A Direct Comparison of the Activities of Two Humanized Respiratory Syncytial Virus Monoclonal Antibodies: MEDI-493 and RSHZ19

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Two humanized monoclonal antibodies, MEDI-493 and RSHZ19, were developed independently as potential improvements over RSV-IGIV for prevention of respiratory syncytial virus (RSV) infection. RSV-IGIV is a polyclonal human antibody preparation for intravenous infusion enriched for RSV neutralizing activity. A phase III clinical trial showed that MEDI-493 significantly reduced hospitalizations due to RSV infection. In a separate trial, RSHZ19 failed to show significant efficacy. In new studies, the in vitro and in vivo activities of MEDI-493 and RSHZ19 were compared to determine whether the different clinical results are related to differences in biologic activity. MEDI-493 was consistently 4- to 5-fold more potent than RSHZ19 in antigen binding, RSV neutralization, and fusion inhibition assays. Although both MEDI-493 and RSHZ19 were effective against A and B subtypes of RSV in the cotton rat model of RSV infection, 2- to 4-fold higher doses of RSHZ19 were required for similar protection. The enhanced activity of MEDI-493 compared with RSHZ19 may, in part, explain its better clinical effect.

Human respiratory syncytial virus (RSV) and related animal viruses are negative-stranded RNA viruses that lack both hemagglutinin and neuraminidase activity [1]. Persons at risk for severe RSV disease include children born prematurely, those with underlying heart or lung disease, and immunocompromised individuals [2–6]. Pathology due to RSV involves both direct cellular injury, including the formation of syncytia, and immunologically induced damage [7]. The potential priming for enhanced immunopathology upon RSV infection has hampered the development of a safe and effective prophylactic vaccine [8, 9]; however, considerable progress has been made in the area of passive immunization.

Neutralizing IgG antibody can prevent dissemination of RSV from the upper to lower respiratory tract by limiting viral infection and replication in these tissues [10]. Neutralizing antibodies are directed primarily at the F and G surface glycoproteins [1]. The F glycoprotein is responsible for the fusion of RSV to the cell after attachment of the virus via the G glycoprotein. RSV also spreads by cell-cell fusion, again utilizing the F glycoprotein in this process. In clinical studies, RSV-IGIV (RespiGam), a polyclonal human antibody preparation for intravenous infusion enriched for RSV neutralizing activity and licensed by the Food and Drug Administration in 1996, was effective in decreasing hospitalization in at-risk infants [11].

Because of their increased potency compared with the polyclonal product, several monoclonal antibodies (MAbs) to RSV have been developed and investigated. MEDI-493 (Palivizumab) and RSHZ19 (SB 209763) are humanized IgG1 MAbs directed to distinct neutralizing epitopes on the F glycoprotein of RSV [7, 12]. Both of these antibodies have been analyzed for their effectiveness in in vitro neutralization assays and in animal models of RSV prophylaxis. In pivotal clinical trials for both antibodies, MEDI-493 significantly reduced hospitalization for RSV-induced disease [13], while in a separate study, efficacy was not demonstrated for RSHZ19 (D. Burch, personal communication). We sought to determine if the differences in clinical outcome of these trials could be related in part to differences in the relative potencies of these two MAbs. In this study, in vitro and in vivo activities of MEDI-493 and RSHZ19 were directly compared for avidity, neutralization, fusion inhibition, and protection of cotton rats against RSV infection.

Materials and Methods

MAbs. MEDI-493 is an IgG1 (COR)/κ (K102) humanized MAb containing the antigen-binding determinants of murine MAb 1129 [7, 14]. RSHZ19 is an IgG1 (NEW)/κ (REI) humanized MAb containing the antigen-binding determinants of murine MAb 19 [12, 15].

RSV microneutralization assay. Neutralizing activity of the antibodies was determined by microneutralization assay [16]. Ten TCID₅₀ of RSV (Long) was incubated with serial dilutions of an-
virus-only control wells. Cells were fixed 4 days later, and RSV replication was determined by F protein-specific ELISA (y-axis). = RSV-IGIV, = RSHZ19, ▲ = MEDI-493, ■ = virus control, ▼ = uninfected cells.

RSV fusion inhibition assay. The ability of the antibodies to block RSV-induced cell fusion after viral attachment to the cells was determined in a fusion inhibition assay. This assay was identical to the microneutralization assay, except that the cells were infected with RSV (Long) for 4 h prior to addition of antibody [15].

Epitope analysis. Epitope analysis of the MAbs was performed with a biosensor (BIAcore, Piscataway, NJ) [17, 18]. The antigen used for this assay was a truncated RSV (A2) F protein (aa, 1–526) expressed in baculovirus. Purified RSV F protein was covalently coupled to an N-hydroxysuccinimidyl-ethyl-3-[3-dimethylaminopropyl]-carbodiimide-activated CM5 sensor chip according to the manufacturer’s protocol. Unreacted active ester groups were reacted with 1 M ethanolamine. A primary injection of either 1 μM or 10 μM MEDI-493 was followed by a wash step with Hanks’ balanced salt solution (HBSS) and then by a secondary injection of either MEDI-493 or RSHZ19. Sensorgrams were analyzed by using BIA (BIAcore) evaluation software.

Isothermal titration calorimetry: The solution affinity of each MAb for RSV F protein was determined by isothermal titration calorimetry [19]. A 1.4-M solution of 4.5 μM RSV F protein was titrated with 5.5-μL injections of 26 μM MEDI-493 or RSHZ19. After each injection of MAb, the amount of heat given off, which is proportional to the amount of binding, was measured. The antigen used was an RSV (A2) F protein truncate (aa, 25–524) expressed in drosophila cells. Titrations were conducted at 44°C and 55°C to achieve optimal signal-to-noise ratios. Thermal stability of the MAbs and the F protein at these temperatures was demonstrated by circular dichroism-unfolding experiments. Using the integrated van’t Hoff equation [20], we corrected affinities to 37°C for comparison with in vivo data. The van’t Hoff correction is based solely on the F protein binding enthalpy change, which was measured directly by calorimetry. Since the binding enthalpy changes for MEDI-493 and RSHZ19 were similar, the temperature corrections for Kd were nearly identical.

Cotton rat prophylaxis. In vivo efficacy was determined by the cotton rat model [21]. Cotton rats (Sigmodon hispidus, average weight 100 g) were anesthetized with methoxyllumene, bled, and given 0.1 mL of purified MAb by intramuscular (im) injection at doses of 5, 2.5, 1.25, or 0.625 mg/kg body weight or bovine serum albumin control at 5 mg/kg body weight. Twenty-four hours later, animals were again anesthetized, bled for serum MAb concentration determination, and challenged by intranasal instillation of 106 pfu of RSV A (Long) or B (18537) strains. Four days later, the animals were sacrificed and their lungs were harvested. Lungs were homogenized in 10 parts (wt/vol) of HBSS and the resultant suspension was used to determine pulmonary virus titers by plaque assay. Serum antibody titers at the time of challenge were determined by an anti-human IgG ELISA.

Results

Neutralization of RSV either before (microneutralization assay) or after (fusion inhibition assay) attachment to VERO cells was assessed by ELISA to measure viral replication. The results of these assays are shown in figures 1 and 2, respectively. In the microneutralization assay, MEDI-493 (EC50 = 0.11 ±

Figure 1. Respiratory syncytial virus (RSV) neutralizations by MEDI-493, RSHZ19, and RSV-IGIV. 10 TCID50 of RSV (Long) were incubated with various concentrations of antibody (x-axis) for 2 h at 37°C before infection of VERO cells in 96-well format. Cells were fixed 4 days later, and RSV replication was determined by F protein-specific ELISA (y-axis). = RSV-IGIV, = RSHZ19, ▲ = MEDI-493, ■ = virus control, ▼ = uninfected cells.

Figure 2. Respiratory syncytial virus (RSV) fusion inhibition assays of MEDI-493, RSHZ19, and RSV-IGIV. 10 TCID50 of RSV (Long) were used to infect VERO cells in 96-well format. After attachment for 4 h at 37°C, virus was removed and plates were washed, and antibody was added at various concentrations (x-axis). Cells were fixed 4 days later, and RSV replication was determined by F protein-specific ELISA (y-axis). = RSV-IGIV, = RSHZ19, ▲ = MEDI-493, ■ = virus control, ▼ = uninfected cells.
0.04 μg/mL) was ~5-fold more potent than RSHZ19 (EC\textsubscript{50} = 0.49 ± 0.37 μg/mL) and 20-fold more potent than RSV-IGIV (EC\textsubscript{50} = 2.02 ± 0.60 μg/mL). Similar differences were seen in the fusion inhibition assay, in which MEDI-493 had an EC\textsubscript{50} of 0.23 ± 0.03 μg/mL compared with 0.95 ± 0.13 μg/mL for RSHZ19 and 9.26 ± 1.9 μg/mL for RSV-IGIV.

We next determined whether the enhanced neutralization by MEDI-493 would correlate with in vivo protection in a cotton rat prophylaxis model. Various doses of MEDI-493 or RSHZ19 were administered to animals 24 h before challenge with either the A (Long) or B (18537) strain of RSV. The results of these studies are shown in figure 3. The MAb dose administered was compared with the human IgG serum concentration at 24 h after MAb administration (the time of virus challenge) and with the pulmonary virus titer 4 days after virus challenge. The results showed that to achieve a 100-fold (99%, ED\textsubscript{99}) reduction in RSV titer, doses of 2.5 mg/kg (Long) and 1.3 mg/kg (18537) of MEDI-493 were required and that 5 mg/kg of RSHZ19 was required for comparable anti-RSV activity with either type A (Long) or type B (18537) challenge. Notably, even at the highest dose of RSHZ19 tested (5 mg/kg), not all of the animals exhibited a 99% reduction in pulmonary RSV.

In separate studies, cotton rats dosed with 10 mg/kg of RSHZ19 consistently had ≥99% reduction in pulmonary RSV titers [22]. Serum MAb concentrations were variable within groups but were similar, in general, for both MAbs at the single time point evaluated. Serum MAb concentrations corresponding to the cotton rat ED\textsubscript{99} of MEDI-493 and RSHZ19 were 15–21 μg/mL versus 46–53 μg/mL, respectively. Thus, if we assume similar pharmacokinetic properties and pulmonary distribution profiles for both MAbs, MEDI-493 was 2- to 4-fold more potent than RSHZ19 in inhibiting RSV replication in vivo, similar to the differences observed in the in vitro neutralization assay.

Differences in antibody potency in these assays could be related to different antigen affinities of the MAbs or recognition of distinct domains of the F protein that could impede fusion of the virus preferentially. To begin to address these issues, the solution affinities of MEDI-493 and RSHZ19 for RSV F protein were determined by titration calorimetry. In this assay.

![Figure 3](image-url)
binding of the MAbs to F protein was measured with both reactants in solution phase, and binding was assessed at equilibrium. Attainment of equilibrium at each step in the titration was verified visually in real time (figure 4). The calorimetry data for both MAbs were well described in each case by a simple single-equilibrium model [20]. Data for MEDI-493 binding to F protein are shown in figure 4. At both temperatures studied (44 and 55°C), the F protein affinity of MEDI-493 was several-fold tighter than that of RSHZ19. Analysis of the data yielded F protein binding $K_d$ values of $3 \times 10^{-5}$ and $3 \times 10^{-6}$ for RSHZ19 and MEDI-493, respectively, at 37°C.

Kinetic analysis using a BIAcore biosensor revealed 1.4 mM $K_i$ for MEDI-493 and 5.3 mM $K_i$ for RSHZ19. There was a 5-fold difference in the off rates of the two antibodies; MEDI-493 had a $k_{\text{off}}$ of $4.3 \times 10^{-3}$ s$^{-1}$ compared with $2.1 \times 10^{-3}$ s$^{-1}$ for RSHZ19. MEDI-493 also had a significantly faster on rate for the solid-phase bound F protein: $3.04 \times 10^{4}$ versus $4.0 \times 10^{4}$ for RSHZ19. Therefore, the enhanced affinity observed for MEDI-493 is consistent with its increased relative neutralizing activity.

Through the use of antibody-resistant variants of RSV, it was previously shown that the parental MAbs to MEDI-493 and RSHZ19 recognize distinct epitopes on the F protein [23]. In the current study, we used the biosensor to examine whether there was any functional overlap between the epitopes of MEDI-493 and RSHZ19. Biosensor chips with bound F protein were first exposed to MEDI-493, followed by either MEDI-493 or RSHZ19 (figure 5). This order of injection was chosen since MEDI-493 bound to the F protein–coated chip with a higher signal at the same concentration and a slower off rate than RSHZ19. The increase in binding (RU value) was determined after this second exposure and compared with the values obtained after initial binding of MEDI-493. The second administration of MEDI-493 resulted in a net increase of 2%, whereas that of RSHZ19 resulted in a significant RU increase, representing 33% of the RU obtained from a primary injection of RSHZ19. This indicates that MEDI-493 and RSHZ19 recognize distinct epitopes on the F protein antigen, although with some apparent overlap, since complete saturation of RSHZ19 could not be achieved once MEDI-493 was bound to the F protein antigen. Similar results were obtained when either 1 µM or 10 µM of MEDI-493 was used for the primary injection. These results suggest that prebound MEDI-493 either sterically inhibits binding to a structurally proximal epitope of RSHZ19 or makes the epitope for RSHZ19 less accessible through a conformational change in the antigen.

**Discussion**

We compared two humanized MAbs, MEDI-493 and RSHZ19 (SB 209763), that are directed to two distinct neu-
tralizing epitopes on the F glycoprotein of RSV. Each of these antibodies has received intense clinical study for the prophylaxis of lower respiratory tract disease caused by RSV. We conducted laboratory studies to determine whether the differences in the results of the clinical studies could, in part, be related to the neutralization and antigen-binding activities of each MAb.

In a recently completed multicenter, placebo-controlled phase III clinical trial involving 1502 at-risk infants, monthly im administration of MEDI-493 at a dose of 15 mg/kg body weight reduced the incidence of RSV-related hospitalization by 55% \( (P = .00004, \text{Fisher’s exact test}) \) [13]. In contrast, in a separate phase III clinical study involving 800 at-risk infants, RSHZ19 failed to significantly protect when administered im at 10 mg/kg body weight either monthly or bimonthly (D. Burch, personal communication). Although the clinical protocols were dissimilar in other respects (e.g., patient numbers and dosing regimens), results from the current study suggest that the difference in efficacy is due to the greater potency of MEDI-493 compared with RSHZ19. Clinical doses were selected for both MAbs because serum levels achieved in volunteers at the trough of primary dosing (30 days) exceeded the serum levels shown in the cotton rat model of RSV infection to result in \( \geq 99\% \) reduction in pulmonary RSV titers in all animals. In the current comparative studies, the clinical dose of MEDI-493 (15 mg/kg) exceeded the \( ED_{90} \) in cotton rats (2.5 mg/kg) by 6-fold. In contrast, the clinical dose of RSHZ19 (10 mg/kg) was equal to or 2-fold in excess of the \( ED_{90} \) in cotton rats (5 mg/kg), suggesting that a higher dose of RSHZ19 could be clinically effective.

The in vitro antiviral assays used in this study represent formats in which the antibody is added prior to (neutralization) or after (fusion inhibition) addition and attachment of RSV. The results of the assays were consistent, both showing that the \( IC_{50} \) for RSHZ19 is \( \sim 4 \)-fold higher than that for MEDI-493. There was uniform correlation between the increased potency of MEDI-493 compared with RSHZ19 in the studies presented here and in parallel in vitro and in vivo studies (including microneutralization, fusion inhibition, plaque reduction, and cotton rat protection) in other laboratories (W. Gruber, P. Wyde, V. Hemming, personal communications).

A number of studies have addressed the question of whether there is a correlation among virus-neutralizing antibodies between avidity and inhibition of viral replication. In this case, although RSHZ19 and MEDI-493 recognize distinct epitopes, such a relationship is evident in each of the assays: neutralization, fusion inhibition, and cotton rat prophylaxis. The apparent affinity of MEDI-493 for the RSV F protein was 3–37 times greater than that of RSHZ19, depending on the assay; this range could reflect methodologic differences between solution-phase interactions as measured in the calomel cylometry studies versus the BIAcore format in which the F protein is immobilized. Alternatively, subtle differences in the recombinant F proteins used could explain the differences. Nevertheless, both methods indicate the F protein affinity of MEDI-493 is significantly higher than that of RSHZ19. The slower off rate of MEDI-493 may also contribute to its improved efficacy.

The data suggest that, although the epitopes for MEDI-493 and RSHZ19 are distinct, they have a significant functional overlap. This may be reconciled in the fusion inhibitory activity of both antibodies, in that each antibody acts at an apparent distance from the “fusion” domain on the F protein. Fusion-inhibiting antibodies such as these might neutralize RSV by preventing binding of the fusion domain to the target membrane, inhibiting a structural rearrangement of the F protein required for fusion, or by inducing such a structural rearrangement prior to target membrane attachment. The potent fusion-inhibiting activity of both MEDI-493 and RSHZ19 suggests that this property may be responsible for their potency in vivo, although the current data do not exclude alternative mechanisms.

The RSV F protein has structural similarities to influenza hemagglutinin and human immunodeficiency virus gp41/gpl20, including predicted coiled coil structure(s), in and around the fusion domain and may adopt a similar “spring-loaded” conformation [24–26]. It is unknown whether, in addition to its higher avidity for the F protein, MEDI-493 preferentially impedes or otherwise energetically defuses the structural transition required for virus-cell or cell-cell fusion and is therefore more effective in neutralization. Further structural studies are required to distinguish the mechanisms utilized in the neutralization of RSV by these MAbs.

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References

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