and colleagues had developed adjuvanted yeast-expressed recombinant protein SARS and COVID-19 vaccines designed for low- to middle-income countries that are easy to produce and cost-effective.[157–160] In collaboration with the Indian vaccine producer Biological E Ltd, the COVID-19 vaccine marketed under the brand name Corbevax™ in India demonstrated immunogenic superiority over ChAdOxInCoV-19 in a phase 2/3 trial.[158] The technology has been transferred for local production in India, Indonesia, Bangladesh, South Africa, and Botswana. In Indonesia, the vaccine is known as IndoVac, and is being produced as a halal recombinant protein vaccine by BioFarma.

Hotez also addressed the growing antivaccine movement. Antivaccine sentiment is not new. Most recently, however, it has evolved from a focus on vaccines and autism to a politically motivated movement on health and medical freedom that is not limited to the United States. Hotez has led efforts to counter antivaccine activism for decades in his role as both a vaccine scientist and parent of an adult daughter with autism and intellectual disabilities.[c] Hotez posited that antiscience aggression is a leading killer around the world and most certainly contributed to the COVID-19 death toll.[161,162] In the United States, there is a clear divide along political lines in terms of who has received COVID-19 vaccinations. Unsurprisingly, this correlation extends to COVID-19–related deaths: based on analyses conducted by the health analyst Charles Gaba and multiple news outlets, counties that vote more Republican have higher rates of COVID-19–related deaths than those that vote Democrat.[163] Given the thousands of deaths in the United States among the victims of antivaccine activism during the COVID-19 pandemic, Hotez emphasized the urgency to counter antiscience aggression and to not back away from political discourse, although he acknowledged the challenges that the community of physicians and scientists face on this front.

Bekker also challenged the audience with a call to action. Unlike Hotez, who spoke about people who vehemently oppose vaccines, Bekker focused on ways to address vaccine hesitancy among those who are more in the middle. Vaccine hesitancy, defined as the delay in acceptance or refusal of vaccines despite the availability of vaccine services, is not new.[164] In 2019, the WHO listed vaccine hesitancy as one of the top 10 threats to global health and established a vaccines advisory group to understand the reasons behind vaccine hesitancy.[165] Combatting vaccine hesitancy requires identifying who is most likely to refuse vaccines and why. Regarding the COVID-19 pandemic, surveys across developed countries showed that a significant portion of the population does not plan on getting vaccinated,[166] while vaccine acceptance rates were generally higher in low- to middle-income countries than in high-income countries.[167] The determinants of vaccine hesitancy can be boiled down to the three Cs (Figure 2): complacency—when people perceive that the risks of disease are low and do not see the necessity of vaccination; convenience—when getting vaccinated is too burdensome due to time or distance constraints; and confidence—when people lack trust in vaccines, the systems that deliver them, and the policymakers who develop vaccination recommendations.[168] In terms of confidence, belief in the importance of vaccines as opposed to their efficacy or safety had the strongest association with vaccination.[169]

Communication is key to address vaccine hesitancy. Unfortunately, many healthcare workers, who are often at the front lines of communicating health-related information to the public, are themselves vaccine hesitant.[170] Research on promoting vaccine confidence has emphasized stressing the importance of the collective impact of vaccines,

[c] Hoetz wrote the landmark book *Vaccines Did Not Cause Rachel's Autism: My Journey as a Vaccine Scientist, Pediatrician, and Autism Dad*. Johns Hopkins University Press; 1st edition (October 30, 2018).

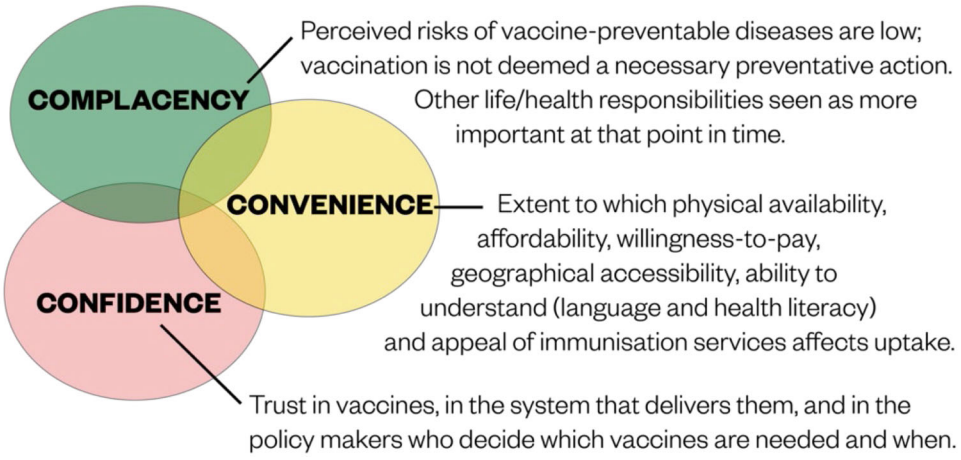

FIGURE 2 The 3C's of vaccine hesitancy (first proposed in 2011).[168]

engaging communities, and establishing trust and partnerships.[171] Vaccine-hesitant individuals may be amenable to changing their vaccination attitudes and behaviors if their concerns are adequately addressed and systemic barriers to accessing health services are removed.[172] Bekker stressed that any vaccine confidence program must be tailored to the audience and incorporate messaging that addresses the audience's main concerns and what drives them to change behavior.[173] Specific interventions identified by the Vaccine Confidence Project, which primarily focused on high-income countries, include advocacy campaigns and reminder systems. The recommendations primarily included combatting misinformation and improving general vaccine knowledge. However, this group stopped short of recommending incentive programs and had few multicomponent approaches.[174] In a more recent systematic review, researchers emphasized the importance of a multidimensional approach that may include community-based interventions, monetary incentives, and health literacy.[175]

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COMPETING INTERESTS

The authors of CureVac AG, Tübingen, Germany, a publicly listed company developing mRNA-based vaccines and immunotherapeutics, may hold shares in the company and are inventors of several patents on mRNA vaccination and use thereof.

The Ellebedy laboratory received funding from Moderna, Emergent BioSolutions, and AbbVie that are unrelated to the data presented in the current study. A.H. Ellebedy has received consulting and speaking fees from InBios International, Inc, Fimbrion Therapeutics, RGAX, Mubadala Investment Company, Moderna, Pfizer, Danaher, Third Rock Ventures, Goldman Sachs, and Morgan Stanley, and is the founder of ImmuneBio Consulting.

L.C. Lindesmith holds patents on norovirus vaccine design and ongoing collaborations with VaxArt, Takeda Vaccines, and HilleVax that are unrelated and do not pose conflicts of interest with this report.

N. Pardi is named on a patent describing the use of nucleoside-modified mRNA in lipid nanoparticles as a vaccine platform. N. Pardi and R. Nachbagauer are named on a patent filed on universal influenza vaccines using nucleoside-modified mRNA. N. Pardi has disclosed those interests fully to the University of Pennsylvania and The Icahn School of Medicine at Mount Sinai, and has in place an approved plan for managing any potential conflicts arising from licensing of the patents.

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