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OPINION PIECE

Nanobodies: an unexplored opportunity to combat COVID-19

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ABSTRACT

Coronavirus disease 2019 (COVID-19) is a highly contagious disease caused by severe acute respiratory coronavirus 2 (SARS-CoV-2). This virus is capable of human-to-human transmission, and is spreading rapidly round the globe, with markedly high fatality rates. Unfortunately, there are neither vaccines nor specific therapies available to combat it, and the developments of such approaches depend on pursuing multiple avenues in biomedical science. Accordingly, in this paper we highlight one such avenue—nanobodies—for potential utility in therapeutic and diagnostic interventions to combat COVID-19.

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Coronavirus disease 2019 (COVID-19) is an emerging disease caused by acute respiratory syndrome coronavirus type 2 (SARS-CoV-2), and is posing serious threats to global public health, and economic and social stability. Since it emerged in December 2019 in Wuhan in China's Hubei Province, SARS-CoV-2 has rapidly spread from its epicenter to create a global pandemic (De Vlieger et al., 2018; Detalle et al., 2016). Patients infected with SARS-CoV-2 generally develop mild symptoms; however, in a minority of cases, the disease leads to severe pneumonia and even death (Dörner et al., 2017). Currently, no effective vaccines or specific drugs exist that can be used to prevent, mitigate, or treat COVID-19 (Ezzikouri et al., 2020; Hanke et al., 2020; Huang et al., 2020). The pandemic is forcing authorities round the world to adopt interventions such as social distancing, self-isolation, quarantine, and lockdown, on a historically unprecedented scale. Such measures are causing significant economic losses, and underline the crucial need for the prompt development of anti-COVID-19 therapeutics and prophylactics. The scientific community is under pressure to devise effective therapeutic and preventive approaches to COVID-19 at breakneck speed. This pressure—coupled with finite resources available for any one approach—means that researchers must be open to exploring a number of different avenues in biomedical science, in the quest for strategies against this disease. In this paper, we highlight one such avenue: nanobodies (Nbs), which have potential utility for both therapeutic and diagnostic interventions to combat COVID-19 (Figure 1). Nbs, also called VHHs, are derived from the heavy-chain variable domains of camelids (llamas, alpacas, dromedaries, and camels) and sharks. They are single-domain antibodies devoid of a light chain component (Huo et al., 2020). The Nbs domain comprises full antigen-binding potential, strong affinity to its cognate antigen, and is considered to possess the smallest naturally occurring (~15 kDa), intact antigen-binding domain. Nbs hold promise as proteins for therapeutic use against infectious diseases due to a number of their characteristics: they are small and have a low molecular weight, they are highly stable and soluble, they can be produced cost-effectively, and they readily penetrate tissue (Ibañez et al., 2011). In a range of studies, Nbs have been proposed as antiviral agents against respiratory diseases such as Middle East respiratory syndrome coronavirus (MERS-CoV) (Jailkhani et al., 2019; Jovcevska & Muyldermans, 2020), respiratory syncytial virus (RSV) (Kupferschmidt & Cohen, 2020), and influenza A virus subtype H5N1 (Mitch, 2018).

The therapeutic potential of Nbs against coronaviruses has been demonstrated in an exciting recent study (Respaud et al., 2015). A pair of potent VHHs were isolated from a llama immunized with prefusion-stabilized coronavirus spike receptor-binding domain (RBD), and successfully used to neutralize MERS-CoV and SARS-CoV-1 *in vitro* (Respaud et al., 2015). One particularly interesting finding in that study was the high-affinity binding of S-directed VHH to the spike RBD, which enabled the neutralization of SARS-CoV-2 (Respaud et al., 2015). Moreover, three VHHs, H11-D4, H11-H4 and Ty1, target SARS-CoV-2 spike receptor binding domain and block interaction with ACE2 have been discovered (Rissiek et al., 2014; Sanaei et al., 2019). This finding suggests a potential role for Nbs as therapeutic agents to be deployed in the

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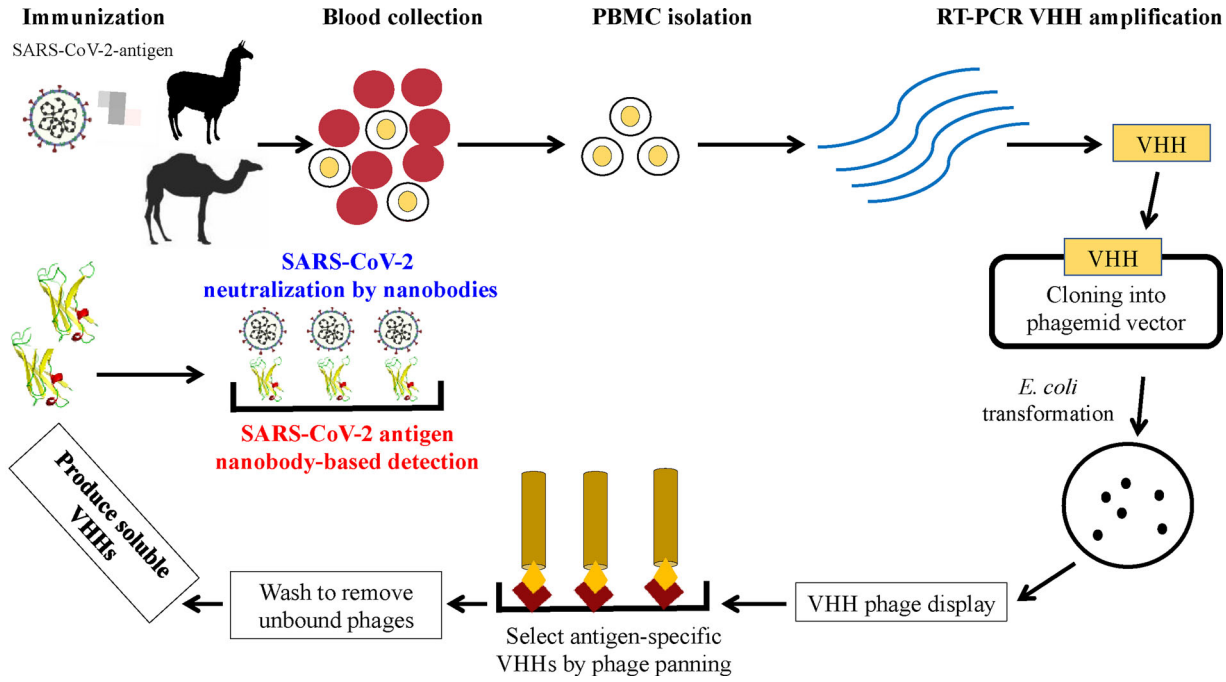

Figure 1. Schematic representation of the heavy-chain variable domains (VHHs) generation process and their potential applications as therapeutic agents and as diagnostic tools for COVID-19 pathology. Blood is collected from SARS-CoV-2 antigen-immunized camelids to isolate PBMCs. RNA is extracted from PBMCs followed by RT-PCR to amplify VHH. The VHH DNA sequence is ligated into a phagemid vector and transformed into *E. coli*. VHH phage display is carried out to isolate SARS-CoV-2 antigen-specific clones. After rounds of panning on the antigen of interest, SARS-CoV-2 antigen-specific VHH coding sequence is selected. The identified VHH coding gene is inserted into a yeast expression vector to produce a soluble VHH.

COVID-19 pandemic (Respaud et al., 2015; Rissiek et al., 2014; Sheridan, 2020).

Nbs have suppressed inflammation in several models, suggesting that they may target inflammatory responses, and could thus mitigate the cytokine storms suggested to be experienced by COVID-19 patients after the development of characteristic lung damage. A further advantage for Nbs concerns their mode of administration; they can be nebulized and, using an inhaler mechanism, delivered directly to the lung, where they may be able to exert anti-inflammatory and anti-viral effects in the context of COVID-19 pathology (Ibañez et al., 2011; Stalin Raj et al., 2018; Thanh Le et al., 2020).

Nbs have also advanced through the drug development process. Recently, the bivalent Nb caplacizumab has received approval as an indication for thrombotic thrombocytopenic purpura in two regions covered by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (Europe and the United States) (WHO. Coronavirus disease \(COVID-19, XXXX). Moreover, the anti-IL-6R Nb vobarilizumab, which also has an anti-human serum albumin domain, has been evaluated for a marketing indication as a rheumatoid arthritis treatment, reaching a clinical phase 2 b study in which it showed safety and "a positive impact on disease activity" (Wrapp et al., 2020). The potential indications of Nbs clearly require further investigation; there are concerns that their fast renal clearance could lead to renal toxicity and failure to achieve an effective concentration in the target tissue. On the other hand, should such issues be overcome, Nbs have a clear development advantage. They can be easily engineered and genetically fused to the fragment crystallizable region (Fc) (CH2-CH3) of different human antibody subclasses to target different viruses (Zhao et al., 2020).

Potential applications of Nbs are not limited to the therapeutic field; they may have utility in diagnostic interventions as well. Challenges are presented in the field of SARS-CoV-2 diagnostics, where several serological kits that detect the relevant immunoglobulin (Ig)M and IgG have been approved but are not being used for general screening due to the lack of the required specificity and sensitivity (Zhao et al., 2018). Nbs represent a potential solution to this problem, as the basis for the rapid antigen tests with high specificity and sensitivity for detecting SARS-CoV-2 infection that are so greatly in demand during the current COVID-19 outbreak (Zhu et al., 2020). Nbs-based ELISAs are already used to detect a range of specific antigens and biomarkers (Ibañez et al., 2011; Sheridan, 2020; Zhao et al., 2020). Nb technology can theoretically be levered to antigen detection in the diagnosis of SARS-CoV-2; however, further investigations are needed.

In conclusion, Nbs are small, stable, and simple to produce, and hold promise as therapeutic proteins to be used in novel interventions for COVID-19. As other coronaviruses are likely to emerge in the future, Nbs indications could be extended to cover such newly emergent coronaviruses as well. Furthermore, the potential utility of Nbs in diagnostics for COVID-19 is an area requiring further study.

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