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Opinion

Inhalable neutralizing antibodies – promising approach to combating respiratory viral infections

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Monoclonal antibodies represent an exciting class of therapeutics against respiratory viral infections. Notwithstanding their specificity and affinity, the conventional parenteral administration is suboptimal in delivering antibodies for neutralizing activity in the airways due to the poor distribution of macromolecules to the respiratory tract. Inhaled therapy is a promising approach to overcome this hurdle in a noninvasive manner, while advances in antibody engineering have led to the development of unique antibody formats which exhibit properties desirable for inhalation. In this Opinion, we examine the major challenges surrounding the development of inhaled antibodies, identify knowledge gaps that need to be addressed and provide strategies from a drug delivery perspective to enhance the efficacy and safety of neutralizing antibodies against respiratory viral infections.

Highlights

Neutralizing monoclonal antibodies against respiratory viral infections are available; however, systemic delivery of antibodies to the airways is ineffective due to the poor transportation of macromolecules across the lung epithelium.

Inhalation is a noninvasive route that allows high concentration of antibodies to be applied directly to the airways for virus neutralization at the sites of infection.

Engineered antibody fragments are suitable for inhalation due to their smaller size and better stability, making them easier to be formulated as aerosols.

To be effective, inhaled neutralizing antibodies must be administered in the early stage of viral infection. While nebulizers can be reserved for inpatients, nasal and dry powder inhalers can be widely distributed to outpatients for early treatment or even prophylaxis in case of an emergency outbreak.

The need for a new strategy to combat respiratory viral infections

Respiratory viruses are frequently causing significant impact on morbidity and mortality globally, as evidenced by influenza epidemics [1] and the current COVID-19 pandemic [2]. Many respiratory viruses, including coronaviruses, influenza virus, and respiratory syncytial virus (RSV), are highly contagious and cause acute respiratory infections and severe disease in infected individuals. Newly emerging viruses are continuously being discovered and evolving into new variants, such as SARS-CoV-2 Omicron variant, resulting in increased transmissibility and drug resistance, and threatening future disease outbreaks [3]. Given the diversity of viruses, the rapid emergence of variants, and their widespread impact on public health, there is an urgent need for safe and effective vaccination and therapeutics. Although vaccines are available against influenza and COVID-19, their efficacies are modest at best as they aim at reducing severe disease and death rates rather than preventing infection, and offer limited protection against new variants [4,5]. Vulnerable populations such as elderly people and immunocompromised patients generally respond poorly to vaccines [4,6], and new therapeutics remain in demand.

Antibodies are proven to be an increasingly important class of therapeutics due to their invaluable advantages such as high specificity and high affinity [7]. They are already widely in use for the treatment of lung diseases such as lung cancer [8] and severe asthma [9]. In response to the COVID-19 pandemic, together with the advances in antibody engineering, the landscape of antibody therapeutics has rapidly revolutionized. However, the administration of antibody therapeutics is currently limited to the **parenteral route** (see Glossary) which present limitations including being invasive, posing increased risks of systemic toxicity such as **cytokine release syndrome** [10], and the requirement of specialized personnel for administration renders it difficult to treat large number of patients outside hospital setting. Moreover, many respiratory viruses that cause acute infection infect locally at the airway epithelium via apical shedding and propagation,

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which leads to manifestation of respiratory symptoms and disease transmission [11,12]. As such, delivery of antivirals through systemic administration is suboptimal in targeting viruses that primarily affect the respiratory tract. Conversely, direct drug administration to the respiratory tract via inhalation offers several advantages such as noninvasiveness, enhanced local effect, minimal systemic exposure, and ease of self-administration for outpatients [13].

We propose that inhalation therapy is a promising approach to improve efficacy and delivery of antibody therapies against respiratory viral infection. We first provide an overview of the protective roles of antibodies against respiratory infection, and highlight and discuss the limitations of recently approved antibody-based therapies against COVID-19 and RSV infection. Also, we present the basics of inhalation therapy (including orally and nasally inhaled) of biological molecules and discuss recent advances in the application of monoclonal antibody and engineered **antibody fragment**-based inhalation therapy for respiratory viruses in preclinical models of influenza, COVID-19, and RSV infection [14]. Finally, we provide insights and direction for the development of an optimal antibody formulation for the treatment and prevention of respiratory viral infections through inhalation. These approaches would allow us to be better equipped to tackle possible future pandemics.

Protective roles of antibodies against respiratory infection

Antibody belongs to the immunoglobulin superfamily, with IgG being the predominant isotype of antibody in research and clinical use [15]. IgG consists of two structural regions, namely the crystallizable fragment (Fc) that is responsible for generating immune response, and the antigen-binding fragment (Fab) that is responsible for antigen recognition. It has a molecular weight of ~150 kDa. Antibodies play a pivotal role in the immune system against infections. There are two major mechanisms of antibody-mediated protection, namely **effector functions** and **neutralization** (Figure 1, Key figure). The Fc portion of IgG participates in the antibody effector functions, including **antibody-dependent cell-mediated cytotoxicity (ADCC)**, **antibody-dependent cellular phagocytosis (ADCP)**, and **complement-dependent cytotoxicity (CDC)** [16]. The Fc domain also plays important roles in enhanced protein stability and extended serum half-life. Conversely, the Fab domain of IgG binds to the **epitope** on the viral surface, thereby inhibiting cellular entry and subsequent infection through neutralization. In addition to IgG, IgM and IgA are also critical defenses of the mucosal immunity. However, due to the lack of reliable recombinant production system at scale for the complex IgM and IgA which are notoriously unstable, IgG remains the focus of investigation as therapeutics against respiratory infections, with several products having already gained approval.

Approved neutralizing antibodies against respiratory viral infections

Prior to the COVID-19 pandemic, the sole approved antibody product indicated for a respiratory viral infection was palivizumab (Synagis). Palivizumab is a **monoclonal antibody** directed against the RSV fusion (F) protein that mediates the fusion of viral and host cell membranes. Administered intramuscularly once a month during the RSV season, palivizumab is indicated for the prevention of severe lower respiratory tract infection caused by RSV in infants with high risk of significant morbidity and mortality [17]. Nirsevimab is a new antibody that also targets the F protein of RSV but at a different binding site to palivizumab, with an Fc-modified region to extend the half-life of antibody. Nirsevimab is yet to be approved, but a clinical study showed that a single prophylactic dose via intramuscular injection reduced lower respiratory tract infections due to RSV in infants [18], although its effectiveness in immunocompromised adults is not known.

Since the COVID-19 pandemic, anti-SARS-CoV-2 antibodies have been rapidly developed. Two antibody cocktails, namely casirivimab/imdevimab [19], and bamlanivimab/etesevimab [20], were among the first antibody therapeutics that gained emergency use authorization by the US Food

Glossary

Aerodynamic diameter: the diameter of a sphere of density 1g/cm^3 that has the same settling velocity in still air as the particle of interest.
Antibody-dependent cell-mediated cytotoxicity (ADCC): an immune response mechanism whereby Fc-receptor-bearing effector cells recognize and kill target cells that have surface antigens complexed with antibody.
Antibody-dependent cellular phagocytosis (ADCP): an immune response mechanism whereby Fc-receptor-bearing phagocytes attack and devour target cells that are complexed with antibody.
Antibody-dependent enhancement (ADE): enhancement of viral infection by antibodies that facilitate viral entry into host cells.
Antibody fragments: a dissected part of an antibody that usually lacks the Fc domain.
Bronchoalveolar lavage (BAL): a procedure that involves instillation of sterile normal saline into the lung, followed by suction and collection of the instilled liquid for analysis.
Complement-dependent cytotoxicity (CDC): an immune response mechanism whereby the complement cascade is activated by antibodies that are bound to target cells, leading to membrane attack complex formation and cell lysis.
Cytokine release syndrome: a life-threatening acute systemic inflammatory response caused by elevated levels of circulating cytokines and is characterized by fever and multiple organ dysfunction.
Effector function: a mechanism of an antibody that involves the engagement with immune cells or complement components to bring about immune responses, including ADCC, ADCP, and CDC.
Epitope: the site on an antigen that specifically binds to the complementary antibody.
Humanization: modification of antibodies derived from nonhuman species to become similar to human antibodies with reduced immunogenicity and preserved efficacy in humans.
Monoclonal antibody: a type of antibody that binds to one specific antigen only.
Mucin: a high molecular weight, glycosylated protein that comprises the major component of mucus.

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Key figure

Mechanisms of action of antibodies against respiratory viral infections

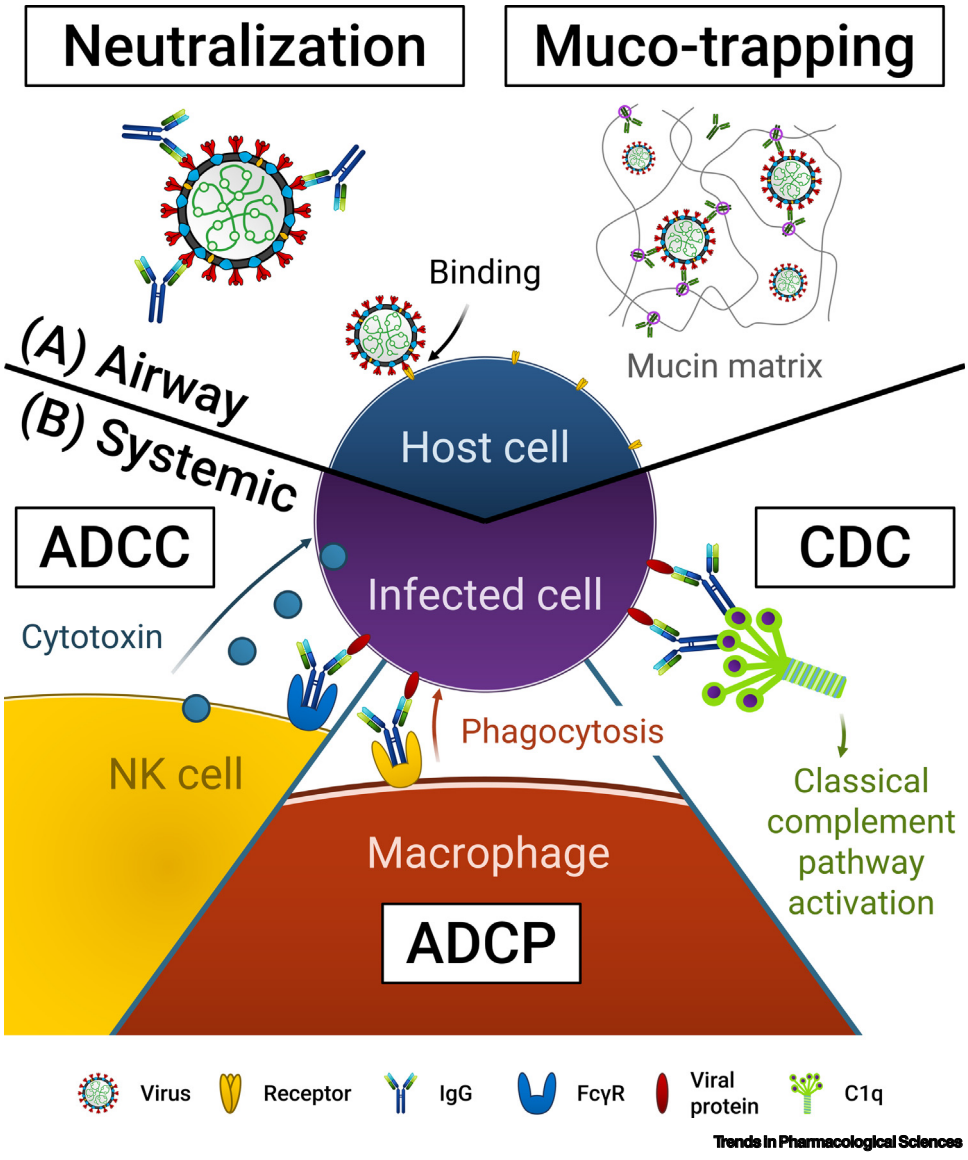

Figure 1. (A) In the airways, neutralizing antibodies bind to the invading viruses directly and prevent the viruses from entering the host cells through receptor binding. Alternatively, muco-trapping antibodies can bind to viruses by the Fab region, whereas the Fc domain of the antibodies crosslink with the mucin matrix (circled), effectively immobilizing the virus particles which will eventually be removed by mucus clearance. (B) As the viruses enter the systemic circulation, effector functions mediated by the Fc region of the circulating antibodies become more dominant. Antibodies conjugated to viral proteins expressed on the surface of infected cells can bind to Fc γ receptors (FcγRs) on different immune cells. Subsequently, effectors such as natural killer (NK) cells secrete cytotoxins, resulting in apoptosis of the nearby infected cells (antibody-dependent cell-mediated cytotoxicity, ADCC), while macrophages phagocytize antibody-conjugated viruses (immune complex) or infected cells directly (antibody-dependent cellular phagocytosis, ADCP). The classical complement pathway is also activated as the complement component 1q (C1q) binds to antibody-bound infected cells, which eventually leads to direct lysis or phagocytosis of the infected cells (complement-dependent cytotoxicity, CDC).

Mutational escape: as majority of RNA viruses replicate with random base mutations in the viral genome, viruses with mutated genes that evade immune cells are preferentially selected under immune selection pressure and result in reduced therapeutic efficacy.
Nanobody: also known as single-domain antibody, an engineered antibody fragment that consists of a single variable antibody domain.
Neutralization: a mechanism of an antibody used to prevent pathogen from infecting the cells, mainly by blocking the interaction between the pathogen and the host cells.
Parenteral route: the administration of drug via injection or infusion, including intravenous (into a vein), intramuscular (into a muscle) and subcutaneous (into fatty tissue under the skin) administration.
Pharmacodynamics: study of the biochemical, physiological, and molecular effects of drugs on the body.
Pharmacokinetics (PK): study of the movement of drugs through the body, describing the time course of the drug's absorption, distribution, metabolism, and excretion.
Receptor-binding domain (RBD): a key part of a virus that specifically binds to endogenous receptors on the host cells to gain entry and lead to infection.
Single-chain variable fragment (scFv): variable region of the heavy and light chain sequences of immunoglobulins are connected using a flexible peptide linker to produce small simple polypeptides with functional antigen-binding domains.

and Drug Administration (FDA) for postexposure prophylaxis of COVID-19 in 2020 and 2021, respectively. By combining two antibodies with nonoverlapping target sites in a single formulation, the cocktails were aimed to minimize **mutational escape**. Thereafter, other single or combined antibody therapeutics were approved for the prophylaxis and/or treatment of COVID-19 (Table 1), with many more still in clinical trials. All these injectable products contain monoclonal antibodies that are designed to bind to epitopes of the **receptor-binding domain (RBD)** of SARS-CoV-2 spike (S) protein, blocking viral entry into host cells [21,22]. Antibody therapeutics demonstrated effectiveness in preventing disease progression of COVID-19 when administered during the early stages of infection [19,23,24]. Unfortunately, due to the emergence of SARS-CoV-2 variants that exhibit multitude of mutations in the S protein, resistance has become a major setback for antibody therapy. For instance, the Omicron subvariants are highly resistant to most neutralizing antibodies that were approved prior to their emergence [25,26], raising concerns over the protective efficacy and sustainability of neutralizing antibodies. However, direct drug administration to the respiratory tract via inhalation offers several advantages such as noninvasiveness, enhanced local effect, minimal systemic exposure, and ease of self-administration for outpatients [13].

Basics of inhalation therapy

Although approved inhaled protein therapeutics are scarce, with recombinant human deoxyribonuclease I (rhDNase; Pulmozyme) and insulin (Exubera and Afrezza) as the only rare examples,

Table 1. Monoclonal antibodies approved against respiratory viral infection and their authorization status (as of November 30, 2022).

Antibodies	Route of administration	Indication	Year of initial approval	Current status
Palivizumab	IM injection	Prevention of serious lower respiratory infection caused by RSV in high-risk infants	FDA (Jun 1998); EMA (Aug 1999)	—
Bamlanivimab	IV infusion	Treatment of mild-to-moderate COVID-19	FDA (Sep 2020)[a]	No longer authorized in the USA (Apr 2021) due to the emergence of resistant variants
Bamlanivimab and etesevimab	IV infusion	Postexposure prevention of COVID-19 in people at high risk for disease progression	FDA (Feb 2021)[a]; EMA (Mar 2021)	No longer in use due to the emergence of resistant variants
Casirivimab and imdevimab	IV infusion or SC injection	Treatment of mild-to-moderate COVID-19	FDA (Nov 2020)[a], EMA (Nov 2021)	Their use is limited by the FDA (Jan 2022) due to the emergence of resistant variants
Tixagevimab and cilgavimab	IM injection	Pre-exposure prevention of COVID-19 in people with weak immunity	FDA (Dec 2021)[a]; EMA (Mar 2022)	—
Sotrovimab	IV infusion	Treatment of mild-to-moderate COVID-19	FDA (May 2021)[a]; EMA (May 2021)	Its use is limited by FDA (Mar 2022) due to the emergence of resistant variants
Regdanvimab	IV infusion	Treatment of COVID-19 in patients who are at risk of disease progression	EMA (Dec 2021)	—
Bebtelovimab	IV injection	Treatment of COVID-19 that retains activity against Omicron variant	FDA (Feb 2022)[a]	No longer authorized in the United States (November 2022) due to the emergence of resistant variants
Amubarvimab and romlusevimab	IV infusion	Treatment of mild COVID-19 [84]	NMPA (Dec 2021)	—

EMA, European Medicines Agency; FDA, US Food and Drug Administration; IM, intramuscular; IV, intravenous; NMPA, National Medical Products Administration of China; SC, subcutaneous.
[a]Emergency use authorization.

drug delivery to the lungs by oral inhalation is a safe and well-established route of administration. Inhalation of small molecules is common in the treatment of lung diseases such as asthma and chronic obstructive pulmonary disease (COPD) [27]. Since proteins are prone to degradation, formulation of antibodies as aerosols is comparatively more challenging [28]. To reach the lower respiratory tract, aerosols as liquid or dry powder need to exhibit an appropriate **aerodynamic diameter** in the range of 2–5 μm [29]. Dry powder inhalers (DPIs) and nebulizers are commonly investigated for delivering proteins to the lungs (Box 1). Both delivery methods require formulation strategies to ensure that the drug-containing aerosols can reach the target site within the respiratory tract while protecting the physically vulnerable biological molecules during formulation preparation and administration. For instance, production of dry powder involves techniques such as spray drying, spray freeze drying, and thin film freezing, the process of which would inevitably expose molecules to different kinds of atomization- or drying-induced stresses such as thermal, shear, and interfacial stresses [30,31]. Use of protective and stabilizing excipients (e.g., sugars, amino acids, and surfactants) and optimization of drying conditions are essential to minimize damage to the molecules [32]. Nonetheless, solid dosage form holds the key advantage

Box 1. Administration by inhalation – DPI, nebulizer, nasal device

A DPI is a handheld device that deliver drugs to the lungs in the form of dry powder aerosol. It is easy to use and highly portable, making it particularly useful and accessible for outpatients. The drug administration time is also short. Most DPIs are breath-activated, thus relying on the patient's inspiration effort to generate sufficient airflow for powder dispersion, rendering them challenging to operate for elderly and young patients, as well as those with compromised lung function [79]. Moreover, the intrinsic resistance of the inhaler device and inhalation flow rate also affect aerosolization and deposition of particles [79].

A nebulizer is a device that delivers drugs to the lungs through a mouthpiece or mask by producing liquid aerosol suitable for inhalation with tidal breathing; a process known as nebulization. A nebulizer generates liquid into aerosols of size range that can effectively reach the lung. Hence, it is suitable for critically ill patients and those with poor lung function [80]. However, the administration time could be lengthy. There are three main types of nebulizers used clinically: (i) jet nebulizers that use compressed air to generate aerosols; (ii) ultrasonic nebulizers that produce aerosols by generating ultrasonic waves directly into liquids; and (iii) vibrating mesh nebulizers that use ultrasonic frequency to vibrate a mesh and aerosols are produced when liquid passes through the vibrating mesh. While traditional nebulizers (e.g., jet nebulizers) are often bulky and are usually limited to hospitalized patients, newer battery-operated handheld nebulizers (e.g., vibrating mesh nebulizers) facilitate patient self-administration and thus minimize healthcare burden [81].

Intranasal administration has been frequently used to deliver drug for treating local conditions such as rhinitis, congestion, bacterial infection as well as for systemic absorption. It is also investigated for delivering drugs to the central nervous system through the olfactory and trigeminal pathways [82]. Recently, the intranasal route of administration has received significant attention for the delivery of antiviral agents as well as vaccines. Nasal spray is the most common dosage form for delivering drug into the nasal cavity. It requires a nasal atomizer to produce a mist of liquid aerosol for nasal deposition. In contrast, nasal powder has the advantage of prolonging drug residence time in the nasal mucosa, but the dispersion of dry powder aerosol for nasal application is more challenging than liquid formulation. The optimal particle size distribution of nasal deposition is substantially larger than that for lung deposition, ranging between 10 and 100 μm. Within the nasal cavity, the region of deposition depends on several factors, such as particle size, inhalation flow rate, and insertion depth and angle of the nasal device [83]. The advantages and disadvantages of different approaches of administration by inhalation and systemic administration are summarized in Table I.

Table I. Comparison between inhalation and systemic delivery of antibodies.

	Inhalation			Systemic
Approach	DPI	Nebulizer	Nasal device	Intravenous or intramuscular
Advantages	• Direct delivery to the lung for neutralization activity • Good stability, avoid cold-chain logistics • Portable, ease of self-administration for outpatients	• Direct delivery to the lung for neutralization activity • Suitable for patients with poor lung function	• Direct delivery to the nasal cavity for neutralization activity • Ease of self-administration for outpatients	• Well-established method of delivering antibody in humans • Allow effector functions (monoclonal antibody) in systemic circulation
Disadvantages	• Not suitable for the young, elderly and patients with poor lung function • Lack of approved excipients for formulation	• Bulky device and requires maintenance • Limited to inpatients • Poor stability in liquid, requires cold-chain logistics • Lack of approved excipients for formulation	• Limited studies on humans in delivering antibodies through the nasal route • Challenges such as removal of antibodies by mucus clearance	• Poor transportation to the respiratory tract for neutralization activity • Invasive • Require trained personnel for administration • Poor stability in liquid, requires cold-chain logistics

of better storage stability that would allow formulations to have an extended shelf-life and avoid cold-chain logistics. In contrast, nebulizing formulations are usually in solution or suspension that may promote hydrolysis and aggregation of proteins. Similar to drying, generation of liquid aerosol during nebulization also exposes the molecules to various kinds of stresses that may jeopardize the structural integrity of biological molecules, resulting in loss of activity [33]. Formulation and nebulizing parameters therefore need to be optimized in parallel to maintain protein stability [33,34].

While DPIs and nebulizers are designed to deliver medication to the lower respiratory tract, many viruses that cause acute respiratory infections also affect upper respiratory tract including the nasal cavity [35]. In the case of COVID-19, the angiotensin-converting enzyme (ACE)-2 receptors that are used by SARS-CoV-2 for cellular entry [36] are abundantly expressed in the nasal cavity. The nasal epithelium is not only the portal of entry but a main target of SARS-CoV-2. Hence, the nasal cavity also represents an important target site of antiviral drugs, in addition to the lung, rendering intranasal administration (Box 1) another important route to deliver antiviral agents to the upper airways.

Advances in antibody-based inhalation therapy for respiratory viruses

In clinical setting, antibody is almost exclusively administered through injection or infusion. Since infection of respiratory viruses typically starts from the apical side of the epithelium, sufficient level of neutralizing antibodies must be present in the airway lumina to exert their activity. **Pharmacokinetics** (PK) studies in mice revealed that when monoclonal antibodies were administered through the inhalation route (nebulization), the concentration of antibodies in the pulmonary epithelial lining fluid (ELF) was ~100-fold higher than that in serum [37]. In contrast, when antibodies were administered systemically (intravenous), the concentration in the ELF was ~50-fold lower than that in serum. Similarly, earlier PK studies in primates showed that following systemic administration of antibodies, only around 0.2% of the dose administered could be recovered in the **bronchoalveolar lavage (BAL)** fluid, suggesting poor transportation of macromolecules across the lung epithelium [38]. The mismatched delivery route could partly explain the lack of antibody therapeutics for the treatment of respiratory viral infection. By delivering antibodies directly into the respiratory tract, a high antibody level at the site of infection could be achieved. This potentially improves antibody efficacy and reduces the required dosage, thus lowering treatment costs, which is a prevailing consideration associated with biological therapies [39]. Antibody therapy against respiratory viral infections could be directly delivered by inhalation in the form of monoclonal antibodies or engineered antibody fragments.

Inhalation of monoclonal antibody

Advances in the delivery of therapeutic antibodies against influenza and COVID-19 support inhalation of monoclonal antibodies as a therapy against respiratory viral infections.

Influenza

A recent study investigated the antiviral efficacy of monoclonal antibody against influenza. Three antibodies that recognize the highly conserved epitopes located within the stalk of influenza haemagglutinin were combined with the aim of providing broad coverage against multiple influenza strains [40]. The combined antibodies, CF-404, were delivered via inhalation route (intranasal or nebulization) or systemic route (intraperitoneal or intravenous), and the two routes of administration were compared in mouse influenza models. The inhalation route was found to be substantially more effective in treating influenza caused by four different viral strains, with the inhalation route relying on the neutralization mechanism, whilst the systemic route was dependent on the effector function of the antibodies [40]. An earlier study in animals reported similar findings, showing that

direct administration (intranasal or nebulization) of monoclonal antibody 6F12 increased therapeutic efficacy against influenza compared to the systemic route (intraperitoneal or intravenous), and the dose to achieve prophylactic protection was reduced by tenfold [41]. These studies highlighted the importance of local delivery of antibody to maximize the neutralization effect against influenza.

COVID-19

Several studies have demonstrated the therapeutic efficacy of monoclonal antibody through inhalation in animal models of COVID-19 [42–44]. In one preclinical study, inhalation (nebulization) of 1212C2, a fully human monoclonal antibody derived from a COVID-19 patient, exhibited remarkable therapeutic efficacy in hamsters, and the inhalation route was more efficient than the systemic route (intraperitoneal) [42]. In another study that used neutralizing antibody identified in a phage display human antibody library approach, systemic (intravenous) and inhalation (intranasal) administration offered protection in a hamster model of COVID-19 [44]. As expected, the PK studies revealed that the intranasal route of administration increased the concentration of antibody in the BAL fluid, allowing more rapid local neutralization. Surprisingly, when the antibody was administered intranasally, the olfactory epithelial thickness increased in infected hamsters, prompting a suggestion that local neutralization action may either reduce inflammation or promote cellular repair in the olfactory system, a potential additional benefit of local route of antibody administration [44].

Muco-trapping is an interesting yet under-recognized mechanism of antibody against viral infection on mucosal surfaces (Figure 1) [45,46]. **Mucin** is the major component of mucus that covers the luminal surface of the respiratory tract. Viruses can usually diffuse freely within the mucin fibers. It has been reported that the Fc domain of IgG is capable of crosslinking with mucins, while the Fab domain can bind to the antigens of viruses with high specificity, thereby immobilizing the viruses to the mucin mesh and facilitating viral elimination by mucus clearance mechanisms, subsequently preventing viral entry at an early stage of infection [47]. IN-006, a reformulation of regdanvimab, which is an anti-SARS-CoV-2 neutralizing antibody, demonstrated muco-trapping properties in human airway mucus [47]. Delivery of IN-006 via nebulization in rats resulted in 100-fold higher concentrations in the lungs than in serum, with no loss of activity. A recent Phase 1 study showed that nebulization of IN-006 was well-tolerated at concentrations orders of magnitude above the inhibitory concentration in the respiratory tract [48]. Although still under investigation, this discovery has offered an exciting opportunity for novel inhaled antibody therapy against respiratory infections.

Engineered antibody fragment as an alternative strategy

Most of the work on neutralizing antibody against respiratory infections has been focused on monoclonal antibodies, partly due to their well-established use in the clinics. Cancer and autoimmune diseases are the two main applications of antibody therapeutics; both rely on the engagement of immune cells through the Fc region of the antibody to bring about the effector functions and hence therapeutic effects [49]. By contrast, the antiviral activity of antibodies relies on the Fab region to bind to the target viral protein, thereby preventing viral entry into the host cells through a neutralizing mechanism [40]. The role of Fc domain is less well-defined. Evidence shows that the enhancement of Fc function by antibody engineering could augment antiviral immune response or even produce vaccinal effect in influenza and COVID-19 animal models [50–52]. However, it may also pose the risk of **antibody-dependent enhancement (ADE)** of disease through the Fc receptor and complement component binding [53]; a phenomenon that has been reported in viral infections associated with coronaviruses [54,55]. Although no clinical case of ADE has been reported with monoclonal antibodies against SARS-CoV-2, the risk cannot be totally disregarded. The Fc domain also serves an important function in extending serum half-life when the antibody is intended for parenteral administration to minimize repeated dosing. However, this role is less

significant when antibody is delivered through inhalation; an approach that exploits the local neutralizing activity in the respiratory tract which renders the serum half-life less relevant. In fact, a recent study has demonstrated that even in the absence of Fc functions, the neutralizing potency of antibody against SARS-CoV-2 can be successfully maintained *in vivo* [56].

While the role of Fc domain against viral infection requires further investigation, engineered antibody fragments that lack the Fc domain but retain the targeting specificity of monoclonal antibodies have emerged as attractive candidates for inhaled therapy due to their favorable physicochemical characteristics for inhalation formulation [46,57]. Various kinds of engineered antibody fragments have been approved by FDA for use in diseases such as age-related macular degeneration and rheumatoid arthritis, and their safety in humans has been established [58]. To overcome the challenge of antibody resistance, it is critical to identify the more conserved epitopes across viral variants when designing neutralizing antibody. The smaller size of antibody fragment may facilitate their access to cryptic epitopes that may not be achievable by full length monoclonal antibody [59].

Nanobodies, also known as single-domain antibodies, belong to a type of antibody fragment that is frequently exploited to target respiratory viral infection, partly due to their small size, simple structure, low production cost, high solubility, and good stability as compared with full-length monoclonal antibodies [60]. These heavy-chain-only antigen binding fragments are typically derived from camelid species that make heavy-chain-only antibodies (Table 2). Nanobodies are versatile as they can exist as monomers or joined to form dimeric or multimeric structures. This

Table 2. Different types of antibodies against respiratory viral infection, their structural characteristics, mechanisms of action, and current stage of development for inhalation

Types of antibodies	Structure and MW	Characteristics and Mechanisms of action	Current stage of development	Refs
Monoclonal antibody (IgG)	 ~150 kDa	• Full-length antibody contains both Fc and Fab domains for immune response and viral neutralization activity, respectively • The Fc domain of muco-trapping antibody can crosslink with mucin and contributes to immobilization of viruses	• Approval for use in COVID-19 but limited to parenteral administration • Inhaled monoclonal antibodies against influenza virus and SARS-CoV-2 were evaluated in animal models • Inhaled muco-trapping antibody (IN-006) against SARS-CoV-2 was studied in clinical trial (Phase 1)	[40–44,48]
Nanobody	 ~15 kDa	• Single-domain antibody that contains heavy-chain only antigen binding fragment for viral neutralization activity • Typically derived from camelid species • May require humanization to avoid undesirable immunological responses	• Inhaled trimeric nanobody (ALX-0171) against RSV was studied in clinical trial (stopped after Phase 2 was completed) • Several inhaled nanobodies against SARS-CoV-2 are currently investigated in animal models	[63–68]
Single-chain variable fragment (scFv)	 ~27 kDa	• Contains variable regions of the light-chain and heavy-chain, joined with a peptide linker to allow antigen binding function for viral neutralization activity	• Inhaled scFv against SARS-CoV-2 are currently investigated in animal studies	[57]
Bispecific single-domain antibody	 ~27 kDa	• Contains two single-domain antibodies, each directs against different epitope for viral neutralization activity	• Inhaled bispecific single-domain antibody against SARS-CoV-2 are currently investigated in animal studies	[70]

feature makes nanobodies attractive for targeting viral proteins. Since common targets such as RSV F protein and SARS-CoV-2 S protein are trimeric in nature, multimeric nanobodies could dramatically enhance the neutralizing potency through avidity binding [60–62].

RSV

One example of inhaled nanobody involves the use of a trimeric nanobody, ALX-0171, that targets the F protein of RSV to inhibit viral entry [63]. Inhalation (nebulization and intranasal) of ALX-0171 in cotton rats was highly effective in reducing viral load when delivered prophylactically and therapeutically. The neutralization efficacy was superior to systemic (intramuscular) delivery of palivizumab; a clinically approved monoclonal antibody product against RSV. A Phase 1/2a clinical study showed that nebulized ALX-0171 was well tolerated and reduced nasal RSV viral titers in young children with RSV infection [64]. However, the results from a Phase 2b study were less promising as nebulized ALX-0171 did not improve clinical outcome once lower respiratory tract infection and inflammation were established [64], suggesting that early intervention of nanobodies is necessary for clinically meaningful antiviral effects.

COVID-19

A number of inhalable nanobodies targeting SARS-CoV-2 have also been developed. PiN-21 is a highly potent trimeric nanobody that binds to the RBD of the SARS-CoV-2 S protein [65]. Inhalation (nebulization or intranasal) of PiN-21 prevented and treated SARS-CoV-2 infection in hamsters. In another study, several trimeric nanobodies that targeted the RBD of the S protein were developed. The most potent candidate, C5 nanobody, prevented disease progression in hamsters when it was administered by inhalation (intranasal) or systemic route (intraperitoneal) [66]. Several other studies indicated that structural integrity and hence neutralizing potency of nanobody against SARS-CoV-2 can be retained successfully following aerosolization [67,68], demonstrating the robustness of nanobodies with favorable properties for inhalation.

While nanobodies against viral infection have attracted much interest, they are not without limitations. For instance, camelid-derived nanobodies may require **humanization** procedures to avoid unwanted immunological response, hindering their development as therapeutics [69]. With an attempt to overcome this safety concern and improve efficacy, other types of antibody fragments have also been investigated. **Single-chain variable fragments (scFvs)** are one example. A human scFv antibody 76clAbs, comprised of the variable regions of the light and heavy chains of an antibody (Table 2), was developed to target the S protein of SARS-CoV-2 [57]. Inhalation (intranasal) of 76clAbs counteracted infection caused by SARS-CoV-2 in mice and hamsters. Bispecific single-domain antibodies are another type of engineered antibody fragments that comprise two single-domain antibodies, each directed against different epitopes (Table 2). Bn03 is a human bispecific single-domain antibody based on two human single-domain antibodies and was developed to target two distinct conserved regions of the SARS-CoV-2 S protein simultaneously [70]. Inhalation (intranasal) of bn03 can effectively treat SARS-CoV-2-infected mice. These encouraging results suggest that scFv and bispecific single-domain antibodies are promising antibody fragment candidates for inhaled therapy.

Translation of inhaled neutralizing antibodies into clinical use

Inhalation of neutralizing antibodies is an effective approach to inhibit respiratory viral infections in animals. To further advance inhaled antibody therapy, there are several challenges to overcome that future research should address.

First, there is a lack of comprehensive understanding on the pulmonary PK and **pharmacodynamics** relationship of antibodies following inhalation, which eventually affects the efficacy of

treatment. Our current understanding is heavily based on animal experiments. Although transgenic mice expressing human neonatal Fc receptor and cynomolgus monkeys are useful in predicting PK of antibodies, their applications are focused on intravenous administration of monoclonal antibodies [71,72]. Unfortunately, given the substantial anatomical and physiological distinctions of the respiratory system between humans and animals, the shortage of relevant and reliable animal models often presents a major barrier for the translation of inhaled therapy (both orally and nasally inhaled) to clinical application. There are considerable difficulties in determining deposited dose in the human airways. Furthermore, since antibodies (full-length or fragments) are macromolecules, their absorption into the systemic circulation through the nasal cavity or lungs is expected to be ineffective, leading to a high level of local accumulation. It is important to examine any potential toxic and/or immunogenic reactions in the respiratory tract, especially for monoclonal antibodies. Although both the nasal cavity and lower airways are important sites for virus neutralization activity, it is not clear if antibodies have a preferential site of action, or if there is any difference in efficacy between these two sites. This is an area that remains to be investigated to develop an optimal formulation for the best clinical outcome. The concept of delivering antiviral drugs to the nasal and lower airways simultaneously through intranasal administration has been proposed [73], and this could be explored for neutralizing antibody therapy.

Second, despite the establishment of inhaled therapy for treating respiratory diseases other than lung infections, the development of inhaled antibody formulations remains a challenge due to the lack of approved excipients for pulmonary or intranasal delivery and limited regulatory guidance on inhaled biologics. Antibodies are susceptible to denaturation and aggregation during nebulization or dry powder production, which may not only lead to loss of activity but the denatured or aggregated forms of antibody may be immunogenic [74]. Excipients such as protein protectants and stabilizers are essential for promoting structural integrity and biological activities of antibodies [28]. When existing approved excipients are unsuitable, safety assessment of new excipients must be conducted in the early stage of formulation development. In terms of storage and transportation, DPI is preferable to nebulization due to better stability of antibodies in the solid state than in the liquid state. Nebulizers and DPIs target different patient populations, with the former reserved for inpatients and the latter aimed at outpatients for self-administration. The portable DPIs can be easily distributed to large population of outpatients during an outbreak for prophylaxis or treatment at the early stage of infection. Several studies have already demonstrated the feasibility of formulating antibody into inhalable dry powder using various particle engineering techniques [75–77]. Most of the preclinical work has been focused on nebulization; therefore, more effort should be put into developing dry powder aerosols of antibodies, including nasal powders, which have not been properly investigated. Early administration is critical for neutralizing antibody therapy. As reported in many studies, administration before the establishment of inflammation can dramatically enhance the efficacy of neutralization antibodies [14,24,78]. This means that neutralizing antibody treatment should be aimed at outpatients at an early stage of infection, for which DPIs and nasal powder formulations should be appropriate.

Lastly, rapid development of mutations that confer resistance after exposure to antibodies poses a major problem in antibody therapy against viral infection. Using combination of antibodies and targeting highly conserved epitopes are two key approaches to minimize mutational escape. We should take advantage of the smaller molecular weight and flexible structures of engineered antibody fragments that have a better chance of accessing multiple conserved epitopes simultaneously. With most clinically approved antibodies being monoclonal antibodies, more studies are needed to understand the efficacy and safety of antibody fragments in humans, as well as their scale-up production.

Concluding remarks

Inhaled neutralizing antibody therapy is a promising strategy in combating respiratory viral infections, as demonstrated in many animal studies. With the rapid emergence of antibody-resistant variants, the efficacy of neutralizing antibodies can be boosted by targeting multiple highly conserved epitopes of viruses to minimize mutational escape. Engineered antibody fragments such as nanobodies, bispecific antibodies, and scFvs have become the focus of attention as their small molecular weight allow them to have better access to cryptic epitopes of viruses. Administration at an early stage of viral infection is critical to the success of antibody therapy. To advance inhaled antibody therapy, we need to have a thorough understanding of the PK profile of antibodies following inhalation. Formulation development is essential to ensure that the integrity of antibody is sufficiently preserved for neutralizing activity while it is delivered in a safe and effective manner (see Outstanding questions).

Outstanding questions

What is the PK/pharmacodynamics relationship of different kinds of neutralizing antibodies following inhalation in humans?

What are the roles of the Fc domain of an antibody in viral infections, especially in the respiratory tract?

Are there any differences in the efficacy of neutralizing antibodies between the nasal cavity and the lower respiratory tract?

How do we improve the translation of inhaled antibody therapy from animal studies to clinical applications?

Are there sufficient choices of safe and effective excipients such as protective agents and stabilizers for the development of stable inhaled formulations of antibodies?

What are the potential toxic and immunogenic risks of neutralizing antibodies when they are localized at a high concentration in the airways?

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Declaration of interests

The authors have no interests to declare.

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