Prevention of Respiratory Syncytial Virus Infection: From Vaccine to Antibody

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ABSTRACT Respiratory syncytial virus (RSV) is the leading cause of lower respiratory tract disease in infants and young children. Initial efforts to develop a vaccine to prevent RSV lower respiratory tract disease in children were halted because of serious adverse events that occurred when children were infected with RSV following vaccination, including vaccine-related deaths. Subsequently, a major focus for researchers was to understand what led to these adverse events. Investment in a vaccine for RSV continues, and new strategies are under development. Success to prevent RSV disease was met by the development of immunoprophylaxis, first with intravenous immunoglobulin and then with recombinant monoclonal antibody. The story of immunoprophylaxis for RSV includes the first-in-class use of antibody technology for infectious disease, and palivizumab currently remains the only way to prevent serious lower respiratory tract disease due to RSV infection.

RESPIRATORY SYNCYTIAL VIRUS

Respiratory syncytial virus (RSV) poses a serious and significant health problem. RSV was discovered in 1956 and quickly became recognized as the leading cause of lower respiratory tract disease in infants and young children (1, 2). Preterm infants and young children with bronchopulmonary dysplasia (BPD) or congenital heart disease (CHD) are at high risk of serious RSV infection and may require hospitalizations and stays in the pediatric intensive care unit (3, 4). Although these high-risk groups experience an increased incidence of RSV disease, it is important to note that the majority of infants hospitalized for RSV are previously healthy, nonpremature children (5). In children less than 5 years of age, RSV infections account for 50 to 80% of winter bronchiolitis hospitalizations and 30 to 60% of pneumonia hospitalizations (6, 7). RSV bronchiolitis is reported as the leading cause of hospitalization for infants less than 12 months of age (8, 9, 10, 11). Hospitalization for RSV can reach rates of 1 to 20 per 1,000 infants less than 1 year of age in developed countries (12). Although not common, RSV can be fatal, as 140 to 500 infant deaths are attributed to RSV each year in the United States (13, 14). In addition to infants and young children, another risk group for RSV disease is the elderly. In fact, while mortality in children due to RSV disease has decreased over the years, mortality due to RSV disease among the elderly is still a significant problem (15, 16, 17, 18, 19, 20). Also, immunosuppressed leukemia patients or patients receiving stem cell transplant therapy experience as much as 80 to 100% mortality upon RSV infection and are therefore a high-risk group for RSV disease (21).
RSV is highly infectious and thus highly prevalent; however, RSV disease does not occur in most cases of infection. Two-thirds of infants are seropositive for RSV by their first birthday, and nearly all children have been infected by their second birthday (22). Outbreaks of RSV are seasonal, occurring during the colder months of the year and varying from year to year in peak months. In certain settings during seasonal RSV outbreaks, such as daycare centers, the attack rate of RSV approaches 100% (23). In addition, RSV is considered a major nosocomial problem for already hospitalized infants (24). Reinfection is quite common throughout life; however, it is typically less severe than the primary infection (22, 23, 25, 26). It is reported that reinfection can occur even with a highly similar virus strain (26, 27), which raises questions about the adaptive immunological response to RSV and about RSV seasonality in that acquisition of herd immunity typically slows the rate of infection until a new strain emerges and starts the next seasonal outbreak, as is observed for influenza. However, the two subtypes of RSV tend to alternate in circulation by 1- to 2-year intervals, which suggests that there is at least some lasting herd immunity (28, 29, 30, 31).

Respiratory syncytial virus is classified as part of the Pneumovirinae subfamily of the Paramyxoviridae family of viruses. RSV is an enveloped virus with a single-stranded, nonsegmented, and negative sense RNA genome. By electron microscopy, RSV is a pleomorphic virus that adopts both spheroid and long filamentous structures (32, 33, 34, 35). These spheroid and filamentous structures are decorated with three envelope glycoproteins including G (glycosylated) protein, F (fusion) protein, and SH (small hydrophobic) protein. There is one serotype of RSV and two antigenic subtypes designated as A and B. The sequence diversity of the G protein defines the A and B subtypes, with 53% sequence identity between A and B subtypes; whereas, F-protein sequence identity between subtypes is as high as 89% (36). The G protein and F protein are the major targets for antibody-mediated virus neutralization.

The first steps of virus replication include attachment to the target cell followed by viral and cellular membrane fusion. The G protein mediates the majority of the attachment process to target cells, and the F protein mediates the process of fusion. Recombinant viruses with ablated G-protein and SH-protein expression are still infectious in vitro (in some cell lines), suggesting that the F protein alone can mediate both attachment and fusion; however, viruses with ablated G protein are highly attenuated in vivo (37). The contribution of the SH protein to virus replication is unclear. Upon fusion, the ribonucleoprotein (RNP) complex, consisting of the viral genome coated by the viral N (nucleocapsid) protein along with the P (phosphoprotein) protein, L (RNA-dependent RNA polymerase) protein, and M2-1 protein (transcription factor), are released into the cytoplasm where viral genome replication, transcription, and translation occur (36). Viral genomes and proteins associate at the cell surface to form new virus particles that bud from the plasma membrane (36). In vitro, the F protein can mediate cell-to-cell fusion, called syncytium formation, which is the major cytopathic effect of RSV and can be easily observed by light microscopy. However, the observation of syncytium formation in vivo is minimal and does not appear to play a major role in viral spread. A general RSV infection process of epithelial cells is shown in Fig. 1.

The pathogenesis of RSV begins with infection of the epithelial layer of the upper respiratory tract, resulting in rhinorrhea, possibly fever and otitis media 4 to 5 days later, which generally lasts 4 to 6 days (38). Viral shedding typically lasts 3 to 8 days, but can be longer in immune-compromised individuals (38). In 25 to 40% of infections, the lower respiratory tract becomes infected, which can result in bronchiolitis or pneumonia (22). RSV infection causes necrosis of the epithelial layer of the respiratory tract, infiltration of leukocytes, edema, and excessive mucous, all of which lead to bronchial obstruction (39, 40). To make matters worse, RSV infection impairs cilia function leading to a reduced ability to clear the necrotic debris (41). Infant bronchioles have a more restricted airway resistance, resulting in greater difficulty dealing with the debris and mucous brought on by an RSV infection of the lower respiratory tract. This might explain, at least in part, why prematurity and young age are risk factors for RSV disease (42). The abnormalities in pulmonary function from RSV disease can persist for 10 years and longer (43), although it is unclear if the patients with persistent pulmonary dysfunction had an underlying condition that predisposed them to RSV disease.

The standard of care or treatment of RSV disease is an evolving paradigm. One goal in treatment is to reduce viral load. To this end, ribavirin, a nonspecific antiviral was used historically as a treatment for RSV. However, ribavirin is no longer recommended since it does not appear to provide a clinical benefit (44, American Academy of Pediatrics). Another approach to treating RSV infection is to treat the pathological results of RSV, particularly inflammation. However, treatment with corticosteroids alone has proven to be ineffective.
Because of the clinical similarity between RSV disease and asthma, bronchial dilators were explored as a treatment strategy for RSV infection, but were not efficacious (46). One success story in the treatment of RSV disease is the use of cysteinyl leukotrienes that effectively treat persistent wheezing that can follow bronchiolitis (47).

RSV is unique in its ability to cause reinfections in persons of all ages (22, 23, 25, 26, 27). This observation has led to the hypothesis that RSV infection does not induce a durable immune response. There is evidence that supports the role of both cell-mediated and humoral immunity in clearing an RSV infection. In terms of cell-mediated immunity, most RSV proteins will stimulate a cytotoxic T lymphocyte (CTL) response in seropositive individuals (48). The observation that RSV tetramer+CD8+ cells are reduced in the elderly population (49), a high-risk group for RSV disease, and that these cells do not have a robust proliferation response in RSV recall assays in comparison with young patients, suggests the importance of cell-mediated immunity in clearing RSV (50). The role of humoral immunity is supported by evidence from numerous studies that show antibodies restrict RSV replication and aid faster clearance by cell-mediated immunity (reviewed in reference 36). One supportive example of this is the observation

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**FIGURE 1** The illustration depicts the mechanism of action of RSV-neutralizing mAbs, palivizumab and motavizumab. An RSV virion is illustrated at the top left corner showing that the F and G proteins are located on the surface of the virion and the drawing below depicts G-protein-mediated attachment to cells followed by F protein mediated virus-cell fusion. The steps of fusion are shown on the top right. F protein also mediates cell-to-cell fusion resulting in syncytium formation, depicted in the middle drawing. In the final drawing on the bottom, an RSV-neutralizing antibody, either palivizumab or motavizumab, binds to F protein and blocks virus replication. It was determined that palivizumab and motavizumab do not inhibit RSV attachment, but rather F-protein-mediated virus-cell fusion and syncytia formation as reported recently (99). doi:10.1128/microbiolspec.AID-0014-2014.f1
that maternal antibodies acquired via transplacental passive immunization provide a protective role for neonates (12). Another example, as discussed in detail later in this article, is that high-titer anti-RSV intravenous immunoglobulin (IVIG) and anti-RSV monoclonal antibody administration provides sterilizing immunity to RSV. Finally, there is a direct correlation between the level of neutralizing antibody in the serum and reduced risk and severity of RSV disease (25, 26, 51, 52, 53, 54, 55, 56, 57, 58).

RSV VACCINE

Despite advances in understanding the immunological correlates to protection, a vaccine for RSV is not available. RSV vaccine efforts were greatly stifled by the use of a formalin-inactivated RSV vaccine that resulted in a greater number of vaccinees hospitalized upon infection with RSV in comparison with the placebo control group (80% versus 5%, respectively) due to serious pulmonary dysfunction, and this resulted in some fatalities (59). Upon RSV infection, vaccinees experienced exaggerated lymphocyte proliferation that resulted in a high Th2 response, which is speculated to have contributed to the high degree of lung inflammation (59). The formalin-inactivated RSV vaccine results were recapitulated in animal models with vaccines consisting of recombinant viral proteins (60, 61, 62), which may guide current vaccine development. For instance, the use of recombinant RSV viral protein vaccines might be safer for the elderly population to prevent reinfection, but may not be preferred for the pediatric population to prevent primary RSV infection out of concern for provoking a high Th2 response. Strategies for pediatric vaccine include attenuated RSV or virus vectors expressing RSV antigens. Attenuated virus vaccines will need to be sufficiently attenuated to greatly minimize the chance of reversion to wild-type virus. Virus vectored antigens typically possess low immunogenicity and will need to be engineered to improve the ability to provoke cell-mediated immune responses. All pediatric vaccine strategies will need to address the challenges of immunological immaturity and maternal antibodies that hamper vaccine efforts (63, 64, 65, 66). Further understanding of the correlates of protection against RSV infection and the correlates of risk will greatly benefit vaccine development efforts.

Since the identification of RSV, the need for a vaccine has long been recognized (1). However, the efforts to develop a safe and effective vaccine against RSV have been met with great challenges. This has steered researchers to seek alternative approaches. Early evidence suggested that passive immunization with antibodies may be a viable alternative for the prophylaxis of RSV infection. It was found that the severity of RSV-induced pneumonia was inversely related to the titer of maternal neutralizing antibody in infants (53). Moreover, the level of serum IgG to RSV F (fusion) protein has correlated with the protective effect against RSV reinfection and illness severity (51). However, there was controversial view about antibodies in the early days, since vaccine results seemed to imply that serum antibodies confer little protection and might even exacerbate disease (67). In addition, the incidence of RSV-induced bronchiolitis peaks in infants between 1 and 6 months of age when maternal antibodies are still present (68). Further studies in animal models using monoclonal or polyclonal antibodies have helped delineate the protection role of antibodies and enhanced confidence in the antibody-mediated prophylaxis approach. Two research teams showed that animals (mice and cotton rats) are protected against lung infection by the administration of mouse anti-RSV monoclonal antibodies prior to RSV challenge (69, 70). In addition, it was found that human convalescent antiserum to RSV administered intraperitoneally to cotton rats provided near-complete pulmonary protection upon RSV challenge (71). Furthermore, human IVIG was shown to reduce RSV replication in the lungs of the RSV-infected monkeys (72). Evidence of enhanced pathology due to the administration of anti-RSV antibodies in the presence of RSV was not observed in any of these animal studies, which is in contrast to the results of the formalin-inactivated vaccine administered to humans.

IMMUNOPROPHYLAXIS OF RSV INFECTION: FROM DISCOVERY TO MARKET APPROVAL

Intravenous Immunoglobulin

Since IVIG was shown to protect animals against RSV infection (71, 72), it seemed likely that passive immunization with human IVIG would prevent RSV infection in high-risk children. Human clinical trials were conducted to test this hypothesis (73, 74). It was concluded that monthly infusions of standard immune globulin containing RSV-neutralizing antibodies could be safely administered to high-risk pediatric patients. There was a trend toward less severe RSV illness in IVIG-treated patients compared with the control group, as measured by the length of hospitalization. However, standard IVIG lacked sufficient RSV-neutralizing antibody titer.
to confer full protection against severe RSV illness (74). To improve on efficacy, an effective screening assay to identify plasma-yielding immunoglobulin with high RSV-neutralizing and animal-protective activities was developed (75). Among seven assays tested to identify such RSV antibody activity, a microneutralization assay was found to be most useful. Microneutralization-screened IVIG, so called RSV-IVIG, has 5-fold better activity to neutralize RSV than standard IVIG.

Two major clinical trials were conducted using RSV-IVIG (76, 77). The results from these randomized, controlled trials were very promising; they demonstrated the safety and efficacy of RSV-IVIG in preventing RSV infection in pediatric patients. In the National Institute of Allergy and Infectious Diseases (NIAID) study, 249 children with prematurity, BPD, or CHD were enrolled in the randomized, controlled trials were very promising; they demonstrated the safety and efficacy of RSV-IVIG in preventing RSV infection in pediatric patients. In the National Institute of Allergy and Infectious Diseases (NIAID) study, 249 children with prematurity, BPD, or CHD were enrolled in the National Institute of Allergy and Infectious Diseases (NIAID) study, 249 children with prematurity, BPD, or CHD were enrolled. Monthly infusions at 750 mg/kg resulted in a 63% reduction in RSV hospitalizations and a 97% reduction in the number of days in the intensive care unit. The adverse events were generally mild. The PREVENT study sponsored by MedImmune, Inc. (Gaithersburg, MD) was a larger trial, which enrolled 510 children with BPD and/or a history of prematurity (77). In this study, it was demonstrated that monthly administration of 750 mg of RSV-IVIG per kg reduced RSV hospitalization by 41%. The PREVENT study reported a 53% reduction in the total number of RSV hospitalization days, a 60% reduction in the number of RSV days with increased oxygen requirement, and a 54% reduction in the number of RSV hospital days with a moderate or severe lower respiratory tract illness upon RSV-IVIG administration. Similar to the NIAID study results, RSV-IVIG was shown to be safe and well tolerated in the PREVENT study, as well, with only 1 to 3% of treated children experiencing medically significant adverse events related to RSV-IVIG administration. The safety profile was similar to other IVIG treatments. Based on these trial results, the U.S. Food and Drug Administration (FDA) approved MedImmune’s RSV-IVIG (RespiGam) on 18 January 1996 for the prevention of serious lower respiratory tract infection caused by RSV in children <24 months of age with BPD or a history of premature birth (≤35 weeks gestation). This was a major milestone in the development of effective medicine against severe RSV disease. It validated the hypothesis of passive immunization with antibodies as an effective approach for the prophylaxis of RSV infection in humans. In separate trials, RSV-IVIG was tested to treat RSV-infected infants and young children. Unfortunately, despite giving the very high dose of 1,500 mg/kg, there was no significant therapeutic effect (78, 79).

Monoclonal Antibody

Although RSV-IVIG is a safe and effective immunoprophylaxis against RSV infection, there are some drawbacks. RSV-IVIG is derived from human donors screened for a high titer of RSV-neutralizing activity. Despite the fact that the production of RSV-IVIG utilizes modern viral inactivation methods, concerns about transmission of unsuspected blood-borne pathogens have remained. RSV-IVIG requires monthly intravenous infusion and is time consuming, typically lasting several hours with the administration of a total fluid volume of 15 ml/kg. This could cause fluid overload in some children (76, 77). In addition, RSV-IVIG, similar to other IVIGs, may potentially interfere with routine administration schedules of certain vaccines, like measles, mumps, and rubella (80). Furthermore, in a trial in children with CHD, RSV-IVIG did not show a statistically significant decrease in RSV hospitalization for all children with CHD (although there was a trend). Also, there was a significantly higher frequency of unanticipated cyanotic episodes and poor outcomes after surgery among children with cyanotic CHD in the RSV-IVIG group than in the control group (81). The hyperviscosity caused by 750 mg of RSV-IVIG per kg was speculated to be one of the potential causes.

To improve upon RSV-IVIG, researchers turned toward recombinant monoclonal antibodies (mAbs) as a second-generation product. There were great technology advancements in the antibody field from 1975 to 1990. Several key technologies were invented, including hybridoma, chimeric antibody, antibody humanization, and recombinant antibody expression in mammalian cell cultures. These technologies allowed researchers to produce highly specific mAbs against RSV with high affinity. The humanization approach was a key component in the development of the second-generation RSV product. This approach was used to reshape hybridoma-derived murine monoclonal antibodies to be human-like, and enabled evasion of the human immune system to reduce unwanted immunogenicity. Advances in mammalian expressing technologies enabled high-quantity production of RSV mAbs in a defined medium without the concerns of potential blood-borne pathogens.

During the same period of time, basic knowledge about RSV also greatly accumulated, allowing researchers to identify appropriate RSV antigens as antibody targets. In animal studies (69, 70), researchers found that mAbs against two RSV glycoproteins, F and G, confer protection; however, antibodies against other RSV proteins, such as N protein and P protein, have no significant effect.
The substantially conserved sequence identity of F protein between subtypes, in comparison with G protein (36), suggests that F protein is likely a more ideal target than G protein for developing broad neutralizing mAbs among different RSV strains. Further study by the use of a large panel of neutralizing mAbs against the F protein of RSV A2 strain was conducted to construct a detailed topological and operational map of epitopes involved in neutralization and fusion (82). In this study, researchers immunized mice by sequential infection with RSV A2 and recombinant vaccinia virus expressing RSV F.

In the 1990s, there were three recombinant anti-RSV F-protein mAbs, two IgG1, and one IgA tested in humans. One of the antibodies, a humanized IgG1/κ RSHZ19 (SB 209763) was developed by GlaxoSmithKline. RSHZ19 had demonstrated RSV-neutralization ability in mice (83). In an early trial in healthy men at single ascending doses of 0.025 to 10 mg/kg, RSHZ19 was shown to be safe and well tolerated, and immunogenicity against RSHZ19 was not detected (84). However, RSHZ19 administered at repeat doses (two intramuscular doses, 8 weeks apart) up to 10 mg/kg failed in a pediatric trial to protect infants born prematurely or with BPD against RSV lower respiratory tract infection (85). The trial showed that RSHZ19 had a mean half-life of 32.5 days and did not induce detectable antidrug immunogenicity. The authors suggested that higher doses should be considered in additional trials. However, no further study was reported, and RSHZ19 was never licensed in any worldwide market.

The second antibody HNK20 is a mouse IgA secreted by hybridomas derived from lung lymphocytes of RSV-immunized mice (86) and was developed by OraVax. The rationale of using IgA is that it is the dominant antibody isotype in the upper airway secretions. It is also less likely to induce inflammatory responses at the mucosal surface since it cannot fix complement factors efficiently. HNK20 was developed as a nose-drop treatment with the intent of protecting the site of initial infection, thus preventing infection from spreading to the lungs. HNK20 was shown to protect the upper and lower respiratory tract from RSV infections in mice (86) and in rhesus monkeys (87). In rhesus monkeys, HNK20 administered at ~0.5 mg/kg intranasally once daily for 2 days before RSV challenge and for 4 days after challenge reduced viral load in the nose, throat, and lungs by 3 to 4 log10/ml. After treatment, HNK20 remained at viral neutralization levels in nasal secretions for >24 h. Encouraged by these results, human clinical trials were conducted (88). However, HNK20 did not reduce the RSV hospitalization rate significantly in a phase III trial during which >600 high-risk infants received intranasal prophylaxis treatment. In a subgroup analysis, a trend with reduced RSV hospitalization was observed for infants younger than 4 months at study entry. The overall results were not encouraging, and there was no additional clinical development of HNK20 (88).

The third antibody, palivizumab (also named MEDI-493 and Synagis) was developed by MedImmune, Inc., as described in the following section.

**Palivizumab: the Only Approved mAb for Preventing RSV Infection**

The antibody, mAb 1129, is one from a panel of antibodies derived from hybridomas used to characterize the neutralization epitopes of RSV F protein in a study described earlier (82), and it showed neutralization of a broad spectrum of RSV isolates. This antibody was licensed to and further developed by MedImmune, Inc. for potential applications in humans.

Murine mAb 1129 was humanized by a complementarity-determining region (CDR)-grafting approach (Fig. 2) (89). The light-chain CDRs were transplanted onto the human K102 VL/κ framework regions. The heavy-chain CDRs were transplanted onto the human Cor/CE-1 VH framework regions. Several murine residues on framework 4 regions of VH and VL were retained to potentially maintain the structural integrity of the binding site. In addition, because of an unintended frameshift during the humanization process, the first four residues of the light chain CDR1, SASS, were substituted by four random, nonhuman, nonmouse residues, KCQL. The humanized antibody (IgG1/κ), palivizumab, recognizes a conserved neutralizing epitope on the RSV F protein with a binding affinity ~1 to 2 nM in Kd, which is similar to that of the chimeric derivative of the parental antibody (Fig. 1).

Palivizumab was tested against a panel of 57 clinical isolates of RSV consisting of 34 A and 23 B subtypes, and was shown to have broad neutralization activity against all test isolates. When compared with RSV-IVIG in the microneutralization and fusion-inhibition assays, palivizumab demonstrated a 20- to 30-fold enhanced potency. In a cotton rat prophylaxis study, palivizumab was able to reduce RSV titers in the lung by more than 99% at a dose of 2.5 mg/kg. Furthermore, the administration of palivizumab did not induce increased RSV infection or pathology (89). Supported by the in vitro and in vivo results, palivizumab was further evaluated as an immunoprophylaxis agent against RSV infection in high-risk human infants.
Several clinical trials were conducted to evaluate the safety and efficacy of palivizumab. The most notable trial was the multicenter Phase III IMPact study (90). In this study, 1,502 children with prematurity (≤35 weeks) or BPD were randomly assigned to receive either five monthly intramuscular injections of palivizumab (15 mg/kg) or an equivalent volume of placebo. Palivizumab was shown to reduce RSV hospitalization incidence by 55% (hospitalization occurrence was 10.6% for placebo treated versus 4.8% for palivizumab treated). In addition, palivizumab treatment resulted in fewer days spent in the hospital, less time on oxygen support, reduced moderate/severe lower respiratory tract illness, and a lower incidence of intensive care unit admission due to RSV infection. It was concluded that palivizumab is safe and effective for the prevention of serious RSV illness in premature children and those with BPD. Based on these clinical results, palivizumab was approved by the FDA in 1998 for immunoprophylaxis of serious RSV respiratory disease in premature infants and children with BPD. Subsequently, an additional study was conducted to demonstrate the efficacy of palivizumab in young children with CHD (n = 1,287) (91), and this resulted in FDA approval to use palivizumab as immunoprophylaxis in this patient population. Palivizumab was also approved in Europe in 1999, and in Japan in 2002. Currently, it is licensed in over 60 countries and has been administered to more than one million high-risk infants and children.

Motavizumab and Motavizumab-YTE

The approval of palivizumab validates the approach for targeting RSV F protein. However, despite the use of palivizumab, there still existed an unmet medical need regarding RSV infection. For example, some infants treated with palivizumab still become infected by RSV and require hospitalization. In addition, there are no adequate preventative or treatment measures available for adult immunocompromised patients. A second-generation antibody, motavizumab, was generated (92, 93) and tested in human clinical trials by MedImmune. It is an affinity-optimized, humanized mAb derived from palivizumab. A direct-evolution approach was used to substantially improve the binding kinetics, both $k_{on}$ and $k_{off}$, of the antibody to F protein which is mediated by only 13 amino acid substitutions. Motavizumab binds to RSV F protein with 70-fold higher affinity than palivizumab, and exhibits a ~20-fold improvement in the viral neutralization potency in vitro. In a cotton rat prophylaxis model, motavizumab was found to be more potent in reducing nasal and lung RSV titers than palivizumab.

Multiple clinical trials of motavizumab were conducted. In a pivotal phase III, noninferiority trial, 6,635 high-risk infants and children were enrolled and received 15 mg/kg motavizumab or palivizumab monthly for 5 months (94). Recognizing that it may be challenging to show superiority against an effective agent, this trial was designed to evaluate whether motavizumab was noninferior and potentially superior to palivizumab in reducing the RSV hospitalization rate and other RSV-associated endpoints. The trial results showed that the motavizumab group had a 26% lower RSV hospitalization rate than the palivizumab group, which achieved the noninferiority primary endpoint. In addition, motavizumab was shown to be superior to palivizumab in one of the secondary endpoints for reducing RSV-specific outpatient medically attended lower respiratory tract infection (MALRI) by 50% compared with palivizumab ($P = 0.005$). Overall, adverse events were not significantly different between these two groups. However, the incidence of cutaneous reactions were higher in the motavizumab group (7.2% versus 5.1% with palivizumab; $P < 0.001$). The overall results suggest motavizumab may provide an improved alternative in preventing serious RSV infection in high-risk infants and children. In a separate trial that compared motavizumab with palivizumab in children with CHD (n = 1,236) for safety and tolerability, both molecules were shown to have similar safety profiles with the exception of cutaneous reactions, which occurred more frequently in motavizumab recipients (95).

In 2008, MedImmune submitted a Biologics License Application (BLA) for motavizumab to the FDA for the prevention of serious RSV respiratory disease in high-risk pediatric patients. In 2010, The Antiviral Drugs Advisory Committee to the FDA voted not to recommend approval of motavizumab, citing a concern on the risk/benefit profile. The FDA requested additional safety and efficacy data before considering motavizumab for approval. Subsequently, MedImmune withdrew the BLA and discontinued further development of motavizumab for the prophylaxis of serious RSV respiratory disease.

Both palivizumab and motavizumab have a serum half-life of up to ~3 weeks and require monthly dosing during the RSV season. With the intent to reduce the dosing frequency, the antibody constant region (Fc) of motavizumab was engineered to enhance its binding affinity to neonatal Fc receptor (FcRn). Studies have shown that FcRn plays a key role in maintaining the
FIGURE 2 (A) Palivizumab was generated by CDR-grafting humanization of murine mAb 1129. Murine CDR regions are depicted in ball structure. The remaining regions are human origin. (B) Top view of the six murine CDRs that were grafted to human frameworks. doi:10.1128/microbiolspec.AID-0014-2014.f2
serum IgG concentration. IgG binds to FcRn in a pH-dependent manner, as it binds tightly at acidic pH and has almost no binding at neutral pH. This differential binding mechanism allows efficient recycling of IgG back to circulation during the pinocytosis event. Three mutations (M252Y/S254T/T256E) were introduced to the Fc region of motavizumab, resulting in a new molecule named motavizumab-YTE (also named MEDI-557). These mutations, termed YTE, increase the binding of antibody to human FcRn at pH 6 by ∼10-fold while maintaining its very low or no binding ability to FcRn at pH 7.4. In pharmacokinetic studies in cynomolgus monkeys, the serum half-life and lung bioavailability of motavizumab-YTE were increased by 4-fold compared with motavizumab (96). A randomized dose-escalation phase I human trial in healthy adults (n = 31) was conducted to evaluate the pharmacokinetics, tolerability, and safety of motavizumab-YTE (97). A single dose of motavizumab-YTE or motavizumab (0.3, 3, 15, or 30 mg/kg) was administered intravenously, and the data were collected for 240 days. It was found that the half-life of motavizumab-YTE was 2- to 4-fold longer than that of motavizumab. In addition, motavizumab-YTE remains fully functional during the course of 240 days in circulation, as determined by RSV neutralization activity. Motavizumab-YTE was well tolerated and had an extended half-life of up to 100 days.

**FUTURE OPPORTUNITIES FOR TREATMENT AND PREVENTION OF RSV INFECTION**

There are continued efforts in searching for new approaches to treat or prevent RSV infection. Recently, several very potent anti-RSV antibodies were isolated from peripheral blood memory B cells by genetic programming (98). In microneutralization assay, these human antibodies were ∼2 log more potent than palivizumab. One of the most potent antibodies, D25, was tested in a cotton rat prophylaxis model and achieved full prevention of lung viral replication at a dose of 0.6 mg/kg compared with the required 2 mg/kg of palivizumab to achieve same protection. These potent anti-RSV antibodies are currently licensed to MedImmune for further development. MedImmune is applying its half-life extension YTE technology to these antibodies. The goal is to provide patients with a very potent long-lasting antibody that can be administered less frequently, perhaps once per quarter or once per RSV season. In addition to mAbs, other emerging drug modalities are being explored. Ablynx is currently developing an anti-RSV F protein Nanobody (ALX-0141) to treat RSV infections. Nanobody is a technology based on the camelid VH domain, about one-tenth the size of mAb. ALX-0141 is a trivalent Nanobody and is developed for delivery directly into the lungs by inhalation; it is currently in phase I human trial. In addition, Symphogen had once developed a recombinant oligoclonal antibody approach to prevent RSV infection, Sym003. Sym003 is a mixture of six unique antibodies against RSV and was in preclinical development. However, there are no new reports in recent years, and the program is no longer shown on the Symphogen website. It is likely that this project was suspended for further development. Alnylam has developed an RNAi approach (ALN-RSV01) for the treatment of RSV infection. Its RNAi therapeutic was designed to target the nucleocapsid “N” gene, which is required for RSV replication. The molecule was tested in a phase IIb trial against progressive bronchiolitis syndrome (BOS) in lung transplant patients. A dose of 0.6 mg/kg or placebo was administered by inhalation once daily for 5 days. In all analyses, inhaled ALN-RSV01 was associated with a clinically meaningful reduction in the incidence of BOS, and the drug was safe and well tolerated. However, the study narrowly missed its primary endpoint of reduction in BOS in an intent-to-treat analysis of confirmed RSV-infected patients. There are no new reports on the status of this program.

Palivizumab continues to remain the only approved prophylaxis drug against RSV. Despite the challenges in
developing vaccines or new drugs against RSV, these new approaches offer promises for RSV intervention in the future.

ACKNOWLEDGMENTS
We thank Vaheh Oganesyan for providing the antibody model structures used in Fig. 2.

We declare a conflict of interest: Both authors are employees of MedImmune, which developed and markets palivizumab.

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