Development of a Humanized Monoclonal Antibody (MEDI-493) with Potent In Vitro and In Vivo Activity against Respiratory Syncytial Virus


Neutralizing polyclonal antibody to respiratory syncytial virus (RSV) has been shown to be an effective prophylactic agent when administered intravenously in high-risk infants. This study describes the generation of a humanized monoclonal antibody, MEDI-493, that recognizes a conserved neutralizing epitope on the F glycoprotein of RSV. The affinity of MEDI-493 was found to be equal to or slightly better than an isotype-matched chimeric derivative of the parent antibody. In plaque reduction, microneutralization, and fusion-inhibition assays, MEDI-493 was significantly more potent than the polyclonal preparation. Broad neutralization of a panel of 57 clinical isolates of the RSV A and B subtypes was demonstrated. Pretreatment of cotton rats with MEDI-493 resulted in 99% reduction of lung RSV titers at a dose of 2.5 mg/kg, corresponding to a serum concentration of 25–30 μg/mL. Further, MEDI-493 did not induce increased RSV infection or pathology in either a primary or a secondary challenge.

Respiratory syncytial virus (RSV) is the leading cause of serious lower respiratory tract disease in infants and children [1–3]. The yearly epidemic nature of RSV infection is evident worldwide, but the incidence and severity of RSV disease in a given season vary by region [4]. In temperate regions of the northern hemisphere, it usually begins in late fall and ends in late spring. Primary RSV infection occurs most often in children from 6 weeks to 2 years of age and uncommonly in the first 4 weeks of life during nosocomial epidemics [5]. Children at increased risk from RSV infection include preterm infants [5] and children with bronchopulmonary dysplasia [6], congenital heart disease [7], congenital or acquired immunodeficiency [8, 9], and cystic fibrosis [10]. The fatality rate in infants with heart or lung disease who are hospitalized with RSV infection is 3%–4% [11].

RSV infects adults as well as infants and children. In healthy adults, RSV causes predominantly upper respiratory tract disease. It has recently become evident that some adults, especially the elderly, have symptomatic RSV infections more frequently than had been previously reported [12]. Several epidemics also have been reported among nursing home patients and institutionalized young adults [13, 14]. Finally, RSV may cause serious disease in immunosuppressed persons, particularly bone marrow transplant patients [15].

Treatment options for established RSV disease are limited. Severe RSV disease of the lower respiratory tract often requires considerable supportive care, including administration of humidified oxygen and respiratory assistance [16]. The only drug approved for treatment of infection is the antiviral agent ribavirin [17]. It has been shown to be effective in the treatment of RSV pneumonia and bronchiolitis, modifying the course of severe RSV disease in immunocompetent children [18]. However, ribavirin has had limited use because it requires prolonged aerosol administration and because of concerns about its potential risk to pregnant women who may be exposed to the drug during its administration in hospital settings.

While a vaccine might prevent RSV infection, no vaccine is yet licensed for this indication. A major obstacle to vaccine development is safety. A formalin-inactivated vaccine, though immunogenic, unexpectedly caused a higher and more severe reduction, microneutralization, and fusion-inhibition assays, MEDI-493 was significantly more potent than the polyclonal preparation. Broad neutralization of a panel of 57 clinical isolates of the RSV A and B subtypes was demonstrated. Pretreatment of cotton rats with MEDI-493 resulted in 99% reduction of lung RSV titers at a dose of 2.5 mg/kg, corresponding to a serum concentration of 25–30 μg/mL. Further, MEDI-493 did not induce increased RSV infection or pathology in either a primary or a secondary challenge.

Received 19 February 1997; revised 29 May 1997.
All authors except G.B., W.C.H., and J.K.T. have a material interest in MedImmune Inc. Grant support: NIH (phase I and phase II SBIR grants R44-AI-30300). Reprints or correspondence: Dr. Syd Johnson, MedImmune Inc., 35 W. Watkins Mill Rd., Gaithersburg, MD 20878.
The Journal of Infectious Diseases 1997;176:1215–24 © 1997 by The University of Chicago. All rights reserved. 0022-1899/97/17605-0013$02.00
investigators then examined the ability of hyperimmune serum high titer of RSV neutralizing antibody. This same group of one of the antibodies directed to the A site, MAb 1129. secretions yielded RSV, recovered rapidly after IVIG infusion. In the current study, we describe the construction, expression, of RSV antibody in treatment or prevention of RSV infection. Neutralization studies were performed against a Coelingh [38] conducted an extensive analysis of 18 different vaccinations involving maternal antibody in ferrets [25] and humans the biologic and biochemical properties of these MAbs resulted in the identification of three distinct antigenic sites (designated A, B, and C). Neutralization studies were performed against a panel of RSV strains isolated from 1956 to 1985 that demonstrated that epitopes within antigenic sites A and C are highly conserved, while the epitopes of antigenic site B are variable. In the current study, we describe the construction, expression, and biologic properties of MEDI-493, a humanized version of one of the antibodies directed to the A site, MAb 1129.

Materials and Methods

**Virus and antibodies.** RSV Long and 18537 strains were used as A and B subtype laboratory viruses in these studies. In addition, clinical isolates were obtained from centers in Denver, Houston, Long Beach (CA), Nashville, Rochester (NY), St. Louis, and Washington, DC. The viruses were passaged and titered using HEP-2 cells. MAb 1129 was described by Beeler and Coelingh [38]. A MEDI-493–resistant variant of RSV Long was selected as described [38]. The pan F protein, C-site–specific MAb 133-1H, was used in the F protein–specific ELISA to detect viral replication. This MAb has been described by Anderson et al. [39] and was obtained from Chemicon (Temecula, CA). RSV IVIG, lots 4 and 12, were obtained from Massachusetts Public Health Biologics Laboratory (Boston). These lots had microneutralization titers (5% solution) of 1/7000 and 1/5543, respectively.

**Construction and expression of MEDI-493.** MEDI-493 VH and VL genes were assembled de novo using both polymerase chain reaction and site-directed mutagenesis as described by Kunzel [40]. The VL and VH segments were combined with cDNA segments corresponding to C-kappa and C-gamma-1 (nGm1) and initially expressed transiently in COS-1 cells using a CMV immediate-early promoter. Stable cell lines expressing MEDI-493 from mouse myeloma cells, NS0, were generated as described by Bebbington et al. [41].

**Binding analysis.** Immunoaffinity-purified F protein from RSV A2 was provided by P. Paradiso (Wyeth-Lederle Vaccines and Pediatrics, Rochester, NY). To coat the chip surface with antigen, a 10 nM solution of RSV F protein was injected over an N-hydroxysuccinimide/1-ethyl-3-[3-dimethylaminopropyl]-carbodiimide-activated CM5 sensor chip (Biacore, Piscataway, NJ) [42]. Following this, unreacted active ester groups were reacted with 1 M ethanolamine. Kinetic experiments were carried out by injecting 40 µL of MEDI-493 in HBS (10 mM HEPES [pH 7.4], 150 mM NaCl, 3 mM EDTA) at concentrations of 400 nM, 200 nM, 100 nM, 50 nM, 25 nM, and 12.5 nM or an irrelevant murine MAb, HPV-18 H1, at 400 nM. The solutions were passed over the RSV F protein surface using looped sequential injections at a flow rate of 10 µL/min for 4 min. A 4-min dissociation period...
was followed by a regeneration step consisting of 10 μL of 100 mM HCl. Individual rate constants for the association and dissociation were calculated using Bia-evaluation software (provided by Biacore).

Plaque-reduction assay. Serial dilutions of antibody were prepared in 96-well microtiter dishes using Hanks’ balanced salt solution as diluent. An equal volume of RSV suspension previously titrated to give 50 pfu was added to each well to achieve a 50-μL final reaction mixture. After a 1-h incubation at 23°C, 50 μL of this mixture was then added to each of three wells of a 24-well plate (Costar, Cambridge, MA) containing confluent HEp-2 cells, from which all but 15 μL of medium had been aspirated. After 1 h at 37°C, 1 mL of warm plaquing medium (MEM containing 1% methyl cellulose, 2% fetal calf serum, 0.002 M 1-glutamine, and 50 μg/mL gentamycin) was added, and plates were incubated for 5–6 days at 37°C until plaque formation was evident. The overlay was removed by inversion, and the cell layer was fixed and stained with 0.1% crystal violet in 5% glutaraldehyde for 30–60 min, dried at room temperature, and then counted. The concentration of antibody resulting in 50% reductions in plaque formation relative to the virus control without antibody (EC_{50}) was estimated from an interpolated plot of the data.

Microneutralization assay. The microneutralization assay was a modification of the procedures described by Anderson et al. [43]. Antibody dilutions were made in triplicate in a 96-well plate. Seventy-five TCID_{50} of RSV were then added to each well and incubated for 2 h at 37°C. HEp-2 cells (2.5 × 10^5) were then added to each well and cultured for 5 days at 37°C in 5% CO_{2}. After 5 days, the medium was aspirated, and cells were washed and fixed with acetone. Viral replication was then measured using an F protein–specific ELISA. The amount of viral antigen present in wells at the different antibody dilutions was plotted for each sample, and the data were analyzed using a four-parameter curve fit (Softmax software; Molecular Devices, Menlo Park, CA). The curves were then compared with values from control wells with cells alone and for virus-infected cells without antibody. The antibody concentration for each sample that reduced viral replication to 50% of that in virus-infected control wells was then calculated and expressed as the EC_{50}. For evaluating the neutralization of clinical isolates by MEDI-493, the antibody concentration was fixed at 400 μg/mL and the virus dilution was varied.

Fusion-inhibition assay. The fusion-inhibition assay was run in the same format as the microneutralization assay with the following changes. Seventy-five TCID_{50} of RSV A (Long) strain were added to each microtiter well along with 2.5 × 10^4 HEp-2 cells and incubated for 4 h at 37°C in 5% CO_{2}. The unattached virus was then removed, and the cells were washed. Independent dilutions of antibody were prepared in cell culture medium and added to microtiter wells. After 5 days, viral replication was measured by ELISA as described above.

Cotton rat challenge studies. Cotton rats (Sigmodon hispidus, average weight = 100 g) were given 0.1 mL of each antibody intramuscularly or intravenously at the indicated doses. Control animals were injected with bovine serum albumin. One day later, animals were anesthetized, blood samples were taken, and the animals were challenged by intranasal instillation of 10^5 pfu of the A (Long) or B (18537) strains of RSV. Four days after viral challenge, all animals were sacrificed by carbon dioxide asphyxiation. Lungs were harvested and homogenized in 10 parts (wt/vol) Hanks’ balanced salt solution. The resulting suspension was quantified for virus content by plaque assay using HEp-2 cells. Serum MEDI-493 levels at the time of challenge were determined using an anti-human IgG ELISA.

For pathology, the lung tissues were trimmed, dehydrated through graded alcohols, perfused with xylene, processed through paraffin, sectioned at 5 mm, stained with hematoxylin-eosin, and interpreted microscopically in a blinded manner. Control slides representing uninfected and RSV-infected cotton rat tissues were examined. In addition, control slides from cotton rats vaccinated with a formalin-inactivated vaccine and infected with RSV were also examined. Lesions associated with RSV infection in the cotton rats were characterized by the presence of bronchiolitis and, in some animals, focal interstitial pneumonia. The bronchiolitis involved single or multiple airways and was characterized by both epithelial changes and the presence of inflammatory cell infiltrates scattered around the bronchioles. Normal bronchiolar epithelium had relatively small, basally located nuclei in cells with abundant apical cytoplasm. In contrast, bronchiolar epithelial cells in inflamed areas were hypertrophic and characterized by the presence of karyomegalic nuclei located centrally in the cell. Distinct syn- cytial bronchiolar epithelial cells were observed in an occasional affected area. An apoptotic nucleus was rarely observed; infrequent mitoses were also seen. Inflammatory infiltrates consisted predominantly of mononuclear cells (mostly macrophages with few lymphocytes) and fewer neutrophils around the affected airways and infiltrating the smooth muscle to the mucosa. In the lung, alveolar septa were thickened by proliferation of alveolar epithelial cells and very slight infiltrates of macrophages. The extent of pathology was graded: 0 (normal), 1 (minimal), 2 (mild), 3 (moderate), or 4 (marked).

Results

MAb 1129 was humanized using the human K102 germline VL and the Cor and CE-1 VH framework regions (figure 1) [44]. To design the variable region sequences, the three-dimen- sional structure of the MAb 1129 Fv region was modeled by simple substitution of the residues from the MAb 1129 VL and VH sequence into the coordinates of corresponding residues in the known crystal structure of the MCPC603 antibody [45]. Several residues were left as the mouse residue in order to maintain the structural integrity of the binding site. These residues are 105 and 108 in the VH and 104 in the VL (Kabat numbering). At residues VH 108 and VL 104, a residue identical to the mouse residue can be found in a similar context in human germline J-region sequences other than the one initially chosen. The amino acid at position VH 105 is in a beta bulge, which may be important to maintaining the orientation of VH with respect to the VL structure. Residue VL 100 is in a similar position in the light chain. In addition, due to a frameshift, the first four residues of CDR L1 were constructed randomly and thus are nonhuman, nonmouse residues. All other CDR residues of the VL and VH are identical in human and murine sequences or are murine in origin.

The humanized 1129 IgG1, κ, designated MEDI-493, was initially expressed transiently in COS-1 cells. Its ability to bind

Downloaded from https://academic.oup.com/jid/article-abstract/176/5/1215/831423 by guest on 03 May 2019
Figure 1. Design of humanized VL and VH segments based on murine monoclonal antibody 1129. Human framework regions were derived from K102 for VL (A). VH framework 1 (FR1) region was derived from Cor; remaining framework regions were derived from CE-1 sequence (B). CDR sequences, as defined by Kabat, are underlined.

To RSV F protein was assessed by ELISA in comparison to a similarly expressed chimeric version of 1129. The results of this assay indicated that the affinity of MEDI-493 was equivalent to or slightly better than chimeric 1129 (data not shown). The \( K_d \) of both antibodies was estimated to be 1–2 nM (based on the \( EC_{50} \) of each in the ELISA). Subsequently, a stable MEDI-493 expression cell line was constructed in mouse myeloma cell line NS0 using glutamine synthetase selection and amplification using methionine sulfoximine [41]. Additional studies described below were performed using MEDI-493 purified from this cell line, which produces antibody at \( \sim 1 \) g/L of culture.

To obtain a better understanding of the binding properties of MEDI-493 neutralization of laboratory strains of RSV by MEDI-493. In order to survey the spectrum of MEDI-493, a total of 57 isolates consisting of 34 A and 23 B subtype isolates were analyzed. In this assay, a fixed concentration of MEDI-493 was added to serial dilutions of the untitered viruses. Each of these isolates was neutralized by MEDI-493. Representative assays of A and B subtypes are shown in figure 2. Using these kinetic data, the \( k_{(on)} \) was calculated to be \( 3.0 \times 10^5 \) \( M^{-1} \) s\(^{-1}\), and the \( k_{(off)} \) was 4.3 \( \times 10^{-4} \) s\(^{-1}\) giving a \( K_d \) of 1.4 nM, similar to that observed in the ELISA assay with the COS-1–derived antibody.

Three different in vitro neutralization assays were used to assess the ability of MEDI-493 to neutralize RSV. First, we used a plaque-reduction assay to evaluate the neutralization of either A (Long) or B (18537) strains of RSV. MEDI-493 effectively neutralized both types of RSV in a dose-dependent manner (figure 3). The \( EC_{50} \) for MEDI-493 neutralization of RSV Long and 18537 were similar, \( \sim 2 \) \( \mu \)g/mL in this assay. In addition, MEDI-493 was evaluated in microneutralization and fusion-inhibition assays. In the microneutralization assay, antibody was mixed with virus (RSV Long) prior to infection, while in the fusion-inhibition assay, MEDI-493 was added after cells were infected with RSV. The \( EC_{50} \) for MEDI-493 was calculated to be 0.10 \( \pm \) 0.03 \( \mu \)g/mL in the microneutralization assay and 0.17 \( \pm \) 0.08 \( \mu \)g/mL in the fusion-inhibition assay (average of three assays each \( \pm \) SD). These values compared favorably with \( EC_{50} \) values for RSV IVIG (lot 12) of 2.58 \( \pm \) 0.37 \( \mu \)g/mL and 5.25 \( \pm \) 0.17 \( \mu \)g/mL, respectively, demonstrating a 20- to 30-fold enhanced potency of MEDI-493 compared with the polyclonal product.

These data indicated potent neutralization of laboratory strains of RSV by MEDI-493. In order to survey the spectrum of MEDI-493 neutralization of contemporary clinical isolates of RSV, a total of 57 isolates consisting of 34 A and 23 B subtype isolates were analyzed. In this assay, a fixed concentration of MEDI-493 was added to serial dilutions of the untitered viruses. Each of these isolates was neutralized by MEDI-493. Representative assays of A and B subtypes are shown in figure 4. As a control for this assay, a resistant virus preparation, generated by plaquing and passaging in the presence of MEDI-493, was tested in the same assay and found to be susceptible to neutralization by RSV IVIG but not by MEDI-493.

The cotton rat model of RSV infection was used to demonstrate potency of MEDI-493 in vivo. In each experiment, MEDI-493 was administered 1 day before intranasal challenge with 10\(^5\) pfu of RSV. Figure 5 shows the protection of animals given antibody intramuscularly and challenged with either RSV Long or RSV 18537. MEDI-493 is potent against both subtypes, reducing viral replication by \( >99\% \) at a dose of 2.5 mg/kg in each case. As shown in table 1, \( >99\% \) reduction in pulmonary RSV was achieved also at an intravenous dose of 2.5
Figure 2. Biacore analysis of the binding of MEDI-493 to immobilized RSV F protein. Sensorgrams represent the binding and dissociation phases resulting from injection of MEDI-493 at concentrations of 400 nM, 200 nM, 100 nM, 50 nM, 25 nM, and 12.5 nM. Data from irrelevant antibody HPV-18H1 (400 nM) are also represented.

Figure 3. Plaque-reduction assay of neutralization of RSV by MEDI-493. O = RSV Long (A subtype) + MEDI-493; ▲ = RSV 18537 (B subtype) + MEDI-493; ● = RSV Long + RSV intravenous immune globulin (lot 4).
mg/kg. In this experiment, the serum antibody concentration at the time of RSV challenge was $\sim 30 \mu g/mL$ at the 2.5 mg/kg dose.

Two series of additional cotton rat studies were done to assess potential enhancement of virus infectivity or RSV-induced histopathology after primary or secondary RSV challenge. In the first, a dose-ranging study was performed in the cotton rat model to determine whether the presence of MEDI-493 at noninhibitory levels could enhance viral replication or virus-induced pathology during a primary infection or enable the selection of antibody-resistant variants in vivo. Groups of 4 animals each were given bovine serum albumin or various concentrations of MEDI-493 intramuscularly. At 24 h later, all animals were challenged with $10^5$ pfu of RSV Long. Four days later, the animals were sacrificed, and the lung tissue was analyzed for RSV levels and fixed and prepared for histopathology. The results of this study indicated that no enhancement of viral replication or RSV-specific histopathology was observed on primary challenge in the presence of MEDI-493. In order to test for the presence of antibody-resistant mutants, the lung homogenates were incubated with 0.1 mg/mL MEDI-493 at 4°C for 1 h before addition to HEp-2 cells. Only 1 plaque was observed in the entire experiment, from an animal receiving a 0.0032-mg/kg dose.

A second set of experiments was designed to determine if increased pathologic changes would occur after reinfection in animals that received MEDI-493 prior to primary infection. Cotton rats were divided into 3 groups. Group 1 animals ($n = 12$) received 10 mg/kg bovine serum albumin and group 2 ($n = 12$) received 10 mg/kg MEDI-493 intravenously 1 day prior to challenge with RSV ($10^5$ pfu/Long strain). Group 3 ($n = 8$) was not dosed and was mock-challenged with medium only. Four animals each from groups 1 and 2 were sacrificed 4 days after infection, and pulmonary virus titers were determined. Biweekly blood samples were taken from the remaining animals, and MEDI-493 levels were determined by ELISA. After 8 weeks, when MEDI-493 was no longer detectable, the remaining animals were rechallenged with a low dose of RSV ($10^3$ pfu) and sacrificed on day 4 after challenge. The lower dose was used so that any increased viral replication would be observable. Lungs were divided and prepared for histopathology, and virus was titrated as described above. The results of the challenge and rechallenge are summarized in table 2. Again, no enhancement of viral replication or virus-induced pathology was observed. In fact, animals previously protected from lower respiratory tract infection by MEDI-493 were completely resistant to a subsequent challenge after clearance of antibody, indicating the presence of MEDI-493 does not impede development of a protective immune response to RSV.

**Discussion**

Recent studies have shown conclusively that passive administration of high-titer RSV IVIG reduced both the incidence
and the severity of RSV-related lower respiratory tract infection in at-risk infants [34, 35]. This development was a major step forward in the care of premature infants and others at significant risk for RSV morbidity. However, broad use of this product may be hampered by the time required for intravenous administration of the polyclonal preparation and the large volume of RSV IVIG required to achieve adequate titers to treat adults at risk for RSV. In order to achieve a higher specific activity of RSV-specific antibodies to facilitate alternate routes of administration, we have developed a humanized MAb, MEDI-493.

It is expected that a humanized RSV-specific MAb would need to have several important properties. It would be important to retain the affinity of the parent MAb, have potent neutralizing and fusion-inhibition capacity, and react with the majority of recent clinical isolates of both A and B subtypes of RSV. In addition, antiviral activity should be demonstrated in a relevant animal model of RSV infection, and the antibody should not

**Table 1.** Prophylaxis of RSV infection in cotton rats by intravenous administration of MEDI-493.

<table>
<thead>
<tr>
<th>Compound, dose</th>
<th>n</th>
<th>Concentration of human IgG, μg/mL (mean ± SE)</th>
<th>RSV titer, pfu/g (mean log10 ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine serum albumin</td>
<td>10 mg/kg</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>MEDI-493</td>
<td>0.312 mg/kg</td>
<td>7</td>
<td>2.67 ± 0.60</td>
</tr>
<tr>
<td>0.625 mg/kg</td>
<td>17</td>
<td>5.27 ± 0.27</td>
<td>4.44 ± 0.10</td>
</tr>
<tr>
<td>1.25 mg/kg</td>
<td>18</td>
<td>10.1 ± 0.29</td>
<td>3.52 ± 0.15</td>
</tr>
<tr>
<td>2.5 mg/kg</td>
<td>17</td>
<td>28.6 ± 2.15</td>
<td>2.98 ± 0.17</td>
</tr>
<tr>
<td>5.0 mg/kg</td>
<td>15</td>
<td>55.6 ± 3.43</td>
<td>2.12 ± 0.09</td>
</tr>
<tr>
<td>10.0 mg/kg</td>
<td>18</td>
<td>117.6 ± 5.09</td>
<td>&lt;2.0</td>
</tr>
</tbody>
</table>

**NOTE.** This is a compilation of 3 separate experiments.
induce increased viral infection or lung pathology upon primary or secondary RSV infection. The studies performed demonstrate that MEDI-493 satisfies all of these criteria.

In addition to MEDI-493, several other MAbs have been described that may have potential in RSV prophylaxis or treatment (or both). Tempest et al. [46] developed a humanized MAb to a linear epitope on RSV, while Crowe et al. [47] isolated an RSV-specific human Fab fragment from an Escherichia coli phage display library. Both of these compounds were shown to neutralize RSV. It appears from the available data that these antibodies recognize epitopes on the F protein distinct from each other and from MEDI-493 (1129), which recognizes a conformational epitope. Although we do not believe that more than one MAb is required for prophylaxis, it may be desirable to use a cocktail of MAbs in a therapeutic mode, where the virus burden is likely to be substantially higher, so as to limit the potential for escape mutant generation.

Each of the assays used to evaluate MEDI-493 has been advocated as a surrogate for prophylaxis of RSV disease. However, some of these assays may be more valuable than others in predicting in vivo efficacy. Polyclonal IVIG potent in plaque-reduction assays has been shown to induce protection in cotton rats and at-risk infants; however, some human MAbs, which are effective in this in vitro assay, failed to induce protection in cotton rats (Bansal G, Johnson S, unpublished data). On the other hand, the microneutralization assay has been used to screen donors for manufacture of RSV IVIG [48] and yields fewer false positives. In addition, Taylor et al. [49] observed that a fusion-inhibition assay was most predictive of the ability of certain MAbs to protect mice from RSV. MEDI-493 is potent in a dose-dependent manner in all three assays and in the cotton rat model.

The cotton rat model of RSV infection has proven extremely useful in understanding the biology of RSV infection and in evaluating potential prophylactic or therapeutic agents in terms of potency and certain safety issues. The correlation of a specific neutralizing titer with protection of cotton rats from RSV infection was a key observation in the development of an effective IVIG product for at-risk infants [29, 34, 35, 48]. Because of the monoclonal nature of MEDI-493, we were able to determine a precise serum antibody concentration at the time of RSV challenge and correlate this with animal protection. A >99% reduction of lung RSV titers was observed at serum concentration of 25–30 μg/mL. This provides a useful target that can be maintained in human subjects after infusion of MEDI-493 [50].

In addition to enhanced potency, MEDI-493 does not enhance RSV-induced pathology when infused into cotton rats. Two main mechanisms of immune-dependent enhancement of RSV infection have been proposed. Antibody-dependent enhancement of virus infectivity is a phenomenon in which virus entry into host cells may be increased or facilitated by nonneutralizing antibodies, possibly by cross-linking of Fc receptor molecules on the surface of the target cell [51]. Although this phenomenon has been demonstrated for RSV infection in vitro, its significance to the in vivo infection process remains to be demonstrated [52]. A second, more serious phenomenon, is that of enhanced pathology, which occurred when persons previously immunized with a formalin-inactivated RSV vaccine (FI RSV) were exposed to RSV during a natural infection [19, 20]. This histopathology has been duplicated and examined in animal models, including mice and cotton rats [53, 54]. These studies are consistent with a mechanism of pathology that is T cell-dependent. Immunization with FI RSV appears to lead to the generation of primarily nonneutralizing antibodies and priming of the Th2 subset of CD4 helper T cells [54, 55]. Thus, antibody may play a minor role in this setting.

In this study, antibody-dependent enhancement of viral replication was not observed during a primary infection with subprophylactic doses of MEDI-493 in the cotton rat. Furthermore, no enhancement of histopathology was observed in either a primary infection or upon rechallenge of previously infected animals. In addition, MEDI-493 did not impede the development of protective immune responses, as shown in the rechallenge study. This result differs from results from a recent study using RSV IVIG in a mouse model of infection [56] but is consistent with results from previous studies conducted in the cotton rat model [29].

In summary, MEDI-493 appears to be a potent, broadly reactive neutralizing humanized antibody to a conserved epitope of the F protein of RSV and is substantially more potent than a polyclonal hyperimmune IVIG preparation that protected high-risk infants against RSV infection. Thus, MEDI-493 appears to be a promising candidate for the prevention of RSV. On the basis of the potency and safety of MEDI-493 demonstrated in these studies, clinical trials have been initiated to evaluate the safety and efficacy of this molecule as a prophylactic agent for RSV-related lower respiratory tract infection in infants.

Acknowledgments

We thank B. Graham, R. LaVia, P. Piedro, K. Schnabel, and G. Storch for providing RSV clinical isolates. We also acknowl-

Table 2. Assessment of the potential for immunity and increased histopathology upon secondary infection of cotton rats with RSV after MEDI-493 immunoprophylaxis of a primary challenge.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Primary</th>
<th>Secondary</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10 mg/kg BSA</td>
<td>5.51 ± 0.05</td>
<td>&lt;2 ± 0</td>
</tr>
<tr>
<td>2</td>
<td>10 mg/kg MEDI-493</td>
<td>&lt;2 ± 0</td>
<td>&lt;2 ± 0</td>
</tr>
<tr>
<td>3</td>
<td>None</td>
<td>Mock</td>
<td>2.46 ± 0.14</td>
</tr>
</tbody>
</table>

NOTE. Serum MEDI-493 levels were undetectable prior to secondary challenge with RSV. BSA, bovine serum albumin.
edge the assistance of the following persons in carrying out these studies: Ene Allikmets, Francine Brady, David Carlin, Miriam Darnell, Sally Hensen, Susan Johnson, and Charles Riggin. In addition, we thank Frank Top and Edward Connor for helpful comments and suggestions and Donni Leach for administrative assistance.

References


40. Kunkel T. Rapid and efficient site-directed mutagenesis without pheno-
41. Bebbington C, et al. High level expression of a recombinant antibody from
myeloma cells using a glutamine synthetase gene as an amplifiable
42. Karlsson R, Roos H, Fagerstam L, Persson B. Kinetic and concentration
for respiratory syncytial virus based on an enzyme immunoassay, J Clin
44. Kabat E, Wu TT, Perry HM, Gottesman KS, Foeller C. Sequences of
proteins of immunological interest. 5th ed. Bethesda, MD: US Depart-
ment of Health and Human Services, National Institutes of Health, 1991;
NIH publication no. 91–3242.
45. Padlan E, Davies DR, Pecht I, Givol D, Wright C. Model-building studies
of antigen-binding sites: the hapten-binding site of MOPC-315. Cold
antibody to inhibit human respiratory syncytial virus infection in vivo.
47. Barbas, CF, Crowe, JE, Cababa, D, et al. Human monoclonal Fab frag-
ments derived from a combinatorial library bind to respiratory syncytial
virus F glycoprotein and neutralize infectivity. Proc Nat Acad Sci USA
respiratory syncytial virus immune globulin prepared from donors
of respiratory syncytial virus recognized by murine and bovine mono-
50. Subramanian KNS, Weissman L, Rhodes T, Connor E, MEDI-493 Study
Group. Randomized, double-blind, placebo-controlled, dose-escalation
trial of a humanized respiratory syncytial virus (RSV) monoclonal anti-
51. Porterfield JS. Antibody-dependent enhancement of viral infectivity. Adv
52. Krilov LR, Anderson LJ, Marcoux L, et al. Antibody-dependent enhance-
ment of respiratory syncytial virus infection in two monocyte/macrophage
mines T helper cytokine mRNA Patterns in lungs of mice challenged
histo-pathology is observed in cotton rats immunized with formalin-
inactivated respiratory syncytial virus (RSV) or purified F glycoprotein
and challenged with RSV 3–6 months after immunization. Vaccine
55. Connors M, Giese NA, Kulkarni AB, et al. Enhanced pulmonary histo-
pathology induced by respiratory syncytial virus (RSV) challenge of
formalin-inactivated RSV-immunized BALB/c mice is abrogated by
56. Graham BS, Tang YW, Gruber WC. Topical immuno-prophylaxis of respi-
ratory syncytial virus (RSV)–challenged mice with RSV-specific im-