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Forum

The Challenges of Vaccine Development against a New Virus during a Pandemic

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The rapid emergence of a highly pathogenic, readily transmissible coronavirus has resulted in a global pandemic, affecting millions and destabilizing economies. This catastrophe triggered a clarion call for the immediate deployment of a protective vaccine. We describe the unique challenges of developing a vaccine against SARS-CoV-2 in a pandemic setting.

Historically, human coronaviruses have received limited attention from the research and medical communities. Although infections by human coronaviruses (e.g., HCoV-229E and HCoV-OC43) frequently cause common cold symptoms in healthy individuals, severe clinical illness is relatively rare even in immunocompromised individuals, infants, and the elderly. Considerably more has been known about animal coronaviruses, leading to vaccines to prevent the infection of commercially important domesticated animals (e.g., cats, dogs, and cattle). Experiments over many years with murine hepatitis virus (MHV), a neurotropic and hepatotropic coronavirus, have provided substantial insight into the pathogenesis of viral hepatitis and central nervous diseases, including multiple sclerosis. While these studies enhanced our understanding of the molecular virology and pathogenesis of coronaviruses, only the recent explosive emergence of highly pathogenic coronaviruses in humans has sparked intense interest in developing countermeasures for human viruses in this family.

In 2002, the severe acute respiratory syndrome coronavirus (SARS-CoV) virus emerged from bats and possibly civet cats in China. Within eight months, SARS-CoV spread to 27 countries around the world, resulting in 8,096 human infections and 774 deaths (de Wit et al., 2016). An unprecedented level of containment, made possible by extensive collaboration among international public health organi-

zations, limited transmission and human disease. Ten years later, a second coronavirus, the Middle East respiratory syndrome coronavirus (MERS-CoV), was isolated from an individual suffering from severe respiratory disease and renal failure. As observed with SARS-CoV, MERS-CoV spread via travel and contact with infected individuals, resulting in transmission and mortality within and beyond the Arabian Peninsula (~2,494 human cases, 858 deaths). Serological studies identified dromedary camels as the principal reservoir for MERS-CoV. Since the discovery of SARS-CoV, two additional human coronaviruses that cause lower respiratory tract infections (HCoV-NL63 and HCoV-HKU1) have been discovered. Sequencing studies of bat reservoirs identified a large number of novel coronaviruses with an unknown potential to emerge in humans (Graham et al., 2013).

An appreciation of the considerable threat of coronaviruses to global health prompted remarkable advances in our understanding of the molecular virology and pathogenesis of SARS-CoV and MERS-CoV as well as efforts to develop vaccines. In this Forum, we discuss how the prior study of SARS-CoV and MERS-CoV enabled the extraordinarily rapid development of candidate vaccines that hopefully can curtail the incendiary spread of a novel, highly pathogenic zoonotic coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). However, this unprecedented pace has eclipsed some of the funda-

mental basic and translational studies that customarily guide vaccine development and evaluation under non-pandemic circumstances. Accordingly, we discuss the challenges of rapid vaccine development during a pandemic response.

The SARS-CoV-2 Pandemic

SARS-CoV-2 is a positive-sense single-stranded RNA virus first isolated in Wuhan, China, in December of 2019 from a cluster of acute respiratory illness cases (Zhou et al., 2020). SARS-CoV-2 infection results in a clinical syndrome, coronavirus-induced disease-19 (COVID-19), which is characterized by fever, cough, and shortness of breath that can progress rapidly to respiratory and cardiac failure requiring mechanical ventilation (Guan et al., 2020). The elderly, immunocompromised, and those with co-morbid metabolic, pulmonary, and cardiac conditions are at a markedly greater risk of death from COVID-19. Virtually all countries and territories have been affected, with major epidemics in China, Italy, Spain, Germany, France, Iran, and the United States and epicenters in large urban cities. Most cases are spread by direct human-to-human and droplet contact, with a contribution from transmission in asymptomatic or mildly symptomatic individuals. SARS-CoV-2 has caused a global pandemic with more than 2,400,000 infections to date, 165,000 deaths (as of 19 April 2020), and a case-fatality rate estimated at ~4%. The extensive morbidity, mortality, and destabilizing



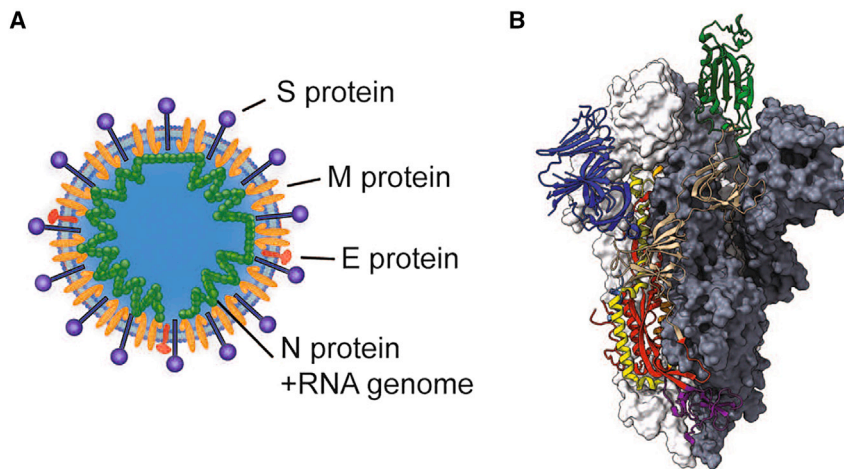


Figure 1. Structure of the SARS-CoV-2

(A) The SARS-CoV-2 is a spherical virus particle that incorporates three structural proteins into its lipid envelope (S, M, and E) and encapsidates a positive-stranded RNA genome in complex with the N protein. (B) The structure of the S protein trimer was solved using cryo-electron microscopy and is shown (PDB: 6VYB) (Wrapp et al., 2020). The RBD (shown in green) may exist in an open or closed (up or down) structural state; the RBD of one of the three S protomers within the trimer is shown in the up position.

social and economic consequences highlight the critical need for the accelerated development of countermeasures. Rapid implementation and deployment of an effective SARS-CoV-2 vaccine has the potential to lessen the deleterious effects of infection, especially in vulnerable populations, curb transmission, end the pandemic, and prevent its return.

Coronavirus Structure and Entry

Coronaviruses are round, ~120-nm-diameter virus particles that derive their name from the incorporation of the viral spike (S) protein in an arrangement that resembles a crown on the virion surface. Three additional viral structural proteins (envelope [E], membrane [M], and nucleocapsid [N]), together with a lipid membrane and ~25–31 kilobase viral genome, comprise the virion (Figure 1A; de Wit et al., 2016). The M and E proteins function during virion morphogenesis, and their expression is sufficient for the formation of virus-like particles, although their value as immunogens may be limited because they lack the S protein thought to be an important target for neutralizing antibodies. Interactions between the M glycoprotein and the other three structural proteins coordinate virion assembly. The small structural E protein has multiple functions including stimulating the membrane curvature required for virion budding and promoting lipid membrane scission of virions once

formed. The nucleocapsid protein binds to the viral genome and facilitates its incorporation into the virus particle.

The S protein exists on virions as homotrimeric spikes that promote coronavirus entry into cells via attachment and membrane fusion (de Wit et al., 2016). In some circumstances, the S protein also promotes the formation of syncytia enabling direct cell-to-cell transfer of the viral genome. The S protein is a class I viral fusion protein related functionally to the entry-fusion proteins of other well-characterized viruses including influenza (hemagglutinin [HA], HIV-1 [gp160], and Ebola [glycoprotein, GP]). This class of viral fusion proteins characteristically regulates fusogenic activity by proteolytic cleavage. Accordingly, the SARS-CoV and SARS-CoV-2 S proteins are cleaved sequentially during the entry process to yield S1 and S2 fragments, followed by further processing of S2 to yield a smaller S2' protein. Cleavage also may occur during viral egress. The S1 fragment includes the receptor binding domain (RBD), whereas the carboxyl terminal S2 portion of the molecule contains regions that drive membrane fusion. The structure of a soluble, stabilized prefusion form of the SARS-CoV-2 S protein was solved to a resolution of 3.5Å using cryo-electron microscopy, revealing considerable similarity to the SARS-CoV S protein (Figure 1B; Wrapp et al., 2020). Both vi-

ruses utilize human angiotensin-converting enzyme 2 (ACE2) as a cellular receptor, consistent with the high conservation of the RBD.

Vaccine Development

The application of strategies that have worked for related viral family members provides a path for rapid vaccine development against a newly emerging virus. For example, several of the antigen targets, vaccine platforms, and clinical trial approaches used for Zika virus (ZIKV) after its emergence in 2015 were based on prior studies and successes with related flaviviruses. Several vaccine strategies against SARS-CoV and MERS-CoV, many focused on the S protein, have been developed and tested extensively in pre-clinical models (Zhou et al., 2018). Although multiple platforms of SARS-CoV and MERS-CoV vaccine candidates were evaluated in human trials (e.g., viral vectored [NCT03615911 and NCT04170829], DNA plasmid [NCT00099463], and inactivated virus [NCT00533741]), none advanced beyond phase 1 safety studies. Similarly, no vaccines have been developed against other human coronaviruses.

Within months of publication of the SARS-CoV-2 genome sequence, work began on dozens of vaccine candidates (<https://www.who.int/blueprint/priority-diseases/key-action/novel-coronavirus-landscape-ncov.pdf?ua=1>), enabled by insights from the SARS-CoV and MERS-CoV vaccine development and well-characterized vaccine antigen expression platforms, including those shown to be safe in humans. Several vaccines, described below, have entered phase 1 clinical trials to evaluate safety in small numbers of subjects (Table 1). However, given the lack of established and tractable pre-clinical disease models of SARS-CoV-2 pathogenesis, these vaccines have advanced to human trials without data in animals establishing efficacy against COVID-19.

mRNA-1273

Modified mRNAs are synthetic molecules optimized for high-level and durable protein expression. This technology has shown promise as a vaccine platform for influenza virus (NCT03076385 and NCT03345043) and in preclinical studies with other infectious disease targets including ZIKV. Developed by the National Institute of Allergy and Infectious

Table 1. Vaccine Candidates in Development against SARS-CoV-2

Vaccine Type	Platform	Protein Target	Stage	Clinical Trial Designation	Company or Institution	Vaccine Name	Status
mRNA	Lipid-encapsulated mRNA	S	Phase 1	NCT04283461 (45 subjects)	Moderna	mRNA-1273	Recruiting
DNA	DNA plasmid	S	Phase 1	NCT04336410 (40 subjects)	Inovio Pharmaceuticals	INO-4800	Recruiting
Human adenovirus (Ad5)	Viral-vectored	S	Phase 1	NCT04313127 (108 subjects)	CanSino Biologics	Ad5-nCoV	Active
Chimpanzee adenovirus	Viral-vectored	S	Phase 1/2	NCT04324606 (510 subjects)	University of Oxford	ChAdOx1 nCoV-19	Planned (4/20)
Spike protein	Bacterially produced soluble protein (oral)	S	Phase 1	NCT04334980 (84 subjects)	Symvivo Corporation	bacTRL-Spike	Planned (4/30)
BCG vaccine	Immune stimulatory	None	Phase 3	NCT04327206 (4170 subjects) NCT04328441 (1000 subjects)	Murdoch Childrens Research Institute University Medical Center, Netherlands	BCG vaccine	Recruiting

Information adapted from <https://ClinicalTrials.gov>.

Diseases (NIAID) in collaboration with Moderna, mRNA-1273 is a lipid-encapsulated modified mRNA expressing a prefusion-stabilized S protein. The design of the S protein antigen encoded by mRNA-1273 was informed by prior structure-guided antigen design studies of the stabilization of class I fusion proteins of MERS-CoV and other coronaviruses (Pallesen et al., 2017). These studies identified stabilizing proline substitutions in the central helix of S2 that reduced the fusion activity and allowed high-level expression of trimeric S in an antigenically native prefusion context. The structure of the stabilized SARS-CoV-2 antigen encoded by mRNA-1273 has been solved (Figure 1B). A phase 1 clinical trial of mRNA-1273 (NCT04283461) was initiated in March of 2020 as an open-label, dose-ranging study in 45 subjects who were administered the vaccine in two doses 29 days apart. Subjects will be followed for 12 months following the second dose for safety, with immunogenicity as a secondary objective.

INO-4800

INO-4800 is a DNA plasmid vaccine candidate encoding the SARS-CoV-2 S protein being developed by Inovio Pharmaceuticals. A similar approach was evaluated in preclinical and clinical trials as a vaccine for MERS-CoV and was shown to be immunogenic (GLS-5300; NCT02670187). Three doses of GLS-5300 elicited S-protein-binding antibodies in a majority (94%) of vaccinated individuals, although neutralizing anti-

bodies were observed less frequently (50% of subjects) and titers waned substantially during the course of study follow-up (Modjarrad et al., 2019). Vaccine-elicited, MERS-CoV-specific T cell responses also were detectable in most recipients. These experiences informed the SARS-CoV-2 vaccine candidate INO-4800, which is now being evaluated in an open-label phase 1 clinical trial. Volunteers will receive two doses of DNA administered intradermally by electroporation 28 days apart.

Adenovirus Vectors

Adenovirus (Ad) vectors, derived from multiple species, have been employed to deliver vaccine antigens for multiple infectious disease targets. Multiple Ad-vectored SARS-CoV and MERS-CoV vaccines have been characterized in preclinical models. These vaccines elicited both humoral and cellular coronavirus-reactive responses and protected against infection. A simian Ad vector expressing the MERS-CoV S protein is being evaluated in a phase 1 clinical trial (NCT04170829). A phase 1/2 study to explore the safety, immunogenicity, and efficacy of a SARS-CoV-2 vaccine using this platform has been initiated by investigators at the University of Oxford (NCT04324606). A similar approach using the human Ad5 serotype is being tested by Beijing Institute of Biotechnology and CanSino Biologics Inc. (NCT04313127).

Immunostimulatory Vaccine

Multiple clinical studies are underway to explore the use of Bacille Calmette-Gue-

rin (BCG) as an immunomodulatory vaccine to protect against COVID-19 disease (Table 1). BCG is a live-attenuated isolate of *Mycobacterium bovis* delivered as a low-cost vaccine to more than 100 million children each year to protect against *Mycobacterium tuberculosis* (TB). Multiple studies in humans and mice suggest BCG administration confers a non-specific protective effect against viral infections (Moorlag et al., 2019). The mechanisms of BCG-mediated cross protections may include enhanced Th1 and Th17 responses to heterologous antigens and the induction of non-specific memory or “trained immunity” in innate immune cells capable of producing inflammatory cytokines when challenged secondarily by virus infection.

Challenges of Vaccine Development during a Pandemic

Despite the expedited design of vaccine candidates against a newly emerging virus in a pandemic setting, there remain substantive challenges for implementation due, in part, to a need to concurrently unravel fundamental aspects of virus biology and immunity along with product development efforts. This approach contrasts with vaccine efforts against viruses that have been studied for many years (e.g., influenza, dengue [DENV], and respiratory syncytial viruses) for which we understand in great detail both the antigenicity of the viral structural proteins and the nature of protective and potentially pathological immune responses.

Correlates of Protection

While multiple vaccine platforms against SARS-CoV-2 focus on the S protein, questions remain regarding the key elements of a protective immune response. (1) Will neutralizing antibodies correlate with protection? Do protective responses require antibody targeting of specific epitopes? Is there a threshold neutralizing antibody response that protects against SARS-CoV-2? (2) Do some of the assays commonly used *in vitro* to measure neutralization (e.g., pseudotyped viruses) faithfully predict protective activity *in vivo*? (3) What is the role of non-neutralizing anti-S antibodies that bind the surface of infected cells? Could non-neutralizing antibodies promote effector-function-mediated virus clearance via Fc- γ -receptor interactions? (4) What is the contribution of mucosal immunity in the respiratory tract for protection against infection or dissemination? (5) Are humoral responses to other viral open reading frames (ORFs) important for immunity, especially those with immune antagonist activity that are displayed on the cell surface or secreted extracellularly? And (6) how important are CD4⁺ and CD8⁺ T cell responses to vaccine-mediated protection? While some of this information can be gleaned from pre-clinical and early vaccine trials with SARS-CoV and MERS-CoV, how certain are we that these correlates of protection will transfer to the pandemic SARS-CoV-2?

Animal Models

The emergence and rapid spread of SARS-CoV-2 has provided a very short window to address knowledge gaps about coronavirus immunity. Indeed, a major challenge has been the absence of high-throughput small animal disease models to facilitate vaccine candidate down-selection and the detailed study of protective immunity. Pre-print manuscripts indicate that conventional laboratory strains of mice are not susceptible to SARS-CoV-2 infection (<https://doi.org/10.1101/2020.02.07.939389>), likely because mouse ACE2 cannot act as a cellular receptor for SARS-CoV-2 (Letko et al., 2020). Transgenic mice that express human ACE2 (hACE2-Tg) develop pneumonia after infection; however, these mice are not yet widely available for vaccine testing. Beyond this, pathogenesis and immune response evaluation studies in different non-human primate species

or other possible animal models (e.g., ferrets and hamsters) still are in early stages of development.

Immune Enhancement

Safety is the primary consideration of any vaccine development program, given the anticipated administration to large numbers of people. Beyond the expected reactogenicity profiles, studies with other distantly and closely related coronaviruses have raised concerns that humoral vaccine responses might potentiate SARS-CoV-2 infection via immune enhancement mechanisms. Immune enhancement might occur via at least two distinct mechanisms, detailed below.

Antibody-dependent enhancement (ADE) of infection describes a phenomenon in which poorly neutralizing antibodies bind viruses and increase attachment, internalization, and infection of myeloid cells expressing Fc- γ or complement receptors. ADE of DENV infection contributes to severe clinical disease in a small fraction of individuals infected for a second time by a heterologous serotype of DENV or in newborns that have sub-neutralizing levels of maternal DENV-immune IgG. ADE is an obstacle for DENV-vaccine development, as illustrated by adverse events experienced by DENV-naive children that received the licensed tetravalent vaccine Dengvaxia. Through a possibly similar, yet undefined, mechanism, immunization of cats with a vaccinia virus-vectored feline infectious peritonitis virus S protein vaccine resulted in greater rates of death upon challenge of cats with this animal coronavirus (Vennema et al., 1990). In cell culture experiments, SARS-CoV entry and infection were enhanced by some anti-S antibodies through both Fc- γ -receptor-dependent and -independent mechanisms (Jaume et al., 2011; Wan et al., 2020). Extensive studies with flaviviruses revealed that ADE assays in cell culture do not correlate directly with *in vivo* outcomes, as enhancement can be demonstrated *in vitro* for many viruses for which there is no immune-enhanced disease *in vivo*. This may be explained by the very reductionist format of traditional ADE assays that do not integrate features of the immune response known to contribute to immunity. The inclusion of complement at physiological concentrations, which is omitted from many *in vitro* assays, reduces enhancement in an IgG subclass-dependent manner.

ADE has been demonstrated using immunodeficient murine models of DENV infection following passive transfer of immune sera, but disease severity is reduced considerably in the presence of antiviral T cells. Coronavirus antibodies that enhance *in vitro* have not been tested for their ability to exacerbate infection and disease in animals. Is the infection of Fc- γ -receptor-expressing myeloid cells, which might be augmented by antibodies, an important contributor to COVID-19 disease severity? Studies to address these concepts await the development of appropriate disease models. For DENV, the most compelling evidence for a role of ADE in pathogenesis comes from epidemiological studies in humans.

A second potential mechanism for vaccine-induced disease enhancement results from skewing of the cellular immune response. Enhanced respiratory disease was observed in the 1960s in some recipients of a formalin-inactivated respiratory syncytial virus (RSV) vaccine. Enhanced disease was characterized by lung infiltration by mononuclear cells, including eosinophils, along with a Th2-directed T cell response (Ruckwardt et al., 2019). Immune complex deposition after the induction of binding, but not neutralizing, antibodies has been proposed as an explanation for this response. While pre-clinical studies of multiple SARS-CoV vaccine candidates demonstrated significant protection from infection, a similar Th2-driven immunopathogenic response was observed in some instances (Bolles et al., 2011). The structure of the immunogen, vaccine formulation including the adjuvant, and age of vaccine recipient may influence the immune response and subsequent outcome of natural infection, although this remains unclear. Based on recent progress in RSV vaccine development, eliciting a neutralizing antibody response focused on the most protective prefusion S epitopes together with a Th1 T cell response may be key features of a safe SARS-CoV-2 vaccine.

Conclusions

The development of an effective viral vaccine is a challenge typically guided by decades of basic research on viral biology and the host response to infection. This traditional path is not feasible for a rapidly emerging, highly virulent pathogen like SARS-CoV-2 for which a dire need for a

vaccine exists. Although behavioral changes may stem the spread of infections, vaccination likely will be required to prevent the establishment of seasonal (or additional) waves of infection and disease. Against the backdrop of the many unknowns about coronavirus immunity, there is some risk associated with expedited vaccine development during a pandemic. However, this uncertainty must be balanced by the enormous cost of inaction. Studies of other pathogenic coronaviruses have enabled the rapid design of candidate SARS-CoV-2 vaccines, provided insight into aspects of immunity that may be important for protection, and identified features that merit attention with respect to vaccine safety. In this setting, carefully designed clinical trials in humans that establish and monitor safety and define correlates and durability of protection, may not only result in an effective countermeasure that curtails the pandemic but also provide key mechanistic answers into immune control of SARS-CoV-2 infection and possibly related highly pathogenic coronaviruses that could emerge in the future.

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DECLARATION OF INTERESTS

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