Coronavirus

Single-domain antibodies make a difference

A double hit with one antibody construct may avoid viral escape

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The spike protein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19, mediates attachment of the virion to the host cell, binding to the receptor, and fusion of the viral and host cell membranes. This releases the viral genomic RNA into the host cell cytoplasm, which is the start of virus replication. Antibodies that interfere with SARS-CoV-2 spike function—in particular, antibodies that prevent the interaction between the receptor binding domain (RBD) of spike and human angiotensin-converting enzyme 2 (ACE2), the canonical receptor on the host cell surface—can neutralize the virus in vitro and are associated with protection from infection in vivo (1). On page 691 of this issue, Koenig et al. (2) describe four SARS-CoV-2-neutralizing single-domain antibodies, or VHHs, and combinations thereof that can disable spike function. This extends the growing list of reports on SARS-CoV-2 spike-specific single-domain antibodies that have been proposed as potential therapeutics for COVID-19 patients.

VHHs are the variable domains of heavy chain–only antibodies, which lack a light chain and the first constant domain of the heavy chain that occur in conventional antibodies. VHHs are present in camelid species, such as dromedaries (Arabian camels) (3). Koenig et al. immunized a llama and an alpaca with recombinant spike RBD antigen and inactivated SARS-CoV-2 virions to select high-affinity VHHs directed against SARS-CoV-2 spike. Structural analysis of the VHHs in complex with RBD or prefusion-stabilized spike revealed their epitopes, explained their capacity to hinder ACE2 binding, and showed that two of the VHHs captured spike in its so-called up-conformation in which the RBD is flipped upward, which is essential for the virus to engage with ACE2. The closed state is the predominant form of spike on the virion (4). ACE2 binding combined with proteolytic activation of spike triggers membrane fusion. The authors propose that some of their VHHs— including biparatropic ones that bind two nonoverlapping epitopes on spike—can destabilize the prefusion conformation of the spike protein and thereby inactivate the virus fusion machinery.

Several other studies have generated single-domain antibodies against spike: Some also started off with an immunized llama or alpaca (5–8), whereas others obtained neutralizing single-domain antibodies from synthetic libraries (9–12). VHH72 cross-neutralizes SARS-CoV, a betacoronavirus that caused the SARS outbreak in 2002–2003, and SARS-CoV-2 through binding of a highly conserved epitope in the spike RBDs of these viruses (8). VHH72 was fused with the Fc domain of human immunoglobulin G1 (IgG1), which imparts an IgG-like long circulatory half-life, enhances potency by making the antibody bivalent, and allows tuning of effector function, for example, to reduce or promote the binding to Fc receptors expressed by different immune cells. A further potency-enhanced engineered derivative of VHH72 is now entering clinical trials to treat COVID-19 patients.

Another way of increasing the valency of VHHs, which increases the binding strength to their target, is by putting them in tandem repeat fusion proteins. This is exemplified by a homotrimERIC VHH construct (5) that, with a median inhibitory concentration (IC50) of 5.4 μM, is one of the most potent SARS-CoV-2–neutralizing VHH constructs reported to date. Another potently neutralizing VHH construct was derived from a yeast-displayed synthetic VHH library, which was screened for pre-
This can be overcome when the VHHs are covalently linked in a bispecific molecule. RBD-ACE2 binding can be prevented by single-domain antibodies, or VHHs. Combined treatment with low antibody titers and high viral loads at the onset of treatment (13, 14). How could VHH-based biologics contribute to the control of COVID-19? The region of the spike RBD that interacts with ACE2 is a hotspot for neutralizing human antibodies; hence, it is expected that SARS-CoV-2 variants may arise with mutations in this immunogenic ACE2-binding RBD region. Indeed, recently there has been rapid spread of SARS-CoV-2 variants, such as the B.1.1.7 variant that emerged in the United Kingdom and the 501YV2 variant that was found in South Africa, that are capable of reducing neutralization potency of some monoclonal antibodies that bind this region and, worryingly, even of human convalescent plasma (15).

Linked single-domain antibodies prevent virus escape

The spike protein of SARS-CoV-2 contains the RBD that binds to ACE2 on host cells, leading to cell entry. RBD-ACE2 binding can be prevented by single-domain antibodies, or VHHs. Combined treatment with VHH1 and VHH2, which bind nonoverlapping regions of the RBD, prevents infection until escape mutants arise. This can be overcome when the VHHs are covalently linked in a bispecific molecule.

The emergence of variants urges the need to develop neutralizing antibodies that bind to epitopes that are under reduced selection pressure by the human antibody response. For this, single-domain antibodies are particularly suited. With their small size, some single-domain antibodies can reach sites in spike that are more difficult to access by conventional antibodies. An additional way of reducing the chance of viral escape from immunity is by merging different VHHs into bipartopic constructs. Using a recombinant vesicular stomatitis virus engineered to use spike for cell entry, but that is more error-prone than SARS-CoV-2 and thus evolves faster in vitro, Koenig et al. showed that tandem repeats of two neutralizing VHHs that bind nonoverlapping epitopes strongly reduced the chance that mutant viruses that escape neutralization were selected in vitro (see the figure).

The recent rollout of COVID-19 vaccines in many countries has brought hope that the pandemic will be gradually curbed. For now, however, the spread of SARS-CoV-2 is not yet slowing down: As of now, more than 10,000 COVID-19 patients die every day globally, with many more requiring hospital admission. Thus, there is a pressing need for antiviral drugs, such as neutralizing single-domain antibodies, that can prevent or reduce SARS-CoV-2 replication in exposed patients with progressive disease. This need will likely persist even after the widespread use of COVID-19 vaccines to treat those for whom the vaccine provided no protection, was not used, or was not available, or, in a most alarming scenario, to provide treatment if vaccine-escape mutants arise. Perhaps, in the future, a positive rapid SARS-CoV-2 test outcome will go hand in hand with an easily administered, affordable, subcutaneous injection or nebulized inhalation of an antibody targeting highly conserved epitopes not recognized by the human immune system.

REFERENCES AND NOTES
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ACKNOWLEDGMENTS
We thank N. Callewaert for helpful discussions. The authors are named as inventors on patent applications related to the use of single-domain antibodies for the prevention and treatment of viral infectious diseases, including disease caused by SARS-CoV-2.
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Science 371 (6530), 681-682.
DOI: 10.1126/science.abg2294