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Snatching the Crown from SARS-CoV-2

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In this issue of Cell Host & Microbe, three papers describe the pseudotyping of vesicular stomatitis virus (VSV) with the SARS-CoV-2 spike. This VSV-CoV-2-S platform allows virus neutralization assays to be performed at BSL-2 and also has applications as a candidate vectored vaccine to elicit protective immunity against SARS-CoV-2.

Despite knowledge of the threat posed by cross-species transmission events with coronaviruses (CoVs) circulating in bats (Menachery et al., 2015), and previous outbreaks of SARS-CoV (Zhong et al., 2003) and MERS-CoV (de Groot et al., 2013; Zaki et al., 2012), the world was vastly underprepared for the emergence of SARS-CoV-2 (Zhou et al., 2020). This virus has gone on to cause a global pandemic resulting in tragic loss of life and has had a devastating economic, political, and social impact. At the outset, the research community lacked specific tools, diagnostics, therapeutics, vaccines, animal models, or sufficient knowledge about this new virus to mitigate its impact. As a result, public health management was limited to physical interventions such as social distancing, mandatory mask wearing, and hand hygiene practices. However, the ensuing response from the research community has been unprecedented, and we now have several vaccines undergoing clinical testing (i.e. NCT04283461, NCT04324606, NCT04313127, NCT04368728, and NCT04368988). This was facilitated by valuable prior work on SARS-CoV and MERS-CoV by established coronavirus investigators, who had at times struggled to secure sustained research investment for these important zoonotic viruses. In contrast, SARS-COV-2 and COVID-19 research is now an international priority.

It is clear that an effective vaccine will be central in bringing an end to this pandemic. A focus of this effort is to develop a vaccine which can elicit protective antibodies (Abs) that neutralize the virus and prevent viral entry into host cells. The major target for neutralizing antibodies (NAbs) against SARS-CoV-2 is the spike (S) glycoprotein (Figure 1A). The S protein is responsible for mediating entry, through an interaction between its receptor-binding domain (RBD) and the human angiotensin-converting enzyme-2 (hACE2) receptor, and subsequent membrane fusion after proteolytic cleavage of S by host serine protease TMRPSS2, or cysteine cathepsins (Hoffmann et al., 2020). Although NAbs can have distinct mechanisms of action, their function is defined by the ability to reduce infectivity by blocking steps in the virus life cycle. Neutralization assays involve incubation of virus with immune sera (or Abs) and measuring a reduction in infectivity after a defined time period. The readout can include titration of output virus, immunostaining for viral antigen, or the use of reporter viruses expressing luminescent or fluorescent transgenes, such as enhanced green fluorescent protein (eGFP). However, an impediment to the widespread application of high-throughput neutralization assays for SARS-CoV-2 is the requirement to conduct these in Biosafety level-3 (BSL-3) containment. This requires extensive training and specialized facilities with restricted access, making it more challenging to implement these assays at many research institutes, hospitals, or vaccine-manufacturing sites. Therefore, the development and distribution of surrogate assays that can authentically measure NAbs to SARS-CoV-2 at BSL-2, bypassing the need to work with live SARS-CoV-2 virus at BSL-3, would make this technique more accessible to the research community and accelerate the identification of therapeutics and vaccines.

In a series of manuscripts from Case and Dieterle published in this issue of Cell Host & Microbe (Case et al., 2020a; Case et al., 2020b; Dieterle et al., 2020), the authors describe the engineering of a replication-competent vesicular stomatitis virus (VSV) eGFP reporter virus, in which the surface glycoprotein (G) has been replaced with the SARS-CoV-2 S glycoprotein (Figures 1B and 1C). VSV is an enveloped, bullet-shaped RNA virus that primarily infects animals. Infections in humans are rare and largely asymptomatic, and as a result VSV has low seroprevalence. VSV has been used extensively as a laboratory tool and is amenable to pseudotyping with glycoproteins from highly pathogenic viruses, facilitating mechanistic studies of viral entry and inhibition. The engineering of replication-competent, multi-cycle VSV-CoV-2-S is an advance on pseudotyping approaches that generate single-cycle viruses. Single-cycle VSVs require in trans provision of the heterologous glycoprotein through a multi-plasmid co-transfection protocol. This could be subject to batch-to-batch variation, and pseudotype production can be hampered by low yield or contamination with unmodified VSV. Furthermore, an interesting consideration is that the single-cycle process is not conducive to forward-genetics studies of the SARS-CoV-2 S glycoprotein (Case et al., 2020b). In contrast, the multi-cycle, replication-competent VSV-CoV-2-S has a higher mutability rate than SARS-CoV-2, potentially allowing it to be exploited to investigate the emergence of escape mutants to monoclonal antibodies (mAbs) or inhibitors (Case et al., 2020b).

A technical challenge in generating replication-competent VSV particles...
Figure 1. Development of a Replication-Competent VSV Vector Pseudotyped with SARS-CoV-2 S Glycoprotein as a Serological Tool and Vaccine Candidate

(A) Schematic diagram of the SARS-CoV-2 coronavirus, highlighting the S surface glycoprotein, which is a major target for vaccine development. Structure shown for S is PDB ID: 6XKL (PMID: 32577660).

(B) Schematic diagram of the bullet-shaped VSV, with major viral proteins highlighted.

(C) Schematic diagram showing the structure of VSV when successfully pseudotyped with the SARS-CoV-2 S glycoprotein (VSV-SARS-CoV-2-S<sub>21</sub>).</p>

(D) In manuscripts by Case and Dieterle (Case et al., 2020b; Dieterle et al., 2020), the authors demonstrate that VSV-SARS-CoV-2-S<sub>21</sub> expressing enhanced green fluorescent protein (eGFP) as a reporter transgene, bearing SARS-CoV-2 S on the surface, can be used as a serological tool to measure NAbs to SARS-CoV-2 in a manner comparable to neutralization assays performed at BSL-3 with SARS-CoV-2 virus.

(E) In a separate manuscript, Case and colleagues show that VSV-SARS-CoV-2-S<sub>21</sub> can also be used as an immunogenic vaccine against SARS-CoV-2, eliciting antibody responses against S and its RBD, as well as NAbs (Case et al., 2020a).

(F) By using a previously established model to sensitize mice to challenge with SARS-CoV-2 by pre-administering a non-replicating adenoviral vector expressing human ACE2 (Hassan et al., 2020), the authors showed that immunization with VSV-SARS-CoV-2-S<sub>21</sub>, or passive transfer of sera from VSV-SARS-CoV-2-S<sub>21</sub>-immunized mice, could protect mice from SARS-CoV-2 challenge. Figures were created with BioRender.
displaying SARS-CoV-2 S lies in differences between their viral assembly pathways. For SARS-CoV-2, structural proteins including S are studded into the membrane of the endoplasmic reticulum (ER), with mature virion assembly taking place in the ER-Golgi intermediate compartment. In contrast, VSV is enveloped by budding from the plasma membrane. Therefore, the largely incompatible localization of SARS-CoV-2 S when expressed in trans would make it difficult to generate VSV particles decorated with S. To address this issue, and redirect SARS-CoV-2 S to the plasma membrane, Case and colleagues pre-emptively altered the sequence of an ER retention signal in the cytoplasmic tail of S (Case et al., 2020b). Although they successfully rescued this modified virus (VSV-SARS-CoV-2-Δ21) after plasmid co-transfection, propagation of autonomously replicating VSV-SARS-CoV-2-SΔ21 was not efficient. To overcome this, both Case and Dieterle subsequently took the approach of forward-genetics based selection, using iterative rounds of passage-acquired mutations and RNA sequencing to identify replication-competent VSV-SARS-CoV-2-S mutants that spread efficiently and produced high titers (Case et al., 2020b; Dieterle et al., 2020). Interestingly, despite some differences in the mutations identified, both approaches converged on a 21 amino acid truncation (Δ21) of the cytoplasmic tail of S. The implication is that this deletion re-localizes SARS-CoV-2 S to the plasma membrane of VSV-SARS-CoV-2-SΔ21-infected cells, facilitating multi-round budding of S-coated VSV.

Both sets of authors comprehensively validated the structural and functional integrity of bullet-shaped VSV-CoV-2-SΔ21 particles by using a range of biochemical and immunological assays. This included confirming the presence of SARS-CoV-2 S on VSV, as well as inhibiting the entry of VSV-SARS-CoV-2-SΔ21 into permissive cells by using soluble RBD or hACE2, anti-hACE2 mAbs, or convalescent sera from COVID-19 patients. The broad applicability of this tool in screening for inhibitors of receptor binding and subsequent steps in S-mediated entry was also demonstrated by the ability to block VSV-SARS-CoV-2-SΔ21 entry (but not wildtype VSV) by using inhibitors of endosomal acidification, cysteine cathepsins, or the TMPRSS2 serine protease, in a manner that authentically recapitulated the inhibition of clinical isolates of SARS-CoV-2. By using a panel of convalescent human sera, the authors demonstrated that surrogate neutralization assays using VSV-SARS-CoV-2-SΔ21 correlated well with SARS-CoV-2 neutralization assays performed at BSL-3 (Figure 1D). Collectively, these data confirm the presentation of SARS-CoV-2 S on the surface of VSV in an antigenically and functionally authentic form that mimics native SARS-CoV-2 S. Therefore, this tool could be used as a standardized assay that is amenable to high-throughput applications: to quantify NAbs elicited by candidate SARS-CoV-2 vaccines (pre-clinical and clinical) and to better understand the durability of immune responses after natural infection. Such assays will be invaluable in enabling the identification of correlates of protection, i.e., a defined titer (or phenotype) of NAb determined by a standardized assay that can indicate the likelihood for protection against infection.

The final manuscript by Case and colleagues (Case et al., 2020a) further expanded on the pseudotyped VSV-SARS-CoV-2-SΔ21 platform by evaluating its potential as a standalone vaccine against SARS-CoV-2 (Figure 1E). Immunization of mice with VSV-SARS-CoV-2-SΔ21 elicited high-titer Abs directed toward S and its RBD after a single shot, with responses increased when used in a homologous prime:boost regimen. Importantly, the authors showed that prime:boost immunization elicited high titers of NAbs. Unfortunately, differences between the hACE2 receptor and its murine ortholog result in mice being non-permissive to infection with clinical isolates of SARS-CoV-2. This could also potentially impact on the true immunogenicity of VSV-SARS-CoV-2-SΔ21 by restricting its capacity for cell-to-cell spread in the absence of hACE2 expression. In support of this, an evaluation of immunogenicity in K18-hACE2 transgenic mice resulted in improved immune responses after a single shot of VSV-SARS-CoV-2-SΔ21. To test efficacy, the authors capitalized on a recently described transient model of SARS-CoV-2 sensitization, whereby mice are administered intranasally with a non-replicating adenoviral vector expressing hACE2 five days prior to challenge with SARS-CoV-2 (Hassan et al., 2020). This model was used to determine that VSV-SARS-CoV-2-SΔ21 could protect from pneumonia and reduce SARS-CoV-2 replication in the lung and limit the induction of pro-inflammatory, anti-viral immune responses. Finally, passive transfer of immune sera also reduced viral replication and virus-induced lung inflammation, suggesting that correlates of protection for VSV-SARS-CoV-2-SΔ21 were predominantly Ab mediated (Figure 1F).

Efforts to develop new vaccines to protect against SARS-CoV-2 are urgently needed. Although data generated with the VSV-SARS-CoV-2-SΔ21 vaccine are promising, the authors outline that these findings are early stage and that this platform will require further development, including attenuation and safety studies in relevant animal models, before it could advance to human trials. Nonetheless, a precedent for use of VSV vectors in humans has already been established following FDA approval of Ervebo, a VSV-based vaccine to protect against Ebola virus. Although a number of vaccines are already in clinical trials, these have not yet demonstrated efficacy in humans, and we currently lack consensus on exactly which immunological parameters will be required for long-lasting protection against SARS-CoV-2. NAbs are the ultimate target, but other aspects of the immune response to SARS-CoV-2, such as innate or cellular immunity, could contribute to efficacy by limiting disease severity, viral shedding, or transmission. Therefore, we should continue to evaluate and advance a range of platforms with the potential for use in heterologous prime:boost or co-administration regimens to improve the breadth, or durability of protective immunity.

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