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Nanobodies: COVID-19 and Future Perspectives

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The COVID-19 pandemic has driven biotechnological developments to provide new and more effective tools for prophylaxis, diagnosis, and therapy. Historically, monoclonal antibodies have been valuable tools; however, the pandemic has shown some weaknesses, such as production limitations at a global scale. An alternative to conventional monoclonal antibodies are nanobodies, recombinant fragments of the variable region of single-domain antibodies derived mainly from the Camelidae family. Nanobodies have multiple characteristic benefits: they are small (15 KDa) and have remarkable refolding capability and unlimited possibilities for modifications due to their recombinant nature. Here, we review the application of nanobodies in diagnosis and treatment of SARS-CoV-2 infection.

Keywords: nanobodies (VHH), SARS-CoV-2, COVID-19, virus, llama (*Lama glama*), alpaca (*Vicugna pacos*), VHH antibody fragment

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INTRODUCTION

Heavy-chain-only antibodies (HCAbs) are found in camelids (e.g., dromedaries, camels, llamas, and alpacas) (Arbabi-Ghahroudi, 2017) and some cartilaginous fish species such as nurse shark (*Ginglymostoma cirratum*), wobbegong shark (*Orectolobus maculatus*), spiny dogfish (*Squalus acanthias*), and smooth dogfish (*Mustelus canis*) (Camacho-Villegas et al., 2013; Cheong et al., 2020; Dooley and Flajnik, 2006; Dooley et al., 2006; Feige et al., 2014; Ohtani et al., 2013; Roux et al., 1998; Stanfield et al., 2004; Nuttall et al., 2001). Within their immune system, type IgG2 and IgG3 lack the CH1 domain due to alternative splicing. Consequently, the light chains do not pair to the final antibody leading to the generation of HCAbs (Hamers-Casterman et al., 1993). IgG2 and IgG3 rely on heavy variable domains or VHH, a small domain of approximately 15 kDa to bind to specific antigens (Muyldermans et al., 1994; Desmyter et al., 1996; Arbabi Ghahroudi et al., 1997; Vu et al., 1997; Hassanzadeh GH et al., 1998; Lauwereys et al., 1998). The key difference between conventional monoclonal antibodies and HCAbs is that the latter are encoded from single genes (**Figure 1A**). Thus, the VHH can be amplified from cDNA, cloned into libraries of diverse natures, and later isolated using conventional molecular biology procedures. Once isolated, the recombinant VHH fragments of HCAbs are known as nanobodies (Muyldermans et al., 1994). Nanobodies conserve selective binding to specific antigens in the ~15 KDa minimal structure, 10 times smaller than conventional antibodies (Arbabi Ghahroudi et al., 1997). Nanobodies are soluble and highly stable to a wide range of pH and temperatures (Bond et al., 2003), along with an outstanding penetrability (Muyldermans, 2013) (**Figure 1B**). Some nanobodies are able to penetrate the blood-brain barrier which places them as promising tools for future diagnosis and therapy for neurodegenerative diseases. Among all the outstanding advantages of nanobodies, one of the

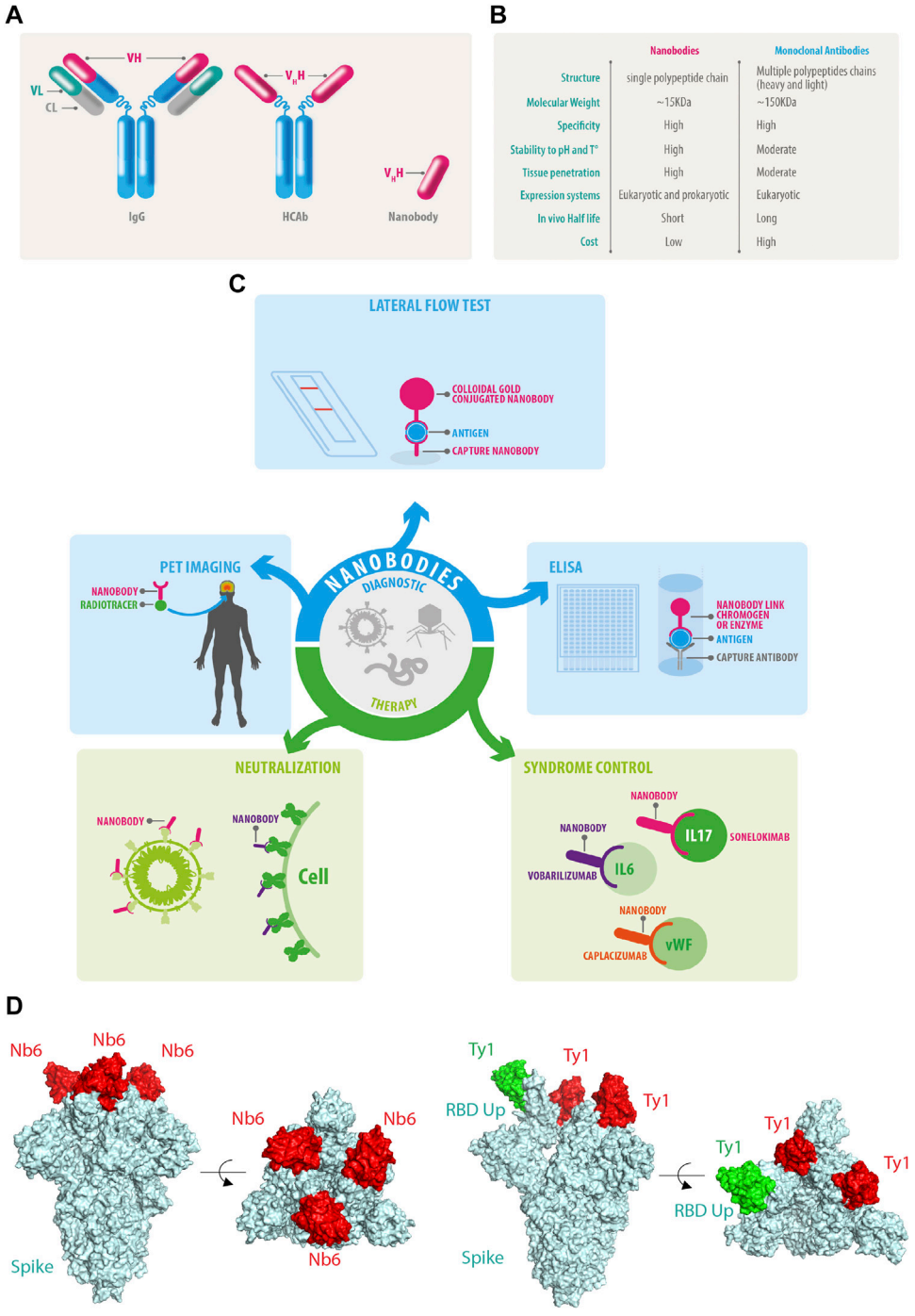

FIGURE 1 | Nanobody features and functions. **(A)** Conventional IgG antibodies contain heavy and light chains, HCAb does not associate with light chains allowing the isolation of the genetic fragments of the Vhh also known as nanobodies. **(B)** Comparative features of nanobodies versus monoclonal antibodies. **(C)** Current and future perspectives of nanobodies. **(D)** Nanobodies bind to the RBD of SARS-CoV-2 and neutralize the infection by different mechanisms: NB6 binds to the RBD of each Spike protomer in a down conformation (PDB ID: 7KKK), while Ty1 allows the upper conformation of one RBD of Spike in green (PDB ID: 6ZXN), in both examples, the nanobodies prevent the engagement of the human ACE2 receptor.

most attractive is the performance achieved through recombinant expression systems. Nanobodies can be produced efficiently in eukaryotic and prokaryotic systems (Arbabi-Ghahroudi et al., 2005; Liu and Huang, 2018; de Marco, 2020) and, because nanobody sequences are obtained during the selection process, the final molecule can be engineered to cope with several applications using conventional molecular biology tools. Moreover, open-source computational pipelines are available for nanobody humanization such as "Llamanade" (Sang et al., 2022).

Nanobodies are remarkable tools for diagnosing and treating various diseases, and the COVID-19 pandemic has driven the generation of several nanobodies against SARS-CoV-2. Here, we summarize some of the current applications and production strategies of nanobodies to help fight viral pathogens.

Nanobody Expression Systems

Nanobodies are compact structures and can be produced in high yields in several expression systems, such as bacteria, yeast, mammalian cells, and plants (Frenken et al., 2000; Arbabi-Ghahroudi et al., 2005; Ismaili et al., 2006).

Production in prokaryotes has a low cost and involves easy handling (Rosano et al., 2019). The traditional way to express recombinant proteins in bacteria is in the cytoplasm; however, the reducing cytoplasmic conditions can negatively affect the formation of disulfide bonds in some nanobodies, which are necessary for the correct folding of its tertiary structure (Govaert et al., 2012; Muyldermans, 2013; Hagihara and Saerens, 2014; Billen et al., 2017). Despite this, there are examples of nanobodies produced under cytoplasmatic conditions, which require the attachment of other proteins (Anderson et al., 2018), or expression in special bacterial strains with an oxidizing cytoplasmatic environment such as SHuffle T7 cells (Li et al., 2019) or co-expression with enzymes such as Erv1p sulfhydryl oxidase (Veggiani and de Marco, 2011; Shriver-Lake et al., 2017). High yields are also obtained when expressing proteins in classical inclusion bodies and further by denaturation in urea-mediated protein extraction (Maggi and Scotti, 2017). Also, there is an interesting alternative of expressing the nanobody coupled to a secretion pathway, such as the hemolysin secretion system (Günaydın et al., 2014; Ruano-Gallego et al., 2019).

Nowadays, the most convenient method for low-scale production of recombinant nanobodies is in *Escherichia coli* periplasm (Salema et al., 2013). Its oxidizing environment ensures correct folding and disulfide bond formation, and the periplasmic extracts enriched with the recombinant nanobodies facilitate subsequent purification (Conrath et al., 2001; Billen et al., 2017). The N-terminal pelB leader sequence drives the protein to the post-translational Sec pathway, the nanobodies are completely synthesized in the ribosome and then released to the Sec-translocase, which enables its carriage through the inner bacterial membrane and accumulates the nanobodies in the periplasm (Keen and Tamaki, 1986; Yoon et al., 2010; Billen et al., 2017). The recombinant proteins that accumulate in the periplasmic space are usually recovered after an osmotic shock which softly breaks the outer bacterial membrane, allowing the release of the proteins and preventing further contamination with *E. coli* cytoplasmatic proteins. Affinity purification can be applied directly after using various affinity matrices, such as hexahistidine (His6)-tag (Salema and Fernández, 2013), maltose-binding protein (MBP), or the Avi-tag, which allows *in vivo* biotinylation of the tagged protein (Hernot et al., 2012; Zhu et al., 2014; Noor et al., 2018; Sun et al., 2018; Du et al., 2019).

Nanobodies can also recognize linear peptides, an example of this is a nanobody called ALFA Selector, which recognizes the short ALFA-tag (SRLEEELRRRLTE) sequence, a very efficient tag for protein purification, including nanobodies themselves (Götzke et al., 2019; Kilisch et al., 2021).

Human or humanized monoclonal antibodies have been approved during the pandemic as emergency treatment measures. However, the production limitation led to the access of being almost exclusive to high-income countries in the northern hemisphere. The production of conventional antibodies occurs in mammalian cells, which is costly and time-consuming. In most cases, single clones of mammalian HEK293 or CHO cells must be isolated and expanded into cell banks, a process that takes several months. In contrast, therapeutic nanobodies and nanobodies fused to the Fc of conventional antibodies (Nb-Fc) can be produced in yeast, such as *Pichia pastoris,* in an endotoxin-free manner and at a very low cost. Interesting technologies based on CRISPR/Cas9 improve the glycosylation homogeneity of *P. pastoris,* which could allow efficient expression for therapeutic technologies using nanobodies (Krainer et al., 2013; Weninger et al., 2016; Schepens et al., 2021).

Nanobodies as Diagnostic Tools

Immunoaffinity techniques are the primary tools for rapid diagnosis, not only in infectious diseases but also in an extensive range of pathologies. Nanobodies are incipient in this field, and some technical difficulties regarding their small size and low retention on nitrocellulose strips remain to be solved. Serological tests have been implemented for parasitic infections such as *Taenia solium* cysticercosis (Deckers et al., 2009; Huang et al., 2010), *Trypanosoma spp* (Saerens et al., 2008a), and bacterial diseases, such as *S. aureus* (Stijlemans et al., 2004; Stijlemans et al., 2017). Indeed, this proof of concept opens up a large field of action for nanobodies in the upcoming years.

Nanobodies have been generated to capture several viral proteins of HIV (Gray et al., 2017); norovirus (Koromyslova and Hansman, 2017), dengue (Fatima et al., 2014) among others. When used for diagnosis, nanobodies can be classified in two groups: the primary nanobodies responsible for the recognition of a pathogen or molecule of interest and the secondary antibodies that bind to primary antibodies and unveil its presence by colorimetric or enzymatic reactions. Nanobodies as recombinant proteins can be modified to accomplish the function of primary and secondary antibodies simultaneously, for instance, nanobody fusion to enzymes such as horseradish peroxidase (HRP) has been used for the detection of anti-Newcastle disease virus (NDV) antibodies in chicken sera (Sheng et al., 2019).

Significant efforts have been made to develop diagnostic techniques for COVID-19, focusing mainly on speed and

accuracy. The first available tools were based on conventional monoclonal IgM and IgG lateral flow immunoassays (Yetisen et al., 2013; Goossens et al., 2017); however, the expression of IgM and IgG against SARS-CoV-2 antigens is only detectable in late disease stages. Therefore, the gold standard diagnostic test for SARS-CoV-2 diagnosis is the reverse-transcription quantitative polymerase chain reaction (RT-qPCR) from nasopharyngeal and oropharyngeal swab samples (Wang et al., 2020a; To et al., 2020). After vaccines were implemented, IgM and IgG lateral flow immunoassays became useful to determine the immune response against vaccines. Recently developed secondary nanobodies showed superior properties for cellular biology studies regarding penetrance, staining accuracy and, furthermore, secondary nanobodies can be premixed with primary antibodies to bypass the primary antibody animal-species limitations (Sograte-Idrissi et al., 2020). Nanobodies can be fused to the Fc of conventional immunoglobulins and produced recombinantly, which complements nanobody binding capabilities with several technologies already available for monoclonal antibodies (Bao et al., 2021; Girt et al., 2021; Valenzuela Nieto et al., 2021) (**Figure 1C**).

Nowadays nanobody-based lateral flow tests can rapidly detect recombinant human interferon α2b (Qin et al., 2021). They have also been used to detect active *Trypanosoma congolense* infections (Pinto Torres et al., 2018). In addition to rapid tests, nanobodies are efficient and sensitive tools for enzyme-linked immunosorbent assay (ELISA), a method allowing the capture and quantitative measure of antigens in the small absorbent surface. In short, nanobodies can be immobilized on the surface of ELISA plates to capture the molecule of interest, and further a second non-competitive nanobody associated to enzymatic activity can be applied to detect the already trapped molecule of interest. Nanobodies covalently coupled to HRP provide sensitive detection of SARS-CoV-2-specific full-length trimeric spike or RBD (Valenzuela Nieto et al., 2021).

In recent years, miniaturization of chips and sensing layers for biosensor equipment linked with microfluidic devices have been proposed as the best option to obtain the most sensitive detection level (Conroy et al., 2009). However, biosensor sensitivity depends on the physical properties of the molecule that binds its target (Saerens et al., 2008b). One of the advantages of nanobodies is their small size, allowing higher molecule density on a surface, and the possibility of easy directional immobilization, which translates into a higher ligand binding capacity leading to improved sensitivity for detecting low analyte concentrations (Huang et al., 2005). Another recently developed system is the use of nanobody-based organic electrochemical transistors (OECTs) which applies a conjugated polymer and a nanobody to detect SARS-CoV-2 spike protein (Guo et al., 2021).

In the field of non-infectious diseases, nanobodies outperform some conventional antibodies, for instance, in the detection of human prostate-specific antigen, an early marker of prostate cancer (Huang et al., 2005; Saerens et al., 2005). Nanobodies have also been conjugated with radioisotopes as modern diagnostic tools for personalized imaging medicine such as positron emission tomography (PET) to detect EGFR, a tyrosine kinase receptor that is highly expressed in most epithelial cancer cells, believed to contribute to tumor malignancy (Penault-Llorca et al., 2006; Gainkam et al., 2008; Tijink et al., 2008). PET imaging is a promising and emergent field for nanobody applications in *in vivo* imaging (**Figure 1C**). It requires the accumulation of radiolabeled nanobodies at the target tissue or tumor and rapid excretion of the remanent circulating nanobodies to distinguish between the real signal and background (Massoud and Gambhir, 2003). Most radioisotopes used for *in vivo* imaging have a short half-life; for instance, Fluorine ^{18}F decays by positron (β+) emission with a half-life of 109.7 min. Conventional antibodies persist in circulation, leading to a high background signal for some applications. In contrast, nanobodies are generally cleared rapidly suggesting a potential extended use for PET diagnostics (Tijink et al., 2008; Harmand et al., 2021). Nanobodies are expected to become important for cancer diagnosis: Molecules including monoclonal antibodies (Frigerio et al., 2021) used for PET diagnostics are useful for directed radiotherapies through the simple exchange of radioisotopes with, for instance, Lutetium ^{77}Lu. These radiotracers used for diagnostics and therapeutical applications are known as Theranostics (Debnath et al., 2022; Woźniak et al., 2022).

Nanobodies show plenty of advantages and overcome some of the problems observed with conventional antibodies, creating many opportunities for future diagnostic applications.

Immunotherapies Based on Nanobodies

Previous to the COVID-19 pandemic, nanobody-based therapeutic approaches were developed against viruses of global concern such as human immunodeficiency virus-1 (HIV-1) (Chen et al., 2008; Gong et al., 2012; McCoy et al., 2012; Matz et al., 2013), influenza viruses (Ashour et al., 2015; Schmidt et al., 2016), hepatitis C virus (HCV) (Tarr et al., 2013), respiratory syncytial virus (RSV) (Hultberg et al., 2011; Schepens et al., 2011), and enteric viruses (Wu et al., 2017). Antiviral nanobodies were also tested in clinical trials against rotavirus and the human respiratory syncytial virus (RSV). Remarkably, ALX-0171, a trivalent nanobody that neutralizes RSV, substantially decreased the viral load in children. Additionally, it is the first nanobody-based treatment delivered by nebulization through the airway (Palomo and Mas, 2016; Stohr and Palomo, 2016; Larios Mora and Gallup, 2018). Nanobodies have significant advantages when used as a therapy (Chakravarty et al., 2014; Jovčevska and Muyldermans, 2020). Several nanobodies were developed to modulate the immune and inflammatory responses. For example, vobarilizumab (ALX-0061) (Dörner et al., 2017) is a bispecific anti-IL6-R nanobody which has been engineered to extend its half-life targeting human serum albumin; secukinumab (ALX-0761) is a trivalent nanobody against IL-17A/F (Langley et al., 2014; De Munter et al., 2018; Svecova et al., 2019; Xie et al., 2019); caplacizumab (ALX-0081 or ALX-0681) is a bivalent humanized anti–von Willebrand Factor (vWF) nanobody (Abdelghany and Baggett, 2016; Peyvandi et al., 2016; Peyvandi et al., 2017; Scully et al., 2019), which has received approval from the European Medicines Agency (EMA) and the Food and Drug Administration (FDA) in the United States, for treating patients with thrombotic thrombocytopenic purpura

TABLE 1 | Nanobodies and SARS-CoV-2.

Nanobodies	Source	Method	Target	References
2NSP23; 2NSP90	Llama	Phage display	Nsp9	Esposito et al., 2021 (Esposito et al., 2021)
6ID10 (5; 6; 16; 40; 70; 71; 75; 96; 99; 113)	Shark	Phage display	RBD	Gauhar et al., 2021 (Gauhar et al., 2021)
A8-G11-Fc	Llama (naïve library)	Phage display	ACE2	Lu et al., 2021 (Lu et al., 2021b)
aRBD-2–5; aRBD2-7	Alpaca	Phage display	RBD	Ma et al., 2021 (Ma et al., 2021)
C5; H3; C1; F2	Llama	Phage display	RBD	Huo et al., 2021 (Huo et al., 2021)
H11-D4; H11-H4	Llama (naïve library)	Phage display	RBD	Huo et al., 2020 (Huo et al., 2020)
k-874A	cDNA library	cDNA display	RBD	Haga et al., 2021 (Haga et al., 2021)
KA1; KC1; KC3	Synthetic library	Yeast display	RBD	Zupancic et al., 2021 (Zupancic et al., 2021)
MR3; MR17; SR4; SR31	Synthetic library	Ribosome and Phage display	RBD	Li et al., 2021; Yao et al., 2021 (Li et al., 2021b; Yao et al., 2021)
Nanosota-1	Llama and alpaca (naïve library)	Phage display	RBD	Ye et al., 2021 (Ye et al., 2021)
NB1A7; NB1B11	Camels	Phage display	RBD	Shi et al., 2022 (Shi et al., 2022)
NB1-Nb2-Fc	Synthetic library	Phage display	RBD	Chi et al., 2022 (Chi et al., 2022)
Nb11-59	Camels	Phage display	RBD	Gai et al., 2021 (Gai et al., 2021)
Nb15; Nb56; Nb12; Nb30	Llama; alpaca; dromedary, Bactrian camel	Phage display; Nanomouse	RBD	Xu et al., 2021 (Xu et al., 2021)
NB15; Nb22; Nb31	Alpaca	Phage display	RBD	Wu et al., 2021 (Wu et al., 2021b)
Nb6	Synthetic library	Yeast display	Spike	Schoof et al., 2020 (Schoof et al., 2020)
Nb91-Nb3-hFc	Camel (naïve library)	Phage display	RBD	Lu et al., 2021 (Lu et al., 2021a)
Nbs 89	Llama	MS proteomic	RBD	Nambulli et al., 2021; Sun et al., 2021; Xiang et al., 2020 (Xiang et al., 2020; Nambulli et al., 2021; Sun et al., 2021)
NIH-CoVnb-112	Llama	Phage display	Spike	Esparza et al., 2020 (Esparza et al., 2020)
NM1226; NM1230	Alpaca	Phage display	RBD	Wagner et al., 2021 (Wagner et al., 2021)
P2C5; P5F8; P2G1	Camel	Phage display	RBD	Favorskaya et al., 2022 (Favorskaya et al., 2022)
Re6H06; Re9B09; Re5D06; R28	Alpaca	Phage display	RBD	Güttler et al., 2021 (Güttler et al., 2021)
S1-49; S1-1; S1-23; S1-46; RBD-9; RBD-35; S2-10; S2-40	Llama	MS proteomic	RBD	Mast et al., 2021 (Mast et al., 2021)
S14	Alpaca	Phage display	RBD	Li et al., 2021 (Li et al., 2021c)
saRBD-1	Alpaca	Phage display	RBD	Weinstein et al., 2022 (Weinstein et al., 2022)
Sb14; Sb16; Sb45; Sb68	Synthetic library	Ribosome and Phage display	RBD	Ahmad et al., 2021 (Ahmad et al., 2021)
Sb23	Synthetic library	Phage display	RBD	Custódio et al., 2020 (Custódio et al., 2020)
SP1b4; SP1D9; SP3H4	Synthetic library	Phage display	RBD	Stefan et al., 2021 (Stefan et al., 2021)
SR6v15; Nb21; SR6	CeVICA	Ribosome display	RBD	Chen et al., 2021 (Chen et al., 2021)
TB202-1; TB202-3; TB202-63	Synthetic library	Phage display	RBD	Yuan et al., 2022 (Yuan et al., 2022)
Ty1; Fu2	Alpaca	Phage display	RBD	Hanke et al., 2020, 2022 (Hanke et al., 2020a; Hanke et al., 2022)
VHH-E; VHH-U; VHH-V; VHH-W	Alpaca and llama	Phage display	RBD	Koenig et al., 2021 (Koenig et al., 2021)
VHH-Fc	Llama (naïve library)	Phage display	Spike	Dong et al., 2020 (Dong et al., 2020a; Dong et al., 2020b)
VHH72*	Llama	Phage display	RBD	Schepens et al., 2021 (Schepens et al., 2021)
W25	Alpaca	Bacterial display	RBD	Valenzuela Nieto et al., 2021 (Valenzuela Nieto et al., 2021)
WNb2; WNb7; WNb10	Alpacas	Phage display	RBD	Pymm et al., 2021 (Pymm et al., 2021)

**An enhanced version of this Fc-linked nanobody in clinical study phase II is called XVR011. (www.clinicaltrials.gov).*

(Jovčevska and Muyldermans, 2020), becoming the first nanobody approved for clinical therapy of a chronic disease (**Figure 1C**).

The primary global response to the COVID-19 pandemic was the creation of efficient vaccines (Li et al., 2020; Li et al., 2021a; Awadasseid et al., 2021; Wu et al., 2021a; Baden et al., 2021; Russell et al., 2021). Nowadays, the global vaccination initiatives cover 56% of the world population with two doses. Global vaccination success was limited by inaccessibility to vaccines and refusal to be vaccinated for personal reasons. In addition, significant efforts were placed in the study of repurposing drugs that may limit mortality and ameliorate COVID-19 symptoms (Wang et al., 2020b; Canedo-Marroquín et al., 2020; Valle et al., 2020). There are more than 2000 clinical trials registered on www.clinicaltrials.gov with various topics ranging from contact tracing, dietary supplements, anti-viral therapies, and drugs that have been in the spotlight, such as chloroquine, hydroxychloroquine, and ivermectin.

The current development of antibodies against SARS-CoV-2 focuses on neutralizing antibodies against the spike protein (Chi et al., 2020a; Shi et al., 2020). In the early days of the first wave of the COVID-19 outbreak, plasmapheresis of convalescent SARS-CoV-2 patients was implemented to supplement antibodies to those at risk due to the lack of alternatives (Jiang et al., 2020; Liu et al., 2020; Longueira et al., 2021) (**Table 1**).

The first neutralizing nanobodies against SARS-CoV-2 targeted SARS-CoV-1 and MERS-CoV RBDs, but fortunately, they also exhibited a remarkable cross-reactivity and neutralization capability against SARS-CoV-2 (Wrapp et al., 2020). These were followed by nanobodies identified

from synthetic libraries, "sybodies", against SARS-CoV-2 (Dong et al., 2020a; Custódio et al., 2020; Walter et al., 2020). The framework regions of the synthetic library were partially humanized to decrease the immune response if administered to humans.

Other reported strategies were the generation of a platform to develop SARS-CoV-2-specific single-domain antibodies of human origin (Wu et al., 2020), humanizing the nanobody backbone, and reducing potential immune recognition. Other studies reported successful isolation of nanobodies and their fusion to the human IgG1-Fc region, improving their binding and neutralizing capabilities (Chi et al., 2020b; Xiaojie et al., 2020; Lu et al., 2021a; Valenzuela Nieto et al., 2021) (**Figure 1C**).

Also, another nanobody set was isolated by from yeast surface-displayed synthetic library against epitopes of the SARS-CoV-2 spike protein. The researchers used Nb6 to design bivalent and trivalent nanobodies resulting in a 2000-fold increase in inhibitory activity against both pseudo virus and live SARS-CoV-2 in infection assays (Schoof et al., 2020) (**Figure 1D**).

Another group reported the isolation and characterization of an alpaca-derived single domain antibody fragment, Ty1, against spike protein (Hanke et al., 2020a; Hanke et al., 2020b). In this study, Ty1 was fused to an Fc domain, increasing the neutralizing capabilities of the nanobody. Moreover, in contrast to Nb6 that binds RBD in the down conformation (**Figure 1D**), cryo-electron microscopy studies demonstrated that Ty1 binds to an epitope on the RBD, accessible in both the "up" and "down" conformations (**Figure 1E**). Glycosylation sites N165, N234, and N343 on the spike protein shield RBD from conventional antibodies, especially when the RBD is in a "down" conformation (Watanabe et al., 2020). In the RBD-down conformation, the glycan on N165 points towards the Ty1-binding epitope, likely not leaving sufficient space to accommodate a conventional antibody. This indicates that nanobodies most likely recognize more epitopes for SARS-CoV-2 neutralization than conventional antibodies. Interestingly, a group of multivalent nanobodies bind the RBD domain of spike and lock it in the "up" conformation, a state that is typically associated with receptor binding activation. The premature activation of the fusion machinery on virions enhances neutralization in a non-reversible manner (Koenig et al., 2021).

Not only alpacas and llamas have been contributing for the generation of SARS-CoV-2 neutralizing nanobodies, also nanobodies isolated from a semisynthetic shark-derived library have been shown to neutralize SARS-CoV-2 (Gauhar et al., 2021).

DISCUSSION

During the COVID-19 pandemic, new technologies were developed for the rapid isolation of nanobodies. Our team implemented a new procedure for fast, economical, and efficient selection of high affinity nanobodies based on bacterial display and density gradient (Valenzuela Nieto et al., 2021).

Unfortunately, new SARS-CoV-2 variants significantly scape the immune response raised by either vaccination or previous SARS-CoV-2 infections. The most dramatic example are the Omicron variants. Omicron BA1 has become the infectious virus in the history of humanity. Several mutations on the spike protein generated less efficient cleavage of the S1 domain by the furin protease TMPRSS2 at the cell membrane. Consequently, the infection predominantly affects the upper airway but is less severe in human and animal models (Chen et al., 2022; Wrenn et al., 2022). However, due to the substantial number of infected people, death rates increased worldwide. Furthermore, a second subvariant of Omicron, BA2, developed in parallel. BA2 preserves the immune evasion capabilities of BA1, but unfortunately, current reports indicate a higher lethality (Wolter et al., 2022). The mechanisms behind the high severity of Omicron BA2 infection are not well understood.

More than 2 years after the first COVID-19 outbreak, over six million succumbed to the disease. There were four infective waves worldwide, and currently, infection rates are rising again, suggesting we are entering another wave of COVID-19 caused by Omicron subvariants. Vaccines have been beneficial and saved millions of lives; however, we must take new complementary approaches due to immune escape.

Our immune responses as humans are determined by the way our immune system is organized, for instance, we cannot raise single chain antibodies as part of our antibody defense. The pandemic has challenged the human population with a virus that replicates, mutates, evades, and overcomes our immune response. Nanobodies differ from our own defense and provide new possibilities for the generation of effective neutralizing antibodies that we could never develop ourselves.

AUTHOR CONTRIBUTIONS

Conceptualization: GV-N and AR-F. All authors have contributed to writing, reviewing, and editing sections and have agreed to the published version of the manuscript.

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