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BBA - General Subjects 1865 (2021) 129974

Contents lists available at ScienceDirect

BBA - General Subjects

journal homepage: www.elsevier.com/locate/bbagen

Nanobodies as powerful pulmonary targeted biotherapeutics against SARS-CoV-2, pharmaceutical point of view

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ARTICLE INFO

Keywords:
SARS-CoV-2
Covid-19
Nanobody
Pulmonary drug delivery

ABSTRACT

Background

Since December 2019, the newly emerged SARS-CoV-2 virus continues to infect humans and many people died from severe Covid-19 during the last 2 years worldwide. Different approaches are being used for treatment of this infection and its consequences, but limited results have been achieved and new therapeutics are still needed. One of the most interesting biotherapeutics in this era are Nanobodies which have shown very promising results in recent researches.

Scope of review

Here, we have reviewed the potentials of Nanobodies in Covid-19 treatment. We have also discussed the properties of these biotherapeutics that make them very suitable for pulmonary drug delivery, which seems to be very important route of administration in this disease.

Major conclusion

Nanobodies with their special biological and biophysical characteristics and their resistance against harsh manufacturing condition, can be considered as promising, targeted biotherapeutics which can be administered by pulmonary delivery pharmaceutical systems against Covid-19.

General significance

Covid-19 has become a global problem during the last two years and with emerging mutant strains, prophylactic and therapeutic approaches are still highly needed. Nanobodies with their specific properties can be considered as valuable and promising candidates in Covid-19 therapy.

1. Introduction

Coronavirus disease (Covid-19) is an infectious disease caused by SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2, an enveloped, positive-sense single-stranded RNA virus [(+)ssRNA]) which was first appeared in Wuhan, China in December 2019 [1]. Most people infected with the SARS-CoV-2 virus will experience mild to moderate respiratory illness but older people, and those with underlying medical problems like cardiovascular disease, diabetes, chronic respiratory disease, and cancer are more likely to develop serious illness [1]. In fact, Covid-19 rapid spread and pandemic, was a severe challenge for health care system and society [2].

The virus main transmission route is through mucosal tissues: nose, mouth and upper respiratory tract [3], and gains entry to the target cells via the Angiotensin Converting Enzyme 2 (ACE2) receptor by the interaction of the Receptor Binding Domain (RBD) of the Spike protein (as the key target for eliciting persistent neutralizing antibodies [4]) on the viral surface [5]. ACE2 receptors can be found in different tissues specially lung, small intestine, heart and digestive system, which reflect the diversity of symptoms and pathologies associated with Covid-19 [6].

2. Strategies for Covid-19 treatment

Based on the SARS-CoV-2 pathogenesis, different strategies for

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https://doi.org/10.1016/j.bbagen.2021.129974
Received 4 May 2021; Received in revised form 23 July 2021; Accepted 28 July 2021
Available online 31 July 2021

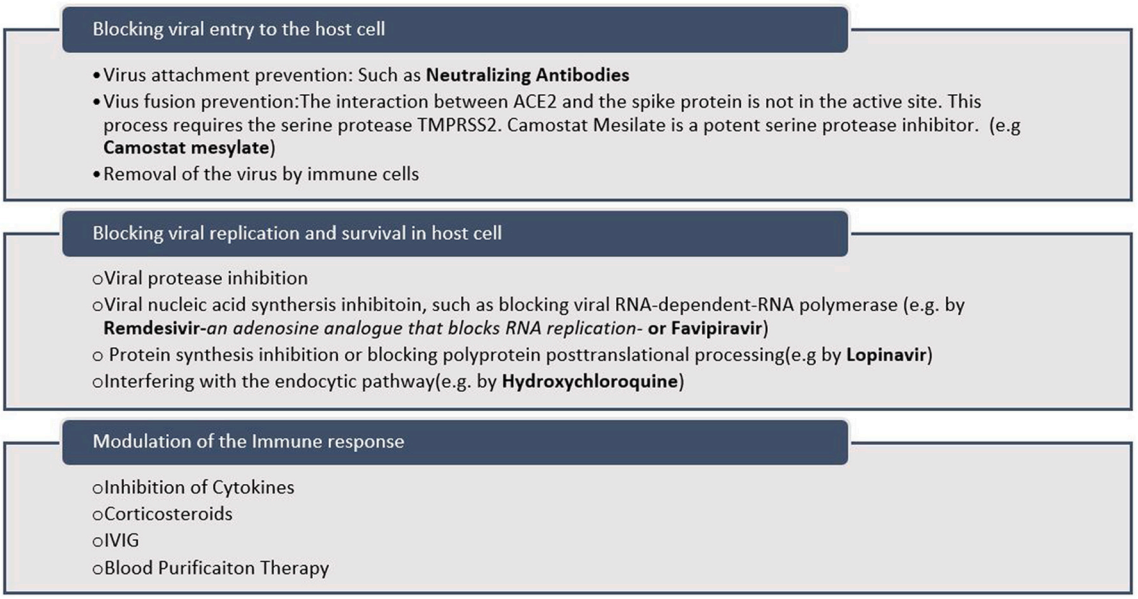

Fig. 1. Summary of strategies for Covid-19 treatment.

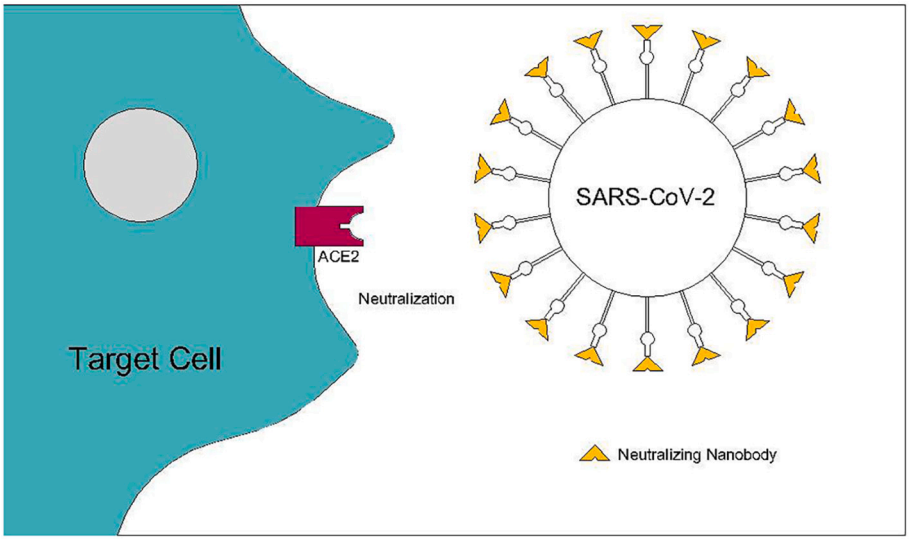

Fig. 2. SARS-CoV-2 neutralization by Nanobodies prevents viral entrance to the target cell. Spike RBD-ACE2 interaction is considered as a therapeutic target for binding and prevention of infection.

Covid-19 treatment are designed [6–8]. These approaches mainly include preventing the virus entrance to the target cell particularly by neutralizing antibodies [9,10] or virus fusion prevention [11], viral replication inhibition specially by targeting the virus proteases [12–14] and reducing the severity of immune response [15] which are summarized in Fig. 1.

It is clear that despite all efforts, still therapeutic and preventive interventions against Covid-19 are an urgent need [16]. A potent target for drug discovery for Covid-19 is RBD-ACE2 interaction that offers a very safe and strong therapeutic target for binding and prevention of infection by antibodies and Nanobodies (Nbs) for researchers who are working on Covid-19(Fig. 2) [17].

Neutralizing antibodies that prevent the virus particles from entering the target cells by inhibiting the RBD-ACE2 interaction, are very interesting anti-viral agents for the treatment of Covid-19 [18–21]. Recently, Zhiqiang Ku et al. have described strategies for the discovery and development of SARS-CoV-2 neutralizing antibodies [22]. However, despite all the potentials, antibodies also show some limitations such as size, stability and possible immunogenicity. While Nanobodies, which are the variable domains of camelid heavy chain-only antibodies, are a promising class of therapeutics and with their impressive characteristics, seems to be very effective molecules in inhibiting the RBD-ACE2 interaction and virus entry into the cells [2,23,24].

3. Nanobodies structure, characteristics and applications

Conventional immunoglobulin-γ (IgG) antibodies assembled from two identical heavy (H)-chains and two identical light (L)-chains. While sera of camelids contain a unique functional heavy (H)-chain antibody (HCAbs) in addition to conventional antibodies. The H chain of these homodimeric antibodies consists of one antigen-binding domain, the VHH, and two constant domains. The smallest intact functional antigen-binding fragment of HCAbs is the single-domain VHH, also known as a Nanobody (Fig. 4C) [25]. Nanobodies can be used in different formats

Table 1
Nanobodies developed against SARS-CoV-2 during the Covid-19 pandemic.

	Nanobody format (name)	Source	Expression System	SARS-Cov-2 target protein	Neutralization or binding ability	Claims	References
1	• Monovalent (Nb11-59)	Immunized Camel with RBD	*Pichia pastoris*	RBD	• Nb11-59 Kd = 21.6 nM • Block the interaction between ACE2 and eight different SARS-CoV-2-RBD variants. • Potent neutralizing ability against authentic SARS-CoV-2 in the Plaque Reduction Neutralization Test (PRNT) at the concentration of 50 and 5 μg/ml	Good stability profile which was not impacted by nebulization.	[48]
2	• Sybody	Synthetic	*E. coli*	RBD	• Six Sybodies can bind to the viral spike protein with double-digit nanomolar affinity • Five Sybodies showed substantial inhibition of RBD interaction with ACE2	Binding small antiviral molecules to the Sybody can increase their antiviral potency due to recognizing secondary epitopes of the virus.	[49]
3	• Monovalent Fc conjugated (Nanosota-1C-Fc)	Naïve library from llamas and alpacas	*E. coli*	RBD	• Tightly bound to the SARS-CoV-2 RBD and completely blocked out ACE2 (Kd = 15.7 pM) • Potently neutralized SARS-CoV-2 pseudovirus entry and authentic infection. • Preventive and therapeutic efficacy of this Nb in hamsters subjected to SARS-CoV-2 infection.	• Affinity maturation with error-prone PCR was done • More than 10-day in vivo half-life efficacy (Fc conjugation) and high tissue bioavailability	[50]
4	• Monovalent (Nb21 or Pin-21)	Immunized llama with RBD	*E. coli*	RBD	• Nb-21 reached 100% neutralization and neutralized the virus in a dose-dependent manner (IC50 values of 0.022 nM) • Subpicomolar affinity in Nb21 (KD < 1 pM)	• Thermostable to 72.8 °C • Soluble after ~6 weeks of storage at room temperature • Resistant to lyophilization and aerosolization • Intranasal delivery of PiN-21 at 0.6 mg/kg protected infected animals from weight loss and substantially reduced viral burdens in both lower and upper airways compared to control.	[41,44,51]
5	• Monovalent (Nb6, 11, 3, mNb6) • bivalent (Nb6-bi) • Trivalent (Nb6-tri, mNb6-tri)	Yeast surface-displayed library of synthetic nanobody sequences	*E. coli*	RBD	• Panel of nanobodies that\bind to multiple epitopes on Spike and block ACE2 interaction via two distinct mechanisms. • Femtomolar affinity for SARS-CoV-2 Spike and picomolar neutralization of SARS-CoV-2 infection in mNb6-tri	• These constructs showed preserved stability and function after aerosolization, lyophilization, and heat treatment. • Aerosol-mediated delivery by neutralizer directly to the airway epithelia.	[52]
6	• Monovalent (Fu2) • Monovalent Fc conjugated (Fu2-Fc) • Dimers	Immunized *Alpaca* with spike protein and RBD	Fu2: *E. coli* Fu2-Fc: Mammalian cell	RBD	• A PRNT using the B.1.351 (501Y·V2)1 variant showed that Fu2 very potently neutralized this novel variant and that the Fu2 dimer and Fu2-Fc improved neutralization efficiency • Prophylactic and therapeutic efficacy assessments in transgenic mice that express human ACE2 as a model were performed and the data showed that the Fu2-Ty1 dimer suppressed SARS-CoV-2 in vivo.		[53]
7	• Monovalent (Nb 12, 15,17,19, 30, 56) • Bivalent • Trivalent	Immunized *Llama* and Transgenic mice (nanomice) with spike protein and RBD	Unknown	RBD	• Dissociation constants more than 30 nM • The Nb monomers displayed nM and sub-nM half-maximal inhibitory concentration (IC50) in in vitro lentiviral particles pseudotyped with the SARS-CoV-2 spike	• Some Nbs are ineffective against viruses that carry substitutions in the RBD, but combining the two Nb classes into heterotrimeric constructs might further improve their efficacy against wild and mutant type of virus. • Nbs were thermostable and can be aerosolized with commercially available mesh nebulizers without losing neutralization activity.	[54]
8	• Monovalent (VHH E, U, V, W) • Bivalent (EE, EV, VE)	Immunized Alpaca and Llama with RBD and formalin-	*E. coli*	RBD	• Dissociation constants between 2 and 22 nM • In monomer form IC50 values ranged from 48 to 185 nM as	• Targeting two independent epitopes on the RBD can prevent viral escape.	[2]

(*continued on next page*)

Table 1 (*continued*)

	Nanobody format (name)	Source	Expression System	SARS-Cov-2 target protein	Neutralization or binding ability	Claims	References
	• Trivalent (EEE)	inactivated SARS-CoV-2			measured by PRNTs. In dimer form IC50 values of 0.7 nM for EV, 1.32 nM for VE, 180 for EE and 170 pM for EEE were achieved.	• Bivalent or trivalent of these nanobodies fusions increased the neutralizing potential in vitro.	
9	• Monovalent (W25)	Immunized Alpaca with spike protein	*E. coli*	RBD	• Sub-nanomolar affinity (KD = 295 ± 84 pM) • Strong neutralizing activity with IC50 values of 9.82 ± 1.92 nM for D614 and 5.09 ± 1.09 nM for G614 SARS-CoV-2 variant	• W25 is a single and monomeric Nanobody that binds to the SARS-CoV-2 RBD variant with sub-nanomolar affinity and efficiently competes with ACE2 receptor binding.	[55]
10	• Monovalent (aRBD2, 3, 5, 47, 41, 42) • Bivalent (aRBD2-5, aRBD2-7) • Homo bivalent Fc conjugated (Nb-TEV-Fc)	Immunized Alpaca with RBD	Mammalian expression system	RBD	• Dissociation constants between 2.60 and 21.9 nM in monovalent form, 1.59–72.7 nM in fusion with Fc and 59.2 pM and 0.25 nM in dimeric form (aRBD2-5 and aRBD2-7) • Nb-Fc fusions and dimeric forms showed enhanced neutralizing potency compared to monovalent form	• Therapeutic and diagnostic potential	[47]
11	• Monovalent (H11-D4, H11-H4) • Homo bivalent Fc conjugated (H11-D4-Fc, H11-H4-Fc)	Naïve llama library	*E. coli*	RBD	• Dissociation constants between 5 and 10 nM in monovalent form • Potent neutralization capacity in PRNT (ND50 of 6 nM for H11-H4-Fc and 18 nM for H11-D4-Fc)	• Passive immunization of severely ill C-ovid-19 patients with a cocktail of laboratory-synthesized neutralizing Nb	[19]
12	• Monovalent Fc conjugated (2A-Fc, 1B-Fc, 3F-Fc) • Trivalent (3F-1B-2A, 1B-3F-2A)	Naïve llama library and humanized synthetic library	Mammalian cells	RBD	• Kd ~ 0.047 nM for tri-specific VHH-Fcs 3F-1B-2A and Kd ~ 0.095 nM for 1B-3F-2A • tri-specific VHH-Fcs were more effective in neutralizing the pseudovirus infection than the combination treatment, with IC50 values of 3.00 nM for 3F-1B-2A, 6.44 nM for 1B-3F-2A	• tri-specific VHH-Fc showed more potent RBD binding, RBD/ACE2 blocking, and SARS-CoV-2 pseudovirus neutralization than the bi-specific VHH-Fc or combination of individual monoclonal VHH-Fc	[56]
13	• Monovalent Fc conjugated (Nb15-Fc, Nb22-Fc, Nb31-Fc) • bi-, tri-, tetra-valent • tri- valent with albumin specific Nb (Nb15-NbH-Nb15)	Immunized Alpaca with spike protein	Mammalian cells	RBD	• Kd from 1.13 to 1.76 nM for monovalent form • Potent neutralization activity with IC50 values in the range of 10–28.8 pM in pseudovirus neutralization assay. bi-, tri- and tetra-valent form of Nb15 exhibited higher neutralization potency than the monomer	• Nb15-NbH-Nb15 provides ultrahigh neutralization potency against SARS-CoV-2 wild 64 type and 18 mutant variants, including the current circulating variants of D614G 65 and N501Y predominantly in the UK and South Africa. • 45 Intranasal administration of Nb15-NbH-Nb15 provided 100% protection for both prophylactic and 46 therapeutic purposes against SARS-CoV-2 infection in transgenic hACE2 mice.	[57]
14	• Sybody (Sb23) • bivalent Fc conjugated (Sb23-Fc)	Synthetic sybody libraries	*E. coli*	RBD	• Sb23 displayed high affinity with Kd = 75 nM • Pseudovirus neutralizing potency with an IC50 of 0.6 μg/ml in monomeric form. The bivalent Sb23-Fc constructs, displays ~100-fold improved neutralization potential, compared to its monovalent counterpart, with an IC50 of 0.007 μg/ml	• Synthetic libraries are an alternative approach to rapid drug development, quickly generating highly specific binders with neutralization potential.	[58]
15	• Monovalent (NIH-CoVnb-112)	Immunized Llama with spike protein	*E. coli, Pichia pastoris*	RBD	• NIH-CoVnb-**112** displayed high affinity with Kd = 4.9 nM • NIH-CoVnb-112 blocked interaction between human ACE2 and some RBD variants with similar EC50 (1.11 nM) compared to its blocking effects on the prototype sequence spike protein RBD • NIH-CoVnb-112 potently inhibits viral transduction in an infection relevant pseudotyped SARS-CoV-2 virus model.	• NIH-CoVnb-112 is resistant to degradation or aggregation during nebulization and is acceptably stable in the presence of plasma. • Low-cost, stable, and safe nanobody-based therapeutics will be developed for inhaled use in the home and outside of formal healthcare environments.	[17]

such as bivalent monospecific, bivalent bispecific, bivalent bispecific, albumin-conjugated, trivalent bispecific and bispecific chimeric antigen receptor (CAR) T cell [23]. Nanobodies (including multi-specific, multivalent and bi-paratopic constructs) are encoded by single genes and are efficiently produced in various prokaryotic and eukaryotic hosts, including bacteria, yeast, and mammalian cells. They can be formulated at high concentrations and maintain low viscosity, enabling multiple routes of administration, including low volume injectables. (https://www.ablynx.com/technology-innovation/Nanobodies-competitive-features/).

Nanobodies show many interesting characteristics in comparison to conventional antibodies; such as [26]:

1. Easily selection by Phage Display
2. Reaching and recognizing unique epitopes due to small size
3. Ease of manipulation
4. High stability in harsh condition (such as chaotropic agents and pH extremes, so the route of administration can be intravenous, oral, intraperitoneal or intratumor)
5. Low Immunogenicity
6. High specificity
7. Easy production and suitable cost

All these properties, plus combining the beneficial properties of small molecules and monoclonal antibodies, make Nanobodies attractive molecules in drug discovery in various fields including Covid-19.

Recently, a list of selected domain antibodies in development and in clinical trials are published [23,27], among them Cablivi™ (Caplacizumab, From Ablynx, a Sanofi company) is approved by FDA and EMA as the first Nanobody for treatment of aTTP (acquired thrombotic thrombocytopenic purpura), a rare blood-clotting disorder [23].

Nanobodies applications (alone or in conjugation with other molecules) can be classified as [26,27]:

1. Therapy
2. Diagnosis
3. Intracellular targeting
4. Molecular Imaging

4. Nanobodies application against viral infections including SARS-CoV-2

Advantageous properties of Nanobodies, as mentioned above, and ease of their engineering and manipulation, make these single domain antibodies an interesting research tool and biotechnological medication [25]. So, researches have used these promising tools to fight many pathologic conditions including different viral infections [28,29], as well as HIV [30–32], Influenza A virus [33,34], Chikungunya virus [35] and HCV [36] and also in viral respiratory infections such as Respiratory Syncytial Virus (RSV) [37,38] and Middle East Respiratory Syndrome Coronavirus(MERS-CoV) [39,40].

According to the brilliant experience in previous viral infections, researches incline to study Nanobodies potential in prevention and treatment of Covid-19 caused by SARS-CoV-2. In addition, Nanobodies with their unique biophysical properties (including small size and thermostability) have this potential to be used as the pharmaceutical form of inhalation which can be directly deliver the therapeutic agent to the target organ, lung, conferring high pulmonary drug concentrations with minimal systemic side effects [41].

Notably, trimeric spike protein is conformationally flexible and allows each of its RBDs to have two different configurations: a “down” conformation that is thought to be less accessible and an “up” conformation that is receptor (ACE2) accessible conformation [2,42]. One of the best strategies to neutralize SARS-CoV-2 entry to the cells is to develop biotherapeutics with the potential of inducing conformational changes in a way that RBD cannot binds to its receptor anymore. Such as

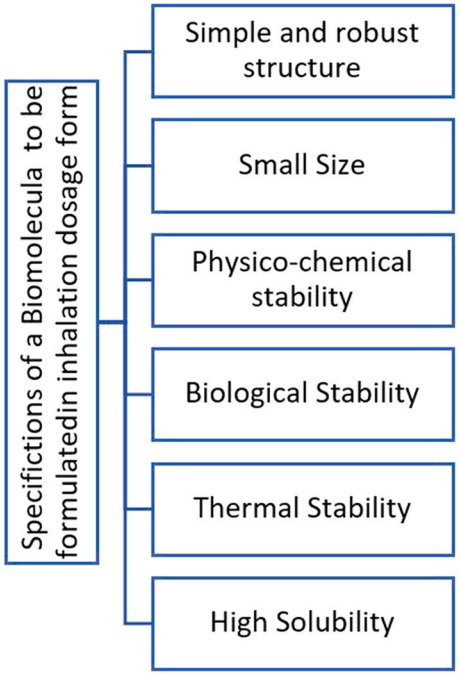

Fig. 3. Key specifications of a biomolecule to be formulated in a pulmonary dosage form (which all can be found in Nanobodies).

bivalent Nanobodies that induce post-fusion conformation of the SARS-CoV-2 spike to neutralize the virus [2,43]. Also, researchers have introduced some conserved epitopes for binding to their designed Nanobodies which were inaccessible to antibodies [44]. During the Covid-19 pandemic, several other studies have done to evaluate Nanobodies potentials for treatment of this syndrome, most of them are designed against SARS-CoV-2 spike RBD [45,46]. Binding kinetics between the SARS-CoV-2 RBD (including association(Ka) and dissociation (Kd) rates) are very important factors that affect the therapeutic outcome of the Nanobody and should be determined accurately [25] which are evaluated in some valuable studies [17,19,41,47]. Some important studies with the aim of development of anti-SARS-CoV-2 nanobodies are summarized in Table 1.

5. Nanobodies production and delivery, pharmaceutical point of view

Local pulmonary delivery of therapeutics may offer benefits for the treatment of lung diseases such as Covid-19. Advantages of pulmonary drug delivery includes the rapid onset of action, reduced systemic side effects, increased therapeutic window and the need for a lower dose to reach the desired therapeutic response, as well as non-invasive administration [59]. Although it is pharmaceutically possible to make different drug delivery systems and drug dosage forms for Nanobodies [26,60], if the target tissue of the drug is lung (for example, in diseases such as the Covid-19, which involves the respiratory system), the pulmonary method seems to be the best route of administration. Previous studies have shown that if the drug is administered systemically, only about 0.2% of the drug reaches the lungs, which means that in order to achieve a therapeutic dose in the lungs, much more concentration of the drug (and more side effects and more manufacturing costs as result) is needed in comparison with local pulmonary route. The same result was achieved in another study where it was shown that the inhalation route potentially offers rapid RSV neutralization, while simultaneously, $t_{1/2}$ of about 20 h allows for once daily dosing [37]. Nevertheless, formulation of biotherapeutics for pulmonary delivery is challenging and requires proteins with favourable biophysical and biochemical properties

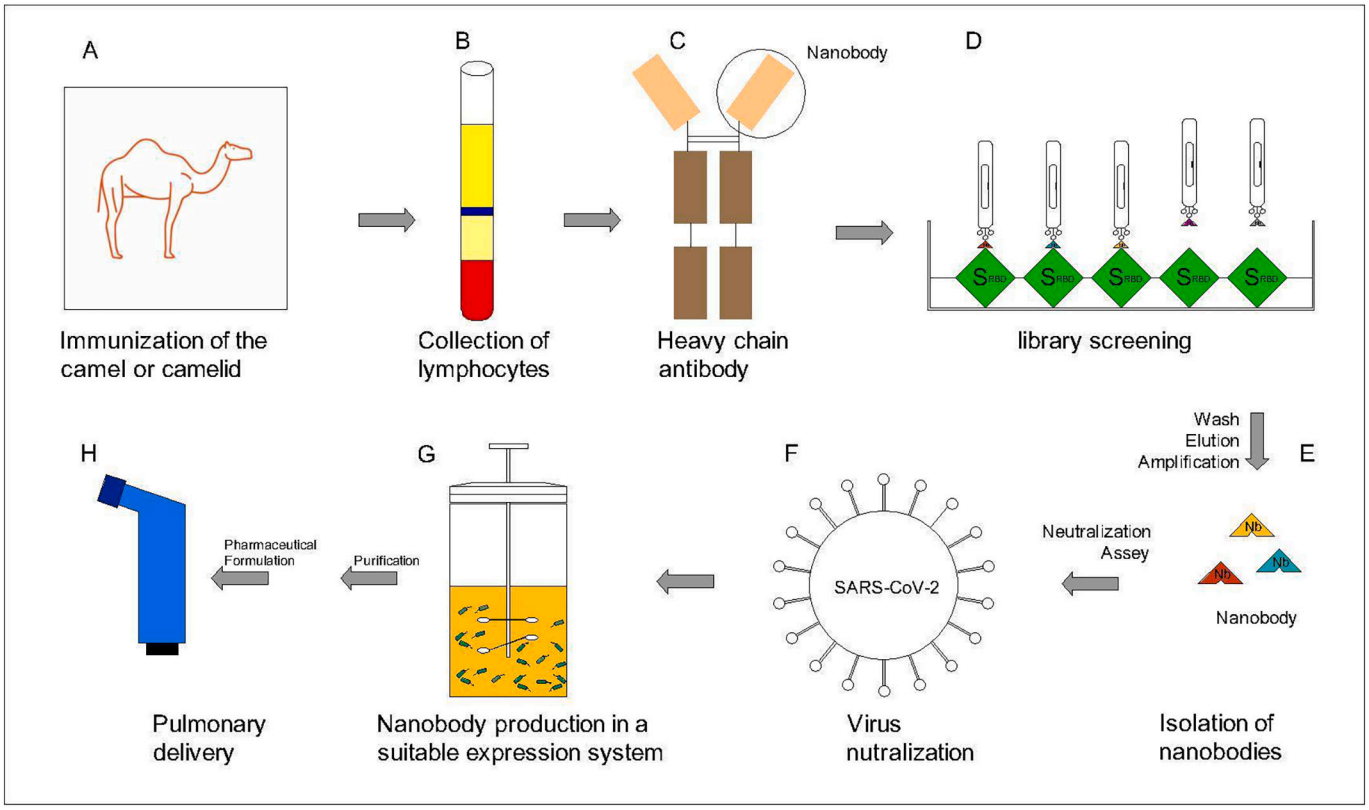

Fig. 4. Schematic process and steps of Nanobody production against SARS-CoV-2 and pharmaceutical formulation in pulmonary dosage form. A. First a Nanobody library should be prepared for example by immunizing the camel or camelid by SARS-CoV-2 spike protein. B. After the defined duration, blood will be collected and lymphocytes are separated. C. The camel blood contains VHH antibody, the smallest functional part of the VHH antibody is called Nanobody. D. The library is screened for Nanobodies against spike SARS-CoV-2 protein (specially the RBD). E. Washing non-specific phages and elution and amplification of the specific-Nanobodies. F. Virus neutralization assay is performed to select the Nanobodies with the most affinity to the SARS-CoV-2 Spike. G. Expression (in *E. coli* or yeast or any other suitable expression systems) and Large-scale production of the selected Nanobody with the highest affinity for the SARS-CoV-2 virus. H. After purification and downstream processes, produced Nanobodies will be formulated in a suitable dosage form for pulmonary delivery.

suitable for inhaled formulation, delivery, and inhalation devices. In other words, since during pharmaceutical development of pulmonary dosage forms, typically multiple buffer systems with different ionic strengths and high heat is used, biomolecules to be formulated into the pulmonary form must have certain properties and specifications. Among all biotherapeutics and in comparison, with conventional antibodies, Nanobodies, are particularly suited for delivery by inhalation due to their small size, simple and robust structure, high thermal stability and solubility, short half-life in the systemic circulation, biological and physico-chemical stability (such as resistance against aggregation and shear forces during manufacturing) (Fig. 3). Furthermore, being only one-tenth the size of a normal immunoglobulin, a single dose of Nanobody packs in ten times more active molecules compared to the same dose of a classic immunoglobulin [59].

To make a suitable inhaled formulation for a biological medicine such as Nanobody, the formulation method and the delivery device should provide not only the proper droplet size (0.5–5 μm aerodynamic diameter for good drug deposition in the lung [59]) but also preserve the structure and activity of the medicine [61]. Depending on the formulation, there are different devices currently used for pulmonary delivery: Nebulizers, pressurized metered-dose inhalers (pMDIs) and dry powder inhalers (DPIs), each of them has its own characteristics and mechanism of action explained elsewhere [62,63]. pMDIs formulations need propellants and as long as most biologics are water-soluble, they do not easily solubilize in propellants and this results in a dose limitation in addition to changes in protein structure. DPIs are not easily applicable in critically ill patients, low consciousness or children; and also drying stage which is necessary in DPIs formulations may affect the protein or Nanobody construct which need extensive studies. Most biological products in the market are generally formulated in either solution or suspension or lyophilized powder. Generally, 75% of inhaled proteins in researches are prepared in the form of liquids for nebulization. Pulmonary delivery by nebulizer does not need a drying step as it may affect the drug properties and it can be used easily by all patients. There are different kinds of nebulizer: air-jet, ultrasonic and vibrating mesh. According to previous researches, It seems that the best method for nebulizing Nanobodies is the vibrating mesh method due to minimizing construct changes and multimerization of the molecules (2% vs 40% in air-jet method) [59,61]. The main advantages of a nebulizer compared to a pMDI or DPI is that oxygen can be administered in combination with the aerosol treatment and higher doses can be administered over a prolonged time. Furthermore, nebulizers can be used without the cooperation of the patient [59], which is a very important factor in hospitalized patients.

Altogether, it seems that nebulizers are the most appropriate pulmonary dosage form for targeted delivery of Nanobodies to the lung. An example of a Nanobody against a respiratory virus that is formulated in inhalation dosage form is ALX-0171 (manufactured by Ablynx company), a potent trivalent Nanobody with antiviral properties against RSV [37] with good safety results in phase 1 clinical trials [59,64]. These successful experiences encouraged researches to use their knowledge for the treatment of new emerging corona virus too.

Physical properties of a nebulizer solution may impact drug product stability and device performance. To resist the shear stress during nebulization and to avoid physico-chemical degradation, high solubility, low viscosity, and physical stability in a physiologic buffer is needed

(a solubility of at least 100 mg/ml and a viscosity below 2 Centipoise for a nebulizer solution) [59]. As shown in some valuable studies, Nanobodies production should be in a way that pre-and post-nebulization, the stability and function of the Nanobody molecule does not changed. For example Esparza et al. demonstrated in their study that their Nanobody (named NIH-CoVnb-112) stability and potent inhibition of SARS-CoV-2 pseudovirus following nebulization did not changed significantly before and after nebulization [17].

The method of design and development of Nanobodies are explained in different review articles [65]. In general, to develop a nanobody, different steps are considered including Nanobody generation, identification, characterization, activity assay and finally formulation and manufacturing. Accordingly, to manufacture a Nanobody against SARS-CoV-2 in pulmonary dosage form (or any other kind of Nanobody and dosage form), it is necessary to first generate a Nanobody library (Immune, synthetic or naive libraries). In immune Nanobody libraries which are very routine, it is necessary to immunize a camel or camelids (Llama or Alpaca) by spike protein of the virus which has strong immunomodulatory properties. After a defined duration (typically 4 to 6 weeks), the blood is collected and lymphocytes are extracted. A phage display library is made and then the Nanobodies with the most affinity to the RBD of the SARS-CoV-2 spike protein will be selected. After assaying Nanobodies for neutralization of SARS-CoV-2 pseudovirus (by Plaque reduction neutralization test (PRNT) which is considered as the "gold standard" for detecting and measuring antibodies that can neutralize the viruses [66]), the next step would be large-scale production in a suitable expression system (bacteria, yeast or mammalian expression system) and downstream process including purification and pharmaceutical formulation as a pulmonary drug dosage form (Fig. 4).

6. Conclusion and suggestions

During the last 2 years after appearance of SARS-CoV-2 in China and Covid-19 pandemic, researches and pharmaceutical companies strongly tried to develop therapeutic and prophylactic candidates against this disease. Among different approaches it seems that VHH, Nanobodies with their promising and outstanding properties truly can be a "magic bullet" against SARS-CoV-2 and Covid-19. It can be suggested that Nanobodies not only can be used as neutralizing agents, but also Nanobodies with anti-inflammatory effects such as anti-IL-6R Nanobody® ALX-0061 which is developed by Ablynx, (Gent, Belgium) primarily for rheumatoid arthritis, probably can be used in Covid-19 with the aim of reducing pulmonary inflammation like Tocilizumab (Actemera®) which is an anti-IL-6R monoclonal antibody with extensive use in Covid-19. According to pathogenesis of Covid-19 and its effects on different body tissues like heart and gastrointestinal system, different Nanobodies with different route of administrations can be designed and produced to help people against this society annoying virus. It should be noted that computational design can also complement experimental Nanobody development to identify new epitopes according to the Nanobodies structural-conformational information [67].

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgment

Authors are grateful to Mrs. Dina Bahrololoumi for her support in graphics. This work was financially supported by Tehran University of Medical Sciences.

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