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Prophylactic and therapeutic testing of Nicotiana-derived anti-RSV human monoclonal antibodies in the cotton rat model

Running title: Testing RSV mAbs in the cotton rat

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Footnote page

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ABSTRACT

Background. Respiratory syncytial virus (RSV) is the single most important cause of severe lower respiratory tract infection during infancy and early childhood worldwide. Synagis® (palivizumab) is a humanized anti-RSV IgG_1 monoclonal antibody (mAb) approved for RSV immunoprophylaxis in at risk neonates. Although Synagis is highly effective, ongoing pharmacoeconomic analyses suggest Synagis may not be cost-effective.

Methods. R19 and F2-5, two human Fabs, and RF-1 and RF-2, two human IgG mAbs, have previously been described as having potent RSV neutralizing activity. We produced these as fully human IgGs in a rapid, scalable, and inexpensive Nicotiana-based manufacturing system. The purified mAbs were tested them in neutralization assays and the cotton rat efficacy model to see if they might provide a cost-effective alternative to Synagis.

Results. All of the mAbs displayed neutralizing activity yet none provided protection *in vivo* comparable to Synagis or Nicotiana-derived Synagis (Synagis-N). Both Synagis and Synagis-N also showed significant ($P<0.05$ and <0.001 respectively) efficacy in a therapeutic protocol.

Conclusions. Synagis remains one of the most potent and efficacious RSV mAbs described to date. Production in plants results in a functionally equivalent molecule (Synagis-N) that offers the potential to reduce manufacturing costs and thereby improve the pharmacoeconomics of RSV immunoprophylaxis and therapy.

Key words: RSV, monoclonal, antibody, prophylaxis, therapy, Nicotiana

INTRODUCTION

RSV, a member of the *Pneumovirus* genus of the Paramyxoviridae family, causes relatively mild common cold symptoms in immunocompetent adults. However, RSV is the single most important cause of both severe lower respiratory tract infection during infancy and early childhood worldwide, and of infant hospitalization in developed countries. Nearly all children have had at least one RSV infection by 2 years of age and nearly one third of infants who have primary RSV-mediated infections develop lower respiratory tract infections. Furthermore, RSV lower respiratory tract illness in early childhood is an independent risk factor for the subsequent development of wheezing in children up to age 11 years [1]. Each year, RSV is estimated to be responsible for up to 125,000 pediatric hospitalizations, with an associated mortality rate of approximately 2%. Among hospitalized infants with chronic lung and congenital heart disease (CLD and CHD), the RSV mortality rate may reach 5%. RSV bronchiolitis is the leading cause of infant hospitalization annually [2]. Up to half of all pediatric admissions for bronchiolitis and one-quarter of admissions for pneumonia are due to RSV. RSV also causes significant morbidity and mortality in certain adult populations. For example, 5-10% of elderly patients in long-term care facilities develop RSV infections per year with rates of pneumonia and death of 10-20% and 2-5% respectively [3]. RSV is also a common lower respiratory infection in hematopoietic stem cell transplant patients. In these patients, the incidence of RSV infection typically ranges from 1-15 % and rates of pneumonia following RSV infection can be as high as 80% with death rates of 70-80% of those who develop pneumonia [4]. There are an estimated 175,000 adults hospitalized annually in the U.S. alone with RSV infection [5]

The MedImmune product, Synagis® (palivizumab), entered the market in September 1998. Synagis is an intramuscular version of a murine humanized RSV IgG_1 mAb (anti-F-protein) that is routinely used to prevent serious lower respiratory tract disease caused by RSV in pediatric

patients at high risk of RSV disease. This prophylactic treatment consists of monthly injections in the thigh during the course of RSV season. In pivotal clinical trials, Synagis treatment resulted in a 55% reduction in hospitalizations among high-risk pediatric patients [6]. Although Synagis is an effective drug for RSV immunoprophylaxis in at-risk neonates, ongoing analyses on the pharmacoeconomics demonstrate that Synagis is not cost-effective in actual use, nor even when its use is tightly restricted to its labeled population [7-12]. For example, in one study, the cost-effectiveness of passive immunization against RSV was evaluated by assessing the incremental costs incurred to prevent one hospitalization in high-risk children using an individualized monthly approach. In no instance was cost-effectiveness observed [13]. It has been estimated that 16-18 patients must be treated to prevent one RSV-related hospitalization [14,15]. Prophylaxis with palivizumab is not a treatment with adequate cost/benefit based on the incremental cost-effectiveness ratio (ICER) threshold when used unselectively in children without CLD/CHD or children with CLD or CHD [16].

Several fully human mAbs against glycoprotein F (gF) have been described [17-19] that could be alternatives to Synagis. RF-1 and RF-2 were isolated from human SPL-SCID mice [17] and reported to have IC_{50} ranging from 8-165 ng/mL and 30-1000 ng/mL, respectively, against 13 RSV strains (type A and B). Human Fabs 19 and F2-5 were isolated from human phage display libraries and had IC_{60} ranging from 0.2 – 3.0 μg/mL (19 isolates of type A and B) and 0.1-1.4 μg/mL (11 isolates), respectively [18,19]. Both of these Fabs were found to significantly reduce lung viral titer when applied topically to RSV-infected mice [19,20]. None of these 4 antibodies (RF-1, RF-2, Fab 19, F2-5) has yet been tested in the cotton rat model, the model used for the development of Synagis [21].

Manufacturing of mAbs in plants, although first reported in 1989 [22] as a potential means of cheaper production, has been slow to develop as a viable alternative to traditional manufacturing in bioreactors using mammalian cell culture. Recently, Nicotiana-based manufacturing of antibody

products has made large strides due to two major technological advances: (a) viral based transient expression allowing high accumulation of antibody in days [23,24]; (b) transgenic plants with altered glycosylation pathways that can produce antibody with mammalian glycoforms [25]. Although purification costs are similar to mammalian production of mAbs, raw materials and capital expenses are significantly reduced for plant manufacturing of mAbs [26]. Lowering the cost of RSV treatment could expand the patient populations in which immunoprophylaxis would be cost-effective [7,12] and could make immunotherapy pharmacoeconomically viable.

RESULTS

Production of mAbs

mAbs were generated using a viral-based transient expression system (magnICON) and transgenic *N. benthamiana* in which plant-specific glycans (α1,3 fucosyl-transferase and β1,4 xlylosyl-transferase) are eliminated by RNAi inhibition of plant-specific glycosyl-transferases [25]. Approximately 1 kg of plants were infiltrated with the appropriate vectors and after 7-8 days, plants were homogenized with a commercial juicer. Extract was clarified and IgG purified by protein A chromatography (**Table 1**). Purified mAbs were of greater than 95% purity as assessed by SDS-PAGE and HPLC-SEC, and all mAbs except for F2-5 had low levels of aggregate ($\leq$ 3%). An additional purification step was performed on F2-5 resulting in elimination of detectable aggregates (**Table 1**).

Neutralization testing of mAbs

Over the course of these studies, mAbs were tested in three labs using standard neutralization assays against a variety of strains (**Table 2**). All mAbs displayed neutralizing activity, though mAbs RSV19 and RF-1 were in general the least potent. RF-2 displayed activity similar to Synagis and

Nicotiana-derived Synagis (Synagis-N) although its activity wasn't as broad-spectrum. F2-5 had activity similar to Synagis and Synagis-N, though it wasn't tested against as many strains.

Kinetic analyses of mAb binding to recombinant F protein

The dissociation constant (KD) was determined using surface plasmon resonance (Biacore) with an NTA chip capturing HIS-tagged RSV gF (strain A2). The results are shown in **Table 2**. Interestingly, we found the affinities of the mAbs for antigen did not correlate with neutralizing activity. F2-5 was a more potent neutralizer than Synagis of the A2 strain, but had a weaker binding affinity. Similarly, RF-1 and RF-2 had superior binding affinities than Synagis, but poorer neutralizing activity.

Prophylaxis in the cotton rat model

Prophylaxis studies (**Table 3**) followed the protocol used in the development of Synagis [21]. Cotton rats received intramuscular injections of mAb one day prior to challenge (strain RSV/Tracy, subtype A) [27]. Nasal and lung lavage samples were taken upon necropsy on day four post-challenge. Only Synagis and Nicotiana-derived Synagis (Synagis-N) provided significant reductions in the nasal viral titer ($P<0.05$ to $P<0.001$ depending upon the experiment), although these reductions were relatively minor (2-5 fold). In contrast, all of the mAbs tested *in vivo* (RSV19 and RF-1 were not tested in cotton rats due to their poor neutralization activity relative to Synagis) provided significant ($P<0.01$ to $P<0.001$) reductions in lung viral titer compared to untreated controls. The reduction provided by Synagis and Synagis-N was superior to that seen for F2-5 and RF-2. Given its weak in vitro neutralizing activity against the challenge strain (**Table 2**), the weak protection conferred by RF-2 was not surprising. However, F2-5 had similar neutralizing activity (and superior antigen binding activity), so the poor protection observed was unexpected. **Figure 1** combines the results from multiple experiments, and displays the relative reduction in lung titer compared to untreated controls for the mAbs tested at different doses.

Serum pharmacokinetics

Serum neutralizing titers and ELISA testing (data not shown) demonstrated that F2-5 had significantly reduced serum availability compared to Synagis ($t_{1/2}$ ~ 2 days). Synagis and Synagis-N had indistinguishable serum pharmacokinetics as assessed by ELISA (**Figure 2**), with serum half-lives of approximately 7 days.

Therapy

RF-2, Synagis and Synagis-N were tested therapeutically (**Table 4**) to determine whether mAb treatment could have potential use after an infection is established. When delivered one day after viral challenge, 5 mg/kg RF-2 failed to reduce lung titers significantly. In contrast, 5 mg/kg of Synagis and Synagis-N provided significant reductions (8- and 25-fold, respectively). Increasing the concentration demonstrated a dose-dependent reduction in lung titer for Synagis-N (**Figure 3;** it was cost-prohibitive to test Synagis at higher doses) with up to 540-fold reduction at the highest dose tested. Significant reductions ($P<0.001$) were also seen when Synagis-N was administered a full two days after viral challenge.

DISCUSSION

Production of mAbs using transient expression systems in Nicotiana is becoming routine in research laboratories. All of the mAbs expressed had reasonable yields, especially considering that no attempts were made to optimize expression or purification. All of the Nicotiana-derived mAbs tested had significant neutralizing activity *in vitro* and high affinity for recombinant gF. We observed that affinity for recombinant gF did not correlate with in vitro neutralization potency (**Table 2**). This would either suggest a difference between the conformation of recombinant and native gF or that the specific epitope plays a role in neutralization activity. Of the mAbs tested in vivo, all reduced lung viral titer in the cotton rat, but none were as effective as Synagis, with the exception of Synagis-N.

This observation of Synagis-N's functional equivalence to Synagis, combined with previous reports of Nicotiana-derived mAbs having binding activity indistinguishable from parental mAbs [28-31], would suggest the inferiority of the other mAbs tested was unrelated to the expression system. Indeed, the only demonstrated difference to date between mammalian-derived and plant-derived mAbs is glycosylation. For the mAbs produced here, a transgenic *N. benthamiana* line was used that yields mAbs with mammalian glycans predominantly of the GnGn glycoform [25]. Although this glycoform can enhance antibody dependent cellular cytotoxicity activity (ADCC) via higher affinity binding to the FcγRIII receptor [32], it does not influence other effector functions nor the long serum half-life of IgG conferred by binding to the FcRn receptor. This was confirmed by the pharmacokinetics seen in the cotton rat (**Figure 2**) – rodent FcRn is known to be promiscuous in its binding of IgG from other species [33].

In our study, F2-5, had a shorter serum half-life than the other mAbs (2 days vs. 7 days). Interestingly, this is a similar to an observation made by MedImmune in the development of Motavizumab, an affinity matured and more potent neutralizing version of Synagis [34]. Although MedImmune overcame the pharmacokinetic challenge with Motavizumab by introducing point mutations, licensure of the drug was subsequently rejected by the FDA due to its lack of superiority to Synagis and its increased rate of adverse skin reactions at the injection site. Development of the drug by MedImmune/AstraZeneca was subsequently stopped.

Dale Barnard 5/21/12 3:33 PM
Comment [1]: I think this is appropriate.

Larry Zeitlin 5/17/12 1:17 PM
Comment [2]: does this paragraph have value?

Although there are an estimated 175,000 adults hospitalized annually in the U.S. alone with RSV infection [5], data on the therapeutic usefulness of Synagis are very limited [35]. Studies in stem cell transplant patients (where mortality rates from RSV infection are extremely high) are inconclusive and likely have been limited by dosing costs. With current pricing, a 15 mg/kg dose of Synagis for a 50 kg adult costs approximately $125,000. We are unaware of any reports of therapeutic testing of Synagis in the cotton rat model. In this study we found Synagis and Synagis-N both provided

significant reductions in lung viral titer. If, like in the prophylactic context, a 100-fold reduction in titer is the target for clinical development [21], 30 mg/kg and 100 mg/kg were required to provide this level of reduction when delivered 1 and 2 days, respectively, after viral challenge.

A large proportion of the world's population does not have access to Synagis and for those populations that do, the pharmacoeconomic benefit is questionable. Monoclonal antibodies produced in the transient Nicotiana system are expected to reduce costs 2-5 fold compared to mAbs produced in mammalian cell culture [24,36]. In large part this is the result of the comparatively inexpensive infrastructure associated with producing the plant raw material. Within the last several years, Nicotiana-derived mAbs have been made under Good Manufacturing Practices (GMPs) and tested clinically [26,37]. This manufacturing platform may offer an alternative production system for reducing the cost of Synagis and thereby expanding the patient populations that can benefit from this potent mAb product.

METHODS

N. benthamiana expression vectors. RF-1 and RF-2 VRs were synthesized using the published sequences (Heard et. al. Molecular Medicine 5:35-45, 1999). The F2-5 variable regions were originally reported in Crowe et. al. [19] and deposited in GenBank (accession nos. L41061 and L41062). The VH sequence was used as reported. The VL (lambda) FR1 residues 1-3 and 8 were altered to be consistent Kabat. The RSV19 VRs was derived from F19 Fab [18]. The VH sequence was used as reported. The VL (kappa) FR1 residues 1-4 and FR4 residues 103-107 were altered to be consistent with Kabat. Synagis VR sequences were used directly as reported [21]. Genes were codon optimized and synthesized (GeneArt, AG) and subsequently cloned into plant (TMV and PVX) expression vectors (Icon Genetics, GmbH), followed by transformation into *Agrobacterium tumefaciens* strain ICF320 [37].

Production of anti-RSV mAbs in *N. benthamiana*. For transient expression of mAbs *in planta*, we used the ‘‘magnifection’’ procedure [Icon Genetics, Halle (Saale), Germany] as described [23], with minor modifications. Plants grown for 4 weeks in an enclosed growth culture room with 20-23˚C were used for vacuum infiltration. Equal volumes of overnight-grown Agrobacterium cultures were mixed in the infiltration buffer 10 mM MES pH 5.5 and 10 mM MgSO4 resulting in a 1:1000 dilution for each individual culture. The infiltration solution was transferred into a 20 L custom built (Kentucky Bioprocessing, Owensboro, KY) vacuum chamber. The aerial parts of entire plants were dipped upside down into the bacterial/buffer solution. A vacuum of 0.5 bars was applied for 2 min. Post infiltration, plants were returned to the growth room under standard growing conditions. Eight days post-infiltration, the leaf tissue was extracted in a juicer (Green Star, Model GS-1000), using 25 ml of chilled extraction buffer (100mM Tris, 40 mM ascorbic acid, 1mM EDTA) per 100 g of green leaf tissue. The plant-derived extract was clarified by lowering the pH of the extract to pH 4.8 with 1 M phosphoric acid then re-adjusting it to pH 7.5 with 2 M Tris base to insolubilize plant debris, followed by centrifugation at 16,000 x g for 30 min. The supernatant was transferred and re-centrifuged at 16,000 x g for an additional 30 min. The clarified extract was filtered through 0.2 µm prior to concentration via Minim Tangential Flow Filtration System (Pall) then 0.2 µm filtered again before loading onto 5 ml HiTrap MabSelect SuRe (GE Healthcare) Protein A column at 2 ml/min. The column then was washed with running buffer (50 mM HEPES/100 mM NaCl, pH 7.5) and eluted with 0.1 M acetic acid, pH 3.0. The resulting eluate was neutralized to pH 7 using 2 M Tris, pH 8.0 and supplemented with Tween 80 to 0.01%. The mAb solution was then polished via Q filtration (Mustang Acrodisc Q membrane; Pall), aliquoted and stored at -80 °C until used. All mAbs were fully assembled and of greater than 95% purity as determined by SDS-PAGE. Endotoxin levels were measured with Endosafe PTS (Charles River) and were <100 EU/mg.

Kinetic testing of mAb binding to recombinant RSV F protein. The association and dissociation rates of RSV F recombinant protein with mAbs were measured on a Biacore X100. The dissociation constant (KD) was determined using an NTA chip to capture recombinant gF (strain A2). Each mAb (diluted in NTA running buffer) was then flowed over the chip at 5 different concentrations (with the highest concentration having an Rmax between 30-80 RUs) and kinetic analyses using BIAEvaluation software performed (1:1 fit). Fast flow rates and controls (including a flow cell with no receptor, and immobilized receptor with flow of buffer only) were performed to insure against acquiring mass transfer-limited data.

Labs A and C: Microneutralization assay. [Tony, please correct any errors]

Tests were performed in 96-well microtiter plates with HEp-2 cells prepared in 2% FBS-MEM. Plaque purified RSV/Tracy (RSV serotype A) or RSV18537 (RSV serotype B) was used in the microneutralization assay. Serial two-fold dilutions (50 µL) in duplicates starting at 3 $\log_2$ were performed to determine the neutralizing titer for each sample. The neutralizing antibody titer is defined as the serum dilution at which >50 % reduction in viral cytopathic effect (CPE) is observed. CPE is determined visually after the wells are fixed with 10% neutral buffered formalin and stained with crystal violet. Neutralizing antibody titers were categorical log numbers and not continuous values. The lowest detectable titer was 2.5 $\log_2$. Samples with non-detectable titers were assigned a value of 2 $\log_2$.

Lab B: Neutralization assay.

The neutralizing activity of the antibodies was measured by a plaque reduction assay using HEp-2 cell culture. The activity was measured in µg/ml and calculated as the lowest concentration that reduced plaque number by 50%. 125 µl of diluted of RSV calculated to yield 50 plaques per well was mixed with 125 µl of 1:4 dilutions of mAb, and incubated at 37 °C for 60 min. Cell monolayers in 24-well tissue culture plates at 80-90% confluency were inoculated in

triplicate by replacing media in each well with 100 µl of virus-mAb mixture. After incubation at 37 °C for 1 hr, each well's monolayer then was overlaid with 0.75% methylcellulose in Opti-MEM I (Invitrogen) supplemented with 2% FBS, 320 µg/mL l-glutamine, 2.7 µg/mL amphotericin B, and 45 µg/mL gentamicin. Cultures were incubated for 4 days at 37 °C in 5% CO2 after which the overlay was removed and the monolayers were fixed in 80% cold methanol. Plaques were stained and quantified by an immunoperoxidase staining procedure. Plaques for each mAb dilution were counted, triplicates averaged, and mAb dilution vs. plaque number plotted. The plaque reduction neutralizing activity (µg/ml) was determined by regression curve analysis.

Cotton rat testing.

Cotton rats (60-125 gm body weight) were obtained from xxxx and housed yyyy. Groups of 5-6 animals were used in all studies. mAbs were injected either intraperitoneally or intramuscularly (see **Table 3**). Control IgG was a Nicotiana-derived human IgG against *C. difficile* toxin A. Challenge virus (RSV-Tracy, 2.25 x 10^5 PFU) was adminstered intranasally (100 µL) to cotton rats lightly anesthesized with isoflurane. Animals were euthanized 4 days after challenge. Following euthanasia with CO_2, each cotton rat was weighed and the sex and age recorded. The left and one of the large right lobes of the lungs was removed, rinsed in sterile water to remove external blood contamination and weighed. The left lobe was transpleurally lavaged using 3 mL of Iscove's media with 15% glycerin mixed with 2% FBS-MEM (1:1, v:v) in a 3 mL syringe with a 26g3/8 needle and injecting at multiple sites to totally inflate the lobe. Lavage fluid was recovered by gently pressing the inflated lobe flat and used to transpleurally lavage the right lobe following the same technique. The lavage fluid was collected and stored on ice until titered. For nasal washes of the upper respiratory tract, the jaw was disarticulated. The head was removed and 1 mL of Iscove's media with 15% glycerin mixed with 2% FBS-MEM (1:1, v:v) was pushed through each nare (total of 2 mL). The effluent was collected from the posterior opening of the pallet and stored on ice until titered. Samples were frozen before titration which

occured at the end of all sample collections. RSV Tracy lung lavage titers (PFU/gm lung) and nasal wash titers (total PFU) were determined by plaque assay. Plaque assays were performed using 24-well tissue cultures plates containing nearly confluent monolayers (20 to 40 $x10^4$ cells/well) of HEp-2 cells prepared in 10% FBS 24 hr prior to start of assay. At the start of each assay, dilutions (serial log_{10}) were made of the test samples. A 0.2 mL sample of each was added to wells in duplicate and allowed to adsorb for 90 min in a 36°C, 5% CO2 incubator with occasional gentle agitation. After the inoculum was removed, the monolayers were overlayed with 0.75% methylcellulose in 2% FBS-MEM containing antibiotics, vitamins and other nutrients. Tissue culture and positive virus controls were included in each assay. The plates were placed in a 36°C, 5% CO2 incubator. Day 6 + 1 day later, plates were stained with 0.1% crystal violet/10% formalin solution (~1.5 mL/well) and allowed to sit for 24-48 hr at room temperature. Wells were rinsed with water. All of the plaques in wells containing approximately 20 to 80 plaques were enumerated, averaged and the virus titers calculated as total log_{10} PFU for nasal wash fluid or log_{10} PFU/g of tissue for lungs. The lower limit of detection by this method was approximately 0.7 total log_{10} PFU for nasal wash fluid or 1.5 log_{10} PFU/g lung tissue. Comparisons of the groups for each experiment were performed by ANOVA. When ANOVA indicated there was at least one treatment that differed significantly from the control, then a follow up Dunnett's pairwise comparison test was performed. Statistical analyses were performed using Prism 5 for Mac OS X.

Figure 1

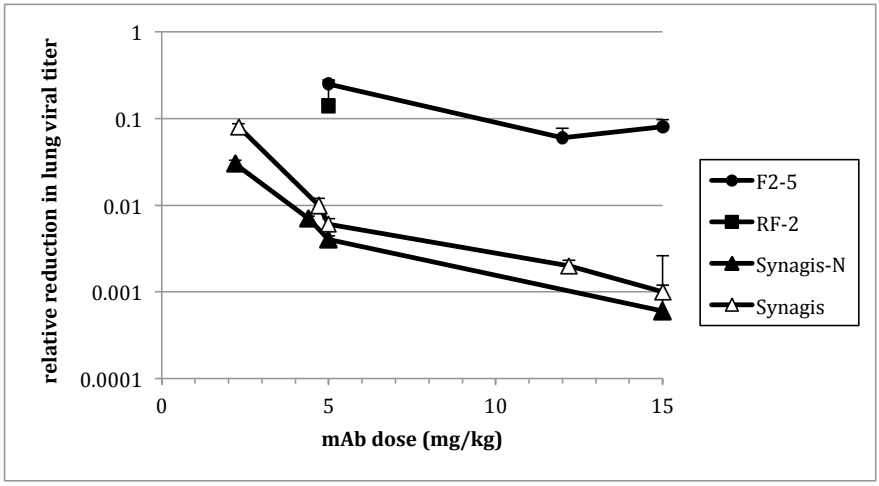

Figure 1. Relative reduction in RSV lung titer in cotton rats receiving mAb prophlyactically. Animals received mAb one day prior to challenge (RSV Tracy) and viral titer was determined four days post-challenge. Error bars denote standard deviation.

Figure 2

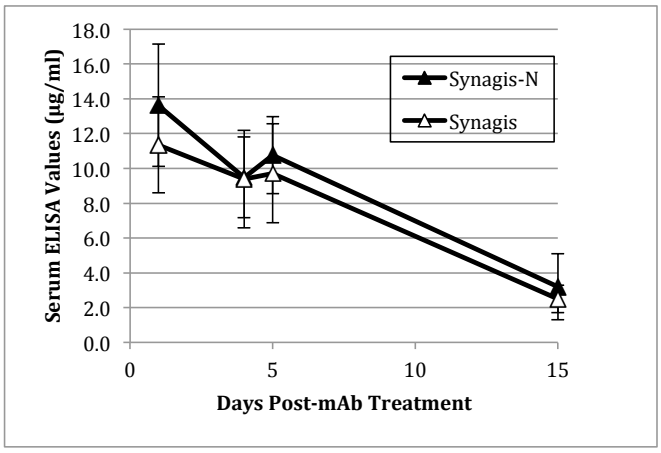

Figure 2. Serum levels of Synagis and Synagis-N in cotton rats. Animals (n=6) were treated with 5 mg/kg of mAb intramuscularly and serum level of mAb assessed by ELISA. Error bars denote standard deviation.

Figure 3

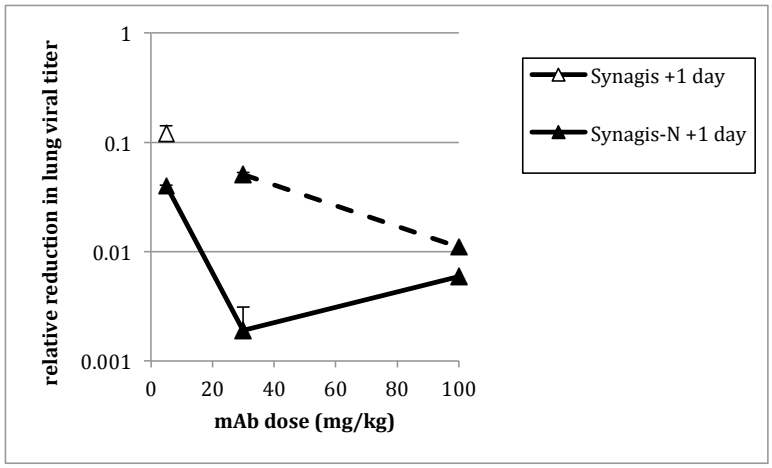

Figure 3. Relative reduction in RSV lung titer in cotton rats treated therapeutically. Animals received mAb (5, 30, or 100 mg/kg) one or two days after challenge (RSV Tracy) and viral titer was determined four days post-challenge. Error bars denote standard deviation.

Table 1. Purification yield and % aggregate of Nicotiana-derived mAbs

	RSV19	F2-5	RF-1	RF-2	Synagis-N
Yield post-protein A (mg/kg)	24	58	117	54	180
% aggregate[1] post protein A	0	15/0[2]	0	0	3

Synagis-N = Synagis amino acid sequence produced in Nicotiana.

[1] as determined by HPLC size exclusion chromatography. [2] Ceramic hydroxyapatite chromatography was used for the F2-5 sample to remove aggregates from the post-protein A preparation.

Table 2. Neutralization activity and antigen binding affinities of the RSV mAbs

		Viral Strain	mAb					
			RSV19	F2-5	RF-1	RF-2	Synagis-N	Synagis
IC_{50} (µg/mL)	Lab A	Tracy	7.3	3.6	190	21	1.9 ± 0.8[1]	1.5 ± 0.8[1]
		18537	5.2	1.8	190	60	3.0	-
	Lab B	A2	3	0.07	-	-	-	0.6
	Lab C	A2	-	-	>10	0.14	0.08	0.08
		9320	-	-	0.3	0.1	0.39	0.15
		F	-	-	1.2	0.15	0.31	0.12
		Long	-	-	>10	1.8	0.23	0.23
KD ($\times 10^{-10}$ M)	Biacore	A2	160 ± 110	4.9 ± 1.7	1.0 ± 0.1	0.8 ± 0.1	1.6 ± 0.2	2.0 ± 0.5

"-" = not tested. [1] Neutralization of these samples was tested 7 times – once before each cotton rat study. Averages and standard deviations are shown.

Table 3. Prophylaxis in the cotton rat

		Nasal Wash Samples					Lung Lavage Samples				
Treatment	Dose (mg/kg)	Nasal titer (log_{10} total PFU)	SD	Change (log_{10})	P-value (vs Control)	Fold reduction from control	Lung titer (log_{10} PFU/g)	SD	Change (log_{10})	P-value (vs Control)	Fold reduction from control
F2-5 IgG (IP)	15	4.95	0.1	0.1			3.96	0.82	-0.93	<0.01	9
F2-5 IgG (IP)	5	4.90	0.14	0.05			4.3	0.21	-0.59		
Synagis	15	4.60	0.26	-0.25	<0.05	2	2.2	0.35	-2.69	<0.001	490
Control IgG (IP)	15	4.88	0.09	0.03			5.12	0.18	0.23		
Control		4.85	0.14								
Synagis-N (IP)	15	4.45	0.2	-0.55	<0.001	4	1.88	0.8	-3.21	<0.001	1600
Synagis	15	4.33	0.16	-0.67	<0.001	5	1.91	0.46	-3.02	<0.001	1000
Control		5.00	0.15				4.93	0.66			
F2-5 IgG	12	4.45	0.58	-0.09			3.7	1.05	-1.21	<0.05	17
Synagis	12.2	4.41	0.46	-0.13			2.25	0.33	-2.66	<0.001	460
Control		4.54	0.41				4.91	0.26			
Synagis-N	2.2	5.01	0.04	0			3.5	0.32	-1.53	<0.001	33
Synagis-N	4.4	4.82	0.2	-0.19			2.85	0.19	-2.18	<0.001	140
Synagis	2.3	4.97	0.09	-0.04			3.93	0.34	-1.1	<0.001	13
Synagis	4.7	4.83	0.18	-0.18			3.03	0.56	-2	<0.001	100
Control		5.01	0.04				5.03	0.23			
Synagis-N	5	4.72	0.22	-0.41	<0.01	3	2.72	0.27	-2.42	<0.001	250
Synagis	5	4.74	0.12	-0.39	<0.01	2	2.9	0.46	-2.24	<0.001	170
RF-2	5	4.95	0.2	-0.18			4.31	0.21	-0.84	<0.001	7
Control		5.13	0.19				5.14	0.06			

IP = intraperitonal, SD = standard deviation. P-values were determined by ANOVA with a follow-up Dunnett's pairwise comparison test.

Table 4. Therapy in the cotton rat

Treatment	Dose (mg/kg)	Day of treatment	Nasal Wash Samples: Nasal titer (log_{10} PFU/g)	SD	Change (log_{10})	P-value (vs Control)	Lung Lavage Samples: Lung titer (log_{10} PFU/g)	SD	Change (log_{10})	P-value (vs Control)	Fold reduction from control
Synagis-N	4.2	+1	4.88	0.17	-0.22		3.57	0.46	-1.39	<0.001	25
Synagis	4.6	+1	5.25	0.26	0.16		4.06	0.7	-0.91	<0.05	8
RF-2	4.6	+1	5.06	0.21	-0.04		4.66	0.44	-0.31		
Control			5.10	0.12			4.97	0.18			
Synagis-N	30	+1	4.78	0.35	-0.09		2.49	0.57	-2.73	<0.001	540
Synagis-N	100	+1	4.91	0.22	0.04		2.99	0.6	-2.23	<0.001	170
Synagis-N	30	+2	4.84	0.22	-0.03		3.92	0.51	-1.29	<0.001	19
Synagis-N	100	+2	4.94	0.37	0.06		3.26	0.65	-1.95	<0.001	89
Control			4.87	0.22			5.21	0.13			

SD = standard deviation. P-values were determined by ANOVA with a follow-up Dunnett's pairwise comparison test.

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