Protein Antibodies in Respiratory Syncytial Infection

Subjects: Virology

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Respiratory syncytial virus infection (RSVI) is an acute medical and social problem in many countries globally. Infection is most dangerous for infants under one year old and the elderly. Despite its epidemiological relevance, only two drugs are registered for clinical use against RSVI: ribavirin (approved in a limited number of countries due to side effects) and palivizumab (Synagis), which is intended only for the prevention, but not the treatment, of infection. Various research are searching for new drugs against RSV, with three main areas of research: small molecules, polymeric drugs (proteins and peptides), and plant extracts.

Keywords: respiratory syncytial virus ; antivirals ; protein

1. Introduction

Human respiratory syncytial virus (RSV, hRSV) is one of the most common viral pathogens affecting the respiratory system. This virus is a member of the *Orthopneumovirus* genus (family *Pneumoviridae*) and contains a single-stranded nonsegmented negative-sense RNA genome. There are 10 open reading frames (ORFs) in its genome, which encode 11 proteins. They are: one membrane protein (M1), two non-structural proteins (NS1 and NS2), two nucleocapsid proteins (N and P), three surface proteins coating the virion—fusion (F), small hydrophobic (SH) and attachment (G), and an M2 gene which contains two ORFs, resulting in the production of M2.1 and M2.2, and large (L) protein [1].

F and G proteins are two major immunogenic proteins. The fusion protein, F, can exist in a prefusion form (pre-F) that undergoes a conformational change to post-F after the membrane fusion. This protein is the target of most neutralizing antibodies and, thus, is used for the most monoclonal antibody preparations and vaccine development [2].

The attachment glycoprotein, G, also plays important roles in RSV infection and in the damage of host immunity. It is less immunogenic than the F protein, eliciting approximately 2 to 10% of human serum neutralizing antibodies [3]. However, the G protein contains a central conserved domain, or nonglycosylated region, that is nearly invariant across different strains of RSV, which is the target of broadly neutralizing antibodies [4].

Due to the variety and severity of clinical manifestations in young children, RSV infection remains a significant medical and social problem today [5][6][7]. In infants and children in the first years of life, RSV infection can cause severe bronchiolitis or even death. Vulnerable groups in relation to RSV are, also, people over 65 years of age, in whom the infection leads to increased hospitalization and mortality, and patients with weakened immunity. The risk of serious illness in adults is increased by the presence of chronic respiratory diseases or circulatory disorders and is associated with a higher viral load.

In children under five years of age, RSV causes more than 30 million cases of acute lower respiratory tract infection per year; 3.2 million of them are severe and require hospitalization [8]. Among children hospitalized with RSV infection, mortality averages 1%, but reaches 37% with a burdened history [9]. In children under three years of age, RSV is the cause of 50–90% of bronchiolitis, 5–40% of pneumonia, and 10–30% of tracheobronchitis. A complicated clinical course is most often recorded in children aged 6 weeks to 9 months [5][10][11]. In 2015, there were about 1.5 million episodes of RSV in older people in industrialized countries (data for developing countries were not available). Of these, approximately 14.5% (214,000) were hospitalized [12].

In 2020, in the context of quarantine and preventive measures aimed at preventing the spread of COVID-19, there were changes in RSV outbreak seasonality. In particular, there was a decrease in the incidence of RSV, as well as other seasonal respiratory viruses in China [13] and Australia [14]. Nevertheless, a 2020 meta-analysis of thirty studies, including 3834 patients, showed that RSV continued to circulate in parallel with SARS-CoV-2 and could join as a concomitant infection in some hospitalized patients. The researchers noted the presence of RSV/bacterial coinfections (7%) and RSV/viral coinfections (3%), with RSV/influenza A representing the most common viral coinfection [15].

Quarantine measures have led to a lack of RSV immunity in children born in 2020–2021. This is associated with a surge in RSV infection in the United States [16] and Japan [17] in July 2021. Data for other countries is not yet widely available, but a survey carried out by the institute (data not yet published) shows the same trend. Thus, due to the widespread prevalence of infection and the possibility of severe consequences, the question of finding a means of prevention and antiviral therapy for RSV infection remains relevant and is currently under active study.

2. Monoclonal Antibodies

Apart from vaccines, the most promising group of compounds for the prevention of respiratory syncytial infection are monoclonal antibodies. When administered to a patient according to a specific regime, they remain in circulation.

Currently, the clinicaltrials.gov website has registered a total of more than 300 studies on the subject of RSV infection [18]. According to PATH (RSV Clinical Trial Tracker) [19], 119 studies of protein compounds have currently been registered, the overwhelming majority of which relate to anti-RSV vaccines. Monoclonal antibodies account for 11 studies (2014–2020) devoted to anti-F mAbs: MK-1654 (Merck, Darmstadt, Germany), REGN2222 (Regeneron Pharmaceuticals, Tarrytown, NY, USA), and MEDI8897 (AstraZeneca, Cambridge, UK/Sanofi Pasteur Paris, France collaboration). Presently, another four mAb preparations are in the preclinical study stage [20]: three anti-F mAbs (Aridis Pharmaceuticals, Los Gatos, USA, Gates MRI, USA, and mAbxience/UCAB, Madrid, Spane collaboration) and one anti-N mAb (Pontificia Universidad Católica de Chile).

The only licensed preparation for this purpose to date is palivizumab (Sinagis), a humanized IgG_{1k} monoclonal antibody that interacts with the A epitope of the fusion protein (F) antigen. The palivizumab molecule consists of human (95%) and mouse (5%) sequences. The preparation was first approved for use in the United States in 1998 [20].

The administration regime consists of five intramuscular injections carried out at one-month intervals during the seasonal rise in RSV incidence (October/November to March). Use of the preparation is very expensive, and its effectiveness has been proven only in a preventive context. Palivizumab exhibits a pronounced neutralizing and inhibitory effect on fusion proteins of subtype A and B RSV strains. Prophylactic administration of palivizumab to newborns during the epidemic season led to a significant decrease in the frequency of hospitalization of patients with RSV infection [21]. However, due to the very high cost [22] of treatment ($20,000 per child over 4–5 doses during the epidemic season) and limited efficacy in children born after 29 weeks of gestation, palivizumab was discontinued in children in the group [23][24][25].

Evidence suggests that palivizumab has some therapeutic effects. Safety and efficacy results for palivizumab, summarized in a review by Canadian researchers, [26] suggest that it is promising for further clinical trials as a therapy. The data combined by the researchers indicate that 3 out of 25 patients with upper respiratory tract infections, and 5 out of 88 patients with lower respiratory tract infections, who received palivizumab died from RSV. The required palivizumab concentrations were maintained for at least three weeks after intravenous injection at a dose of 15 mg/kg. The use of palivizumab led to a significant decrease in the RSV content in tracheal secretions (but not in nasal lavage) on days one and two of therapy [27].

There have been many attempts to create a more effective, longer acting, and less expensive analogue of palivizumab. A number of candidate mAb studies, which have not yet reached the stage of clinical trials, have been published. Binding to different F protein epitopes has been shown to be effective against RSV in vitro and/or in vivo. Palivizumab (MEDI-493) appeared in the process of searching for solutions to increase the effectiveness of the RSV-IGIV, a polyclonal human Ab preparation for intravenous infusion. Felvizumab (RSHZ19), also a humanized mAb, was developed for the same purpose [28].

A comparative study showed that palivizumab significantly reduced the number of hospitalizations with a diagnosis of RSV, while felvizumab did not show significant efficacy [25]. To determine if different clinical results were associated with differences in biological activity, additional comparative studies in vitro and in vivo were carried out. Palivizumab was four- to five-fold more effective than felvizumab in binding antigens, neutralizing RSV, and inhibiting fusion. Although both preparations were effective against RSV subtypes A and B in the cotton rat model, two- to four-fold higher doses of felvizumab were required for similar protection. Felvizumab (RSHZ19) studies appear to have been discontinued at this time [28].

Motavizumab (Numax, MEDI-524), a humanized second-generation mAb from AstraZeneca, has become one of the most promising developments in the F protein binding mAb group [29]. Motavizumab differs from palivizumab by 13 amino acid residues, and it also targets the viral F protein. The preparation showed good results in an experiment with BALB/c mice intranasally infected with RSV A2. Palivizumab or motavizumab was administered once: 24 h before or 48 h after RSV

inoculation. Regardless of the time of administration, viral loads in bronchoalveolar lavage samples were significantly reduced. The amount of virus in the lungs of mice on days 5 and 28 after infection was significantly reduced only when motavizumab was used one day before the introduction of the virus. The same scheme showed the best results when analyzing the histopathological portrait in the lungs; airway obstruction and post-methacholine airway hyperresponsiveness were significantly reduced in mice in the group compared with the other groups and controls. Motavizumab was superior to palivizumab in reducing viral replication as measured by inflammatory and clinical markers of illness severity and its effect on long-term pulmonary abnormalities [30].

More than ten motavizumab clinical trials have been conducted, including phases I to III [31], with quite promising results. It was well tolerated, did not cause significant side effects, and was similar in pharmacokinetics to palivizumab (five doses). It reduced the number of RSV hospitalizations by 83% compared with the placebo and 26% compared with palivizumab [32]. It also reduced the incidence of lower respiratory tract secondary infections in outpatients. However, the FDA finally refused to register motavizumab due to a lack of advantages over palivizumab [33], as well as due to the discovered side effects in the form of allergic reactions [34][35].

Palivizumab and motavizumab recognize an F protein epitope that is unchanged during the fusion process; they do not prevent its initial conformational changes. Further searching for effective mAbs was carried out towards the development of those capable of blocking the F protein before the fusion stage. Antibodies that can lock F protein conformation into its prefusion state have increased efficiency and longer serum half-lives [22].

These compounds include nirsevimab (MEDI-8897, AstraZeneca, Cambridge, UK), which recognizes the antigenic site Ø [36]. Due to the described advantages, a single MEDI8897 injection is sufficient for protection during the epidemic season, as opposed to four or five needed with previous generation preparations. In healthy, premature infants, a single nirsevimab injection contributed to a decrease in the number of hospitalizations with RSV, compared with the placebo, during the entire epidemic season [37]. Specifically, 70.1% fewer cases of RSV-associated lower respiratory tract infection were detected with nirsevimab prophylaxis than with the placebo: 2.6% (25 infants) versus 9.5% (46 infants) ($p < 0.001$). The hospitalization rate was 78.4% lower: 0.8% (8 infants) versus 4.1% (20 infants) ($p < 0.001$). These differences were consistent over the 150-day post-dose period across geographic regions and across RSV subtypes. RSV-neutralizing activity and the ability to maintain protection against RSV during the 5-month epidemic season, after a single intramuscular injection, were shown [38]. Thus, the preparation was safe and could protect healthy, premature babies from RSV. AstraZeneca is currently in phase III clinical trials with nirsevimab [39].

MK-1654 (Merck, Darmstadt, Germany), another mAb targeting F protein, has shown pronounced efficacy in the cotton rat RSV model. Prophylactic administration showed strong, dose-dependent antiviral activity (RSV-A, RSV-B) in the lungs and nasal passages (material taken 4 days after infection). Lung EC_{50} values were 1.1 µg/mL and 1.9 µg/mL for RSV-A and RSV-B. In the upper respiratory tract, these values were 9.9 µg/mL and 8.5 µg/mL, respectively. The first phase of clinical trials showed that the overall safety profile of MK-1654 was similar to that of the placebo, and there were no side effects [40]. In 2019, it was shown to be ineffective in a clinical trial [41].

Another preparation, suptavumab (REGN-2222, Regeneron Pharmaceuticals NY, USA), specific for antigenic site V, successfully reached phase III clinical trials, but did not pass due to insufficient efficacy in healthy, premature infants [42]. In the last decade, researchers' attention has switched to mAbs directed at the G protein. Several experimental studies have been carried out on the G protein's roles in viral penetration, virus neutralization, and RSV-mediated pathology. In an experiment in mice, data were obtained using mouse mAb 131-2G (a reagent for enzyme immunoassays sold by such manufacturers as Merck) against the G protein. In RSV-infected mice, it caused reductions in weight loss, the number of cells in bronchoalveolar lavage, reactivity of respiratory pathways, and Th2 cytokine production. These effects were faster than palivizumab therapy [43].

The prophylactic and therapeutic administration of two mAbs specific to the G protein, 2B11 and 3D3, have also been studied in a model of RSV infection in BALB/c mice. Both anti-G mAbs reduced viral load, leukocyte infiltration, IFN-γ expression, and IL-4 expression in cell-free supernatants in bronchoalveolar lavage. This makes such compounds promising candidates for the prevention and treatment of RSV [44].

3. Nanoantibodies

Preparations developed based on nanoantibodies can be distinguished into a separate subgroup. Nanoantibodies are isolated variable domains of heavy-chain antibodies (aka VHH "single-domain antibodies") that can function in the absence of other domains [45]. Such antibodies have a number of advantages over classical mAbs, including: their small

size (15 kDa), which facilitates penetration into cells and tissues; flexibility of hypervariable regions, which facilitates access to previously inaccessible epitopes; high solubility; thermal stability; resistance to extreme pH; easy additional modification to enhance therapeutic and diagnostic potential; and cost-effective production in bacterial systems. Thanks to these features, nanoantibodies have found application in the development of anti-RSV preparations. Currently, all these studies are still at the stage of preclinical trials.

Thus, ALX-0171, a trimeric nanoantibody that binds the F protein antigenic site II, was more active in neutralizing RSV in vitro than palivizumab. It was shown to completely block replication below the detection limit for 87% of the viruses tested, while palivizumab blocked replication for 18% of the viruses at a fixed concentration. ALX-0171 also caused a significant decrease in RSV titers, in both the nose and lungs, when administered prophylactically or therapeutically directly into the lungs of cotton rats. The researchers believe that ALX-0171 has significant potential for the treatment of RSV-mediated illnesses [46].

However, a 2018 phase IIb, double-blind, randomized, placebo-controlled study of inhaled ALX-0171 showed different results [47]. The use of ALX-0171 did not affect infection outcome, compared with the placebo, in children hospitalized with RSV lower respiratory tract infection. Picomolar concentrations of another nanobody-based preparation, F-VHHb, protected BALB/c mice from RSV infection and associated pneumonia. Therapeutic administration of these nanobodies after RSV infection reduced viral replication and decreased viral pneumonia [48].

Thus, to date, the search for passive immunoprophylaxis continues with a focus on the synthesis of various types of mAbs or nanoantibodies specific to various viral proteins (mainly F and G). However, none of the candidates have yet been licensed. The closest to this stage is nirsevimab. However, evidence of the presence of escape mutations that allow the virus to escape from therapeutic agents targeting the F or G proteins [49][50][51][52], and the lack of effective yet inexpensive preparations, dictate the need to search for fundamentally new candidate agents for the therapy and prevention of RSV infection.

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