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SARS-CoV-2: A New Song Recalls an Old Melody

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The viruses causing the SARS outbreak of 2002–2003 and current COVID-19 pandemic are related betacoronaviruses. What insights were learned from SARS that can inform SARS-CoV-2 vaccine development? Focusing on important lessons from SARS vaccine development and two SARS vaccines evaluated in humans may guide SARS-CoV-2 vaccine design, testing, and implementation.

Neutralizing Antibody Protects from Infection

When mice were infected intranasally with SARS-CoV, virus replication was detected in their upper and lower respiratory tract (Subbarao et al., 2004). The mice cleared the virus in 7 days and developed neutralizing antibody in the serum. The mice that recovered from primary infection were protected from reinfection, and, importantly, passive transfer of serum from the mice that recovered from primary infection to naive mice conferred protection from challenge (Subbarao et al., 2004). With this set of experiments, we established the very important principle, that neutralizing antibody alone was sufficient to protect mice from SARS-CoV infection.

This finding was confirmed in a series of independent studies in which human monoclonal antibodies isolated from immortalized B cells from a recovered patient, phage display library, or transgenic mice with active human immunoglobulin genes, administered to mice or hamsters protected them from subsequent challenge infection (representative reference, Traggiai et al., 2004).

The Spike Protein Is the Only Viral Protein that Induces Neutralizing Antibody

In an elegant experiment, Buchholz et al. demonstrated that the spike glycoprotein of SARS-CoV is the sole structural protein of SARS-CoV that is necessary and sufficient to induce a neutralizing antibody response and protection from challenge (Buchholz et al., 2004). They engineered each structural protein of SARS-CoV-2 alone and in combination, into a bovine-human parainfluenza virus type 3 (BHPIV3) vaccine vector, immunized golden Syrian hamsters, and challenged them with SARS-CoV. The hamsters that were immunized with the spike protein alone or combinations of proteins that included the spike protein developed a neutralizing antibody response and were protected from infection on subsequent challenge (Buchholz et al., 2004). Subsequent work narrowed down the region of the spike protein that was critical for neutralizing activity to the receptor binding domain.

Vaccines that Induce Neutralizing Antibodies Protect Animals from Challenge

We tested several candidate SARS-CoV vaccines in mice (representative reference, Yang et al., 2004), hamsters (representative reference, Roberts et al., 2010), and non-human primates (reviewed in Roberts et al., 2008). The vaccines included whole inactivated virus vaccines (Roberts et al., 2010), purified expressed spike protein, DNA vaccine encoding the spike protein (Yang et al., 2004), and several vectored vaccines (MVA, VSV, BHPIV3) expressing the spike protein (Buchholz et al., 2004); all of the vaccines elicited neutralizing antibodies and protected the immunized hosts from infection on subsequent challenge with wild-type virus. We demonstrated that the antibody response and protective efficacy lasted 6 or more months with some vaccines (Roberts et al., 2008, 2010).

These findings are relevant to SARS-CoV-2 because the spike proteins of the SARS-CoV and SARS-CoV-2 are related (73%), and they share binding specificity to the human receptor, ACE2 (Zhou et al., 2020). Patients who have recovered from SARS-CoV-2 infection develop neutralizing antibodies (Wölfel et al., 2020). Golden Syrian hamsters that were experimentally infected with SARS-CoV-2 developed neutralizing antibodies and were protected from reinfection. Furthermore, as was the case with SARS-CoV, passive transfer of serum from previously infected hamsters to naive hamsters conferred protection from subsequent challenge (Chan et al., 2020).

Summary of the Two SARS Vaccines that Were Evaluated in Phase 1 Clinical Trials

The first SARS-CoV vaccine was an inactivated whole virus vaccine, developed by Sinovac Biotech, that was tested in 36 SARS-CoV seronegative healthy adults, aged 21–40 years, in a randomized, double-blinded, placebo-controlled trial in China (Lin et al., 2007). The vaccine was derived from a clinical strain of SARS-CoV, propagated in Vero cells, inactivated with beta propiolactone and subsequently purified. The antigen content of the vaccine was reported in SARS-CoV units (SU), measured in a passive indirect hemagglutination assay rather than by quantitation of protein. The inactivated vaccine was adsorbed to aluminum hydroxide and the control group received saline injections with aluminum hydroxide. The safety of the vaccine had been evaluated in animals, and the dose range and need for adjuvant was determined from preclinical evaluation in mice, rats, and rhesus monkeys. The study subjects were divided into three groups of 12 (6 males and 6 females) that received 16 SU or 32 SU of vaccine or placebo, given as two doses 28 days apart administered by intramuscular injection. The vaccine

was well tolerated, with only mild local reactogenicity and transient transaminase elevation in 3 vaccinees and 1 placebo recipient. The immunogenicity endpoint of seroconversion in 85% of the vaccines on day 56 was achieved in both of the vaccine groups; 100% of the 16 SU group, 91.1% of the 32 SU group, and none of the placebo group seroconverted on day 56. The neutralizing antibody titers were reported in units with respect to a reference serum. The frequency of seroconversion and antibody titers improved with the second dose of vaccine; titers peaked 2 weeks after the second dose of vaccine and decreased 4 weeks later. Titers achieved in the vaccinees were about half of those observed in convalescent patients. The authors concluded that the vaccine was safe and immunogenic and warranted further evaluation to optimize the dose and schedule and to assess its efficacy (Lin et al., 2007).

The other vaccine that was evaluated in humans was a SARS recombinant plasmid DNA vaccine encoding the SARS spike glycoprotein (Martin et al., 2008) designed, produced, and evaluated at the Vaccine Research Center, National Institutes of Health. An open-label study of the safety, tolerability, and immunogenicity was conducted in 10 subjects, aged 21–49 years, who received three doses 28 days apart. The vaccine was administered by injection into the deltoid muscle using a Biojector 2000 Needle-Free Injection Management System. The vaccine was previously shown to be highly effective in preclinical studies (Yang et al., 2004). Mild injection site and systemic symptoms were reported, but no moderate or severe adverse events were reported. SARS-spike-protein-specific ELISA antibody responses were detected in 8 of the 10 subjects, and neutralizing antibody responses were detected with a pseudotyped lentiviral vector reporter neutralization assay in 8 of the 10 subjects, at one or more time points. Titers peaked between weeks 8 and 12, with detectable antibody in 6 subjects at week 32. However, neutralizing antibodies were not detected in an infectious virus assay. $CD4^+$ T cell responses to the spike protein were detected in all 10 subjects and $CD8^+$ T cell responses were detected in 2 of 10 subjects; these responses followed the same kinetics as the pseudovirus neutralization assay.

Neither of these vaccines nor any of the numerous SARS vaccines that showed promise in preclinical studies were tested further because SARS did not re-emerge. However, the experience is relevant to SARS-CoV-2 vaccines. Vaccine development efforts for SARS progressed rapidly but the pace for COVID-19 is much faster. The first clinical trials of SARS-CoV-2 vaccines began within 4 months of the first report of the new virus. Notably, phase 1 clinical trials have been undertaken without prior demonstration of vaccine efficacy in a preclinical model. The absence of an animal model for COVID-19 disease juxtaposed against the urgency of the rapid spread of the pandemic has altered the conventional sequence of vaccine evaluation.

What Should SARS-CoV-2 Vaccine Developers Look for in Animal Models?

First, the immunogenicity of SARS-CoV-2 vaccines can and should be demonstrated in laboratory animals such as mice, hamsters, ferrets, or non-human primates. Second, protective efficacy should be assessed in animal models that support replication of SARS-CoV-2, ideally with associated clinical signs of disease. Early reports suggest that human ACE-2 expressing mice, hamsters (Chan et al., 2020; Kim et al., 2020), ferrets (Kim et al., 2020), and non-human primates (Rockx et al., 2020) can be infected with SARS-CoV-2, but clinical signs of disease are absent or mild. It is clear that hamsters develop more significant lower respiratory tract involvement with pneumonitis than ferrets (Chan et al., 2020; Kim et al., 2020). Reliance on radiographic changes in infected animals as the main measure of vaccine efficacy is not ideal.

We used a creative approach to demonstrate that hamsters were affected by SARS-CoV infection; a Nalgene activity wheel (Nalge Nunc International, Rochester, NY) equipped with a magnetic switch and an LCD counter that records revolutions was placed in the hamster cages overnight. The average number of revolutions/h that the hamsters ran on the wheel was recorded. Baseline activity level for hamsters was between 700 and 1,000 revolutions/h and decreased by 10-fold or more, to 61 ± 23 revolutions/h following infection with SARS-CoV, returning to normal at 9 to 10 days post-infection (Roberts et al., 2008). This activity measure was applied in assessing the efficacy of a live-attenuated SARS vaccine. Serial passage of the virus in animals can lead to a disease model, as we did with SARS-CoV (Roberts et al., 2007) and others have previously done for influenza and Ebolavirus. Older mice infected with SARS-CoV displayed clinical disease while young adult mice did not (Roberts et al., 2005; Subbarao et al., 2004). Similar avenues for animal model development are being explored for SARS-CoV-2. In the absence of a disease model, the ability of a candidate vaccine to elicit a neutralizing antibody response against infectious virus associated with restricted replication of challenge virus in the respiratory tract should be demonstrated. Concerns about the safety of coronavirus vaccines were raised with SARS and are now being discussed with SARS-CoV-2. Efforts are underway to design studies in animal models and to develop clinical case definitions to identify vaccine-associated adverse events if they occur.

What Studies Can Be Done Now, in the Midst of the Pandemic, that Will Be Valuable for Vaccine Development?

First, the neutralizing antibody response in recovered patients should be characterized, including the average titers after asymptomatic, mild, and severe disease as well as titers in different age groups, kinetics, and longevity of neutralizing antibody. The presence of cross-reactive antibodies against human coronaviruses may differ in different age groups. This information will help us place vaccine studies in context and in comparison to the inactivated SARS vaccine from China, which was immunogenic but achieved post-vaccination titers that were half those seen in convalescent patients (Lin et al., 2007). Second, we can undertake studies in animal models to define a protective neutralizing antibody titer. Human sera from recovered patients with a range of neutralizing antibody titers can be pre-administered to a SARS-CoV-2 animal model, followed by challenge infection. The ability of human serum of a specific titer to prevent productive infection in the animal can identify a target titer for vaccines.

The speed with which SARS-CoV-2 has spread around the world and its toll in numbers of cases, severe illness, and death has been staggering. However, technological advances have made rapid vaccine development possible. We have to ask ourselves what new vaccines should aim to achieve—prevent all infection or prevent severe disease and death? In which age group(s)? What effect will vaccines that address these choices have on subsequent epidemics?

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