

## Forum

## Rational Vaccine Design in the Time of COVID-19

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As scientists consider SARS-CoV-2 vaccine design, we discuss problems that may be encountered and how to tackle them by what we term “rational vaccine design.” We further discuss approaches to pan-coronavirus vaccines. We draw on experiences from recent research on several viruses including HIV and influenza, as well as coronaviruses.

The COVID-19 pandemic is impinging upon the lives of billions of people worldwide. Meanwhile, laboratories across the globe are working intensively on developing small-molecule drugs, antibodies, and vaccines to counter the virus SARS-CoV-2. It is likely that while antibodies to the virus will be available relatively quickly, vaccines will take much longer, and the availability of small molecule drugs is more uncertain. However, most agree that the most affordable long-term solution to the problem posed by the virus is the development of a safe and effective vaccine. The development of such a vaccine could be straightforward, perhaps being solely antibody-based and requiring only the presentation of the surface S protein as a recombinant molecule, a genetic construct, or expressed from a suitable viral vector to induce a long-lived protective antibody response. It is also possible that development will encounter roadblocks that dictate greater sophistication in the design of immunogens and immunization strategies. As a single example of the kind of roadblock that can be encountered, the development of a vaccine for respiratory syncytial virus (RSV) has been held back more than 50 years, fundamentally because of a lack of understanding of the appropriate conformation of the surface F glycoprotein to be presented to the immune system, which has only recently resolved from detailed molecular data. Even if a straightforward approach is effective for a SARS-CoV-2 vaccine, ideally, we would like to develop a vaccine capable of containing multiple betacoronaviruses or at least sarbecoviruses (i.e., “pan-coronavirus” vaccines).

Such vaccines would hopefully be effective in reducing disease not only due to current known coronaviruses but also to those that may emerge or re-emerge in the future. This approach would undoubtedly require a great deal of immunogen design work, but there are some hopeful indications from antibody responses to SARS-CoV-1 and SARS-CoV-2.

### The COVID-19 Vaccine Landscape

Currently, more than 70 vaccine candidates to SARS-CoV-2 are at some stage of development. Many seek to induce neutralizing antibodies (nAbs) to the spike (S) protein on the surface of the virus, given the association of nAbs with protection for many successful viral vaccines (Figure 1). For a respiratory pathogen such as SARS-CoV-2, a vaccine might seek to induce systemic nAbs and prevent lower respiratory tract infection, as for respiratory syncytial virus (RSV) antibodies and vaccines. The prevention of upper respiratory tract infection, likely mediated by mucosal Abs, may be more difficult to sustain through vaccination. A number of factors may contribute to the development of a successful nAb-based vaccine, including 1) the ability of the vaccine to induce nAbs in most vaccinees, 2) the level of nAbs required to provide protection from disease, 3) the durability of the vaccine-induced nAb response, 4) the durability of memory B cells that might differentiate into Ab-producing cells upon virus exposure, 5) the dependence of nAb protection on the ability of vaccine-induced Abs to activate Fc-mediated effector functions, 6) complicating adverse events that may be associated with induction of weakly or non-neutralizing antibodies (antibody-

dependent enhancement [ADE] or enhanced respiratory disease [ERD]), and 7) the ability of the vaccine to induce cellular immunity that may be required, together with nAbs, to provide optimal protection.

Data on these factors is expected to accumulate rapidly as human vaccine trials progress. Meanwhile, preliminary animal protection studies provide some evidence of protection against re-infection with SARS-CoV-2 (Bao et al., 2020). For SARS-CoV-1 and MERS, animal models provide evidence of vaccine protection, including in nonhuman primates (NHPs) (Wang et al., 2015). There is data for SARS-CoV-1 showing antibody enhancement of infection in NHPs, mice, ferrets, but in almost all cases, vaccination is associated with greater survival and reduced virus titers (Roper and Rehm, 2009).

Overall, there are currently grounds for optimism that one of the strategies being investigated to generate a SARS-CoV-2 vaccine will be effective, at least in the shorter term. However, there are some potential red flags, including the apparent failure of some individuals to generate nAbs following natural infection (frequently, but not always, natural infection is more effective at the induction of nAbs than vaccination), the durability of nAb responses observed in other coronavirus infections, and some observations of immune enhancement effects in coronavirus infections.

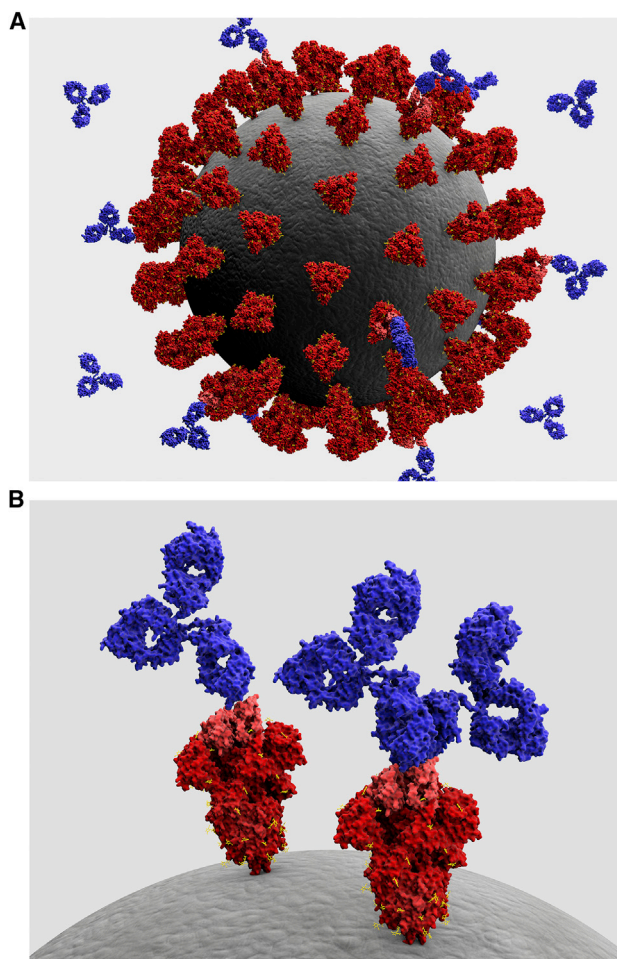
### A Rational Approach to a SARS-CoV-2 Vaccine

If current approaches to a SARS-CoV-2 vaccine are less than optimal, rational vaccine design strategies could be



employed to direct the immune response toward protective epitopes on the S protein. In principle, focusing the B cell response toward epitopes associated with potent neutralizing activity would lead to longer term vaccine protection due to the lower concentrations of antibody required for neutralization. This strategy would also minimize the induction of non- or weakly-neutralizing antibodies, which would mitigate the potential for immune enhancement. Given that the vast majority of anti-CoV nAbs have been shown to target the receptor binding domain (RBD), one way to accomplish this may be to immunize only with the RBD rather than the whole S protein. Indeed, this approach seems to show initial success in induction of nAbs in an animal model (Quinlan et al., 2020).

Another approach would begin by identifying potent nAbs from naturally infected donors and structurally defining the epitopes recognized by these antibodies. This process of designing vaccine antigens based on an exploration of the interactions between potent nAbs and their target epitopes has been termed “reverse vaccinology 2.0” (Burton, 2017). However, for many viruses, one historical hurdle to this approach has been the difficulties associated with generating potent nAbs from naturally infected donors. Studies have shown that such antibodies, particularly the most potent, can be rare in anti-viral memory B cell repertoires, and their identification has often required (1) screening of many donors to identify those that have mounted the most potent neutralizing serum responses and (2) high-throughput single B cell technologies that enable deep mining of antigen-specific memory B cell repertoires (Walker and Burton, 2018). Over the past decade, these two advances have led to the discovery of highly potent,



**Figure 1. Graphical Visualization of Antibodies Binding to Coronavirus Spike Proteins on the Virion Surface**

(A) Coronavirus particle studded with S glycoprotein molecules (red) and antibody IgG molecules (purple), bound and free. The E and M proteins are not shown in this representation.

(B) Two S glycoprotein molecules on the virus surface, one with one IgG molecule (purple) bound, one with two IgG molecules bound. Only the first two glycan residues of each glycan chain are shown.

and in some cases broadly cross-reactive, nAbs to a plethora of viral pathogens (Corti and Lanzavecchia, 2013). It is too early (mid-April 2020) to definitively state how difficult it will be to isolate potent nAbs to SARS-CoV-2, but early data suggests this may not be as difficult as it has been for some viruses.

Once panels of potent nAbs are identified, structural studies of these antibodies in complex with full-length or sub-domains of S will be required to inform the generation of immunogens that optimally present these neutralizing epitopes to the immune system. Various antigen engineering strategies have been employed to focus the antibody response on protec-

tive epitopes, including germline targeting, epitope-based protein scaffolds and structure-guided stabilization of full-length envelope proteins. The latter antigen engineering strategy, first applied to RSV F protein, was employed to generate a stabilized MERS S protein, which induced higher titers of neutralizing antibodies than wild-type S in a mouse model (Pallesen et al., 2017). Recently, these same mutations proved to be effective in stabilizing the SARS-CoV-2 S trimer, which allowed for the rapid determination of the structure by cryo-EM (Wrapp et al., 2020). Immunogenicity studies in animal models and humans will reveal how effective this molecule is in inducing nAbs to SARS-CoV-2. If nAbs are not high in the overwhelming majority of vaccines, then study of the binding of nAbs and non-neutralizing Abs (nnAbs) to the immunogen can help identify potential flaws in design. Various immunogen design strategies could then be employed to reduce the immunogenicity of these non-desired epitopes. For example, in the case of Zika virus, a stabilized E-dimer-based subunit vaccine was recently engineered to abrogate the induction of antibodies to the immunodo-

minant fusion loop and precursor membrane protein, which are antigenic sites associated with weakly neutralizing but enhancing activity (Slon-Campos et al., 2019). Immunization of mice with this stabilized Zika E antigen resulted in the induction of protective antibodies that did not cross-react with DENV or induce ADE of DENV infection. Indeed, ADE has been raised as a potential complication of a SARS-CoV-2 vaccine, although opinion remains sharply divided as to whether this is a real or hypothetical complication. Immune enhancement could be associated with Fc-receptor-enhanced uptake of viruses into FcR-bearing cells, as for flaviviruses (ADE), or

could possibly result from Abs, perhaps particularly nAbs, forming immune complexes with viral proteins and depositing in capillaries of the lung, leading to complement activation and tissue damage (ERD). In either case, more precise immunogen design than typically used in vaccine design would be indicated. Finally, glycan masking is another strategy that has shown some success in limiting antibody responses to non-desired epitopes.

Another scenario that may arise is that traditional SARS-CoV-2 vaccines induce nAb responses that wane rapidly over time. This is of particular concern given previous studies showing that a large proportion of individuals exposed to SARS and MERS failed to generate long-lived nAb responses (Choe et al., 2017; Wu et al., 2007). Furthermore, in the case of MERS, many patients with mild or asymptomatic disease did not even mount short-lived nAb responses, and emerging data suggests the same may be true for SARS-CoV-2 (Choe et al., 2017; Wu et al., 2020). It may then be that a vaccine has to improve upon natural infection. Strategies to improve on the immunogenicity of the viral glycoprotein are being explored for HIV. One example is “slow delivery” immunization, whereby the dose of antigen is escalated over days to weeks. Recent studies have shown that this method of immunogen delivery leads to prolonged retention of antigen in lymph nodes and increased germinal center B cell numbers compared to traditional “single bolus” immunization (Tam et al., 2016). Furthermore, in the context of HIV immunization, slow delivery immunization was shown to enhance nAb development in NHPs by modulating the immunodominance of non-neutralizing epitopes (Cirelli et al., 2019). In another example, it was recently shown that site-specific immobilization of HIV trimer antigens on alum prolonged immunogen bioavailability, resulting in enhanced germinal center and nAb responses compared to traditional alum-absorbed antigens (Moyer et al., 2020). Finally, of course, the development of potent adjuvants such as GSK’s AS01B can greatly enhance Ab responses to glycoproteins.

### A Rational Approach to Pan-coronavirus Vaccines

A new coronavirus will likely emerge in the future, just as the current virus has followed SARS and MERS. The same pattern is true of other pathogens. A Zika virus epidemic

followed the endemic dengue and yellow fever viruses, West Nile virus spread across the United States, and other flaviviruses may emerge. Ebola Bundibungyo emerged after Ebola Zaire and Ebola Sudan had been described. Hantaviruses such as Puumala and Seoul were well known in Eurasia, but then more recently Sin Nombre and Andes viruses emerged in the Americas. The list goes on to reflect the fact that pathogens mutate. Thus, in many ways, many currently envisaged countermeasures to COVID-19 can be viewed as temporary fixes to a long-term problem that has existed for decades.

Ideally, vaccines should provide protection not only against current versions of pathogens but also against those likely to emerge in the future. Are such “pan-pathogen” vaccines possible? The discovery of broadly neutralizing antibodies (bnAbs) first against highly antigenically variable viruses, such as HIV and influenza virus, and then against related paramyxoviruses, flaviviruses, lyssaviruses, orthopoxviruses, and filoviruses provides the proof of principle for pan-pathogen vaccines (Walker and Burton, 2018). It seems that, if one searches hard enough, one can find antibodies that recognize relatively conserved epitopes even against a background of considerable variation. We attribute this observation to the ability of antibodies to recognize every nook and cranny of a pathogen surface, albeit in any given case in a minority of individuals and as a minor part of the response. Early data describing a potent mAb that cross-neutralizes SARS-CoV-2 and SARS-CoV-1 provide support for attempts to design a pan-sarbecovirus vaccine (Pinto et al., 2020). The cross-reactive nAb binds to the receptor binding domain (RBD) of the two viruses, suggesting this domain as a target for vaccine design. The generation of pan-betacoronavirus vaccines is likely to be more difficult. For example, the S proteins of MERS-CoV and SARS-CoV-2 share only 35% identity. Nevertheless, structural studies suggest that the fusion peptide is accessible and highly conserved and may be an appropriate vaccine target (Walls et al., 2016).

Another less appreciated advantage of designing a pan-coronavirus vaccine that elicits cross-reactive antibodies is that such antibodies may be more effective against variants of a single virus compared to strain-specific antibodies.

For instance, a highly cross-reactive antibody against a functional site on both SARS-CoV-1 and SARS-CoV-2 is more likely to target a limited set of critical conserved residues that will show less propensity for mutation than a SARS-CoV-2-specific antibody to that site. This feature might be accomplished by antibodies targeting a smaller conserved footprint within the functional binding site. In any case, there is inevitably concern that a new virus infecting millions, or even billions, of people worldwide will generate variants that will require a vaccine able to induce antibodies with some degree of flexibility in their precise recognition properties.

Finally, it should be noted that the antibodies themselves, initiating the types of vaccine design efforts described above and dubbed “super-antibodies” for their outstanding attributes of cross-reactivity and/or potency, represent promising agents for prophylaxis and therapy of viral infections (Walker and Burton, 2018). High potency means that less antibody is required for efficacy, reducing the frequently quoted high cost of antibodies as drugs. Other factors that can reduce costs include antibody half-life extension and advances in antibody delivery and production. The path to FDA approval is also typically much faster for antibodies than for vaccines and small-molecule drugs. Overall, the combination of these features may enable antibodies to be one of the front-line treatments in responding to future outbreaks of infectious disease.

### Conclusions

An unprecedented effort is ongoing to develop a SARS-CoV-2 vaccine. It is strongly hoped that this effort will be immediately and fully successful. However, some caution is prudent, and the gains that have been made in the last decade in understanding the interplay between the humoral immune system and viruses and in rational vaccine design should be fully exploited without waiting to see the results of the first vaccine efforts. Such endeavors will provide a “Plan B” in the event of problems or complications and, in any case, will likely contribute to an optimal vaccine. Further, the promise of developing pan-coronavirus vaccines to cope with future emerging pathogens should be explored.

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