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Sendai virus-based RSV vaccine protects African green monkeys from RSV infection

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Abstract

Respiratory syncytial virus (RSV) is a serious disease of children, responsible for an estimated 160,000 deaths per year worldwide. Despite the ongoing need for global prevention of RSV and decades of research, there remains no licensed vaccine. Sendai virus (SeV) is a mouse parainfluenza virus-type 1 which has been previously shown to confer protection against its human cousin, human parainfluenza virus-type 1 in African green monkeys (AGM). Here is described the study of a RSV vaccine (SeVRSV), produced by reverse genetics technology using SeV as a backbone to carry the full-length gene for RSV F. To test for immunogenicity, efficacy and safety, the vaccine was administered to AGM by intratracheal (i.t.) and intranasal (i.n.) routes. Control animals received the empty SeV vector or PBS. There were no booster immunizations. SeV and SeVRSV were cleared from the URT and LRT of vaccinated animals by day 10. Antibodies with specificities toward SeV and RSV were detected in SeVRSV primed animals as early as day ten after immunizations in both sera and nasal wash samples. One month after immunization all test and control AGM received an i.n. challenge with RSV-A2. SeVRSV-vaccinated animals exhibited reduced RSV in the URT compared to controls, and complete protection against RSV in the LRT. There were no clinically relevant adverse events associated with vaccination either before or after challenge. These data encourage advanced testing of the SeVRSV vaccine candidate in clinical trials for protection against RSV.

Keywords

Respiratory Syncytial Virus; Parainfluenza Virus; Jennerian vaccine; Protective Immunity

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INTRODUCTION

RSV causes serious lower respiratory tract infections in infants and is responsible for an estimated 160,000 deaths annually worldwide [1;2]. Antibody injections can provide effective prophylaxis against RSV disease, but due to high costs and logistical difficulties, these are not available to most infants, particularly in developing countries [3]. Vaccination remains the single best method for prevention of viral disease and yet there is no licensed vaccine despite more than 50 years of dedicated research [2;4–6].

Sendai virus (SeV) is a mouse parainfluenza virus type 1 (PIV-1) that has been previously developed as a vaccine for human PIV-1 (hPIV-1) [7;8]. SeV is an attractive Jennerian (xenogeneic) vaccine candidate, because it is sensitive to human interferon (IFN)-associated innate immunity [9], and there has never been a confirmed case of SeV-associated disease in humans. In non-human primates, it safely induces a protective immune response against hPIV-1 [7].

SeV elicits high-titered antibodies as well as robust CD8⁺ T cell activities [10;11] in small animals. Pre-clinical research has also demonstrated that virus-specific antibody forming cells and CD8⁺ T cells appear in the upper respiratory tract (URT), the site of expected pathogen exposure, within days after a single immunization. Responses are sustained for the lifetime of the animal and are associated with protective immunity [10;11]. These features emphasize the attraction of SeV-based vaccines for the induction of a protective immune response.

Recently, SeV has been used as a reverse genetics vector to produce recombinant vaccines against a variety of respiratory pathogens. Constructs have expressed genes from pathogens such as human PIV-2, PIV-3, and RSV. In cotton rats, these vaccines can be used individually or in combination to protect against respiratory virus challenge [12–16]. When three vaccines were combined, expressing foreign genes from PIV-2, PIV-3 and RSV, four different pathogen infections could be prevented (hPIV-1, hPIV-2, hPIV-3 and RSV). For RSV, when the fusion protein F gene was presented in the context of recombinant SeV, protection was elicited against both A and B RSV isolates [16].

The study described in this report examined SeVRSV as a vaccine against RSV in African green monkeys. Results showed that immune responses were mounted soon after vaccination. The response was associated with safe and complete protection against RSV infection of the LRT.

MATERIALS AND METHODS

Animals

African green monkeys (AGM) were feral caught and pre-screened to ensure sero-negativity against RSV and SeV. Twelve AGM were housed for vaccinations at BioQual (Rockville, MD) under BSL2 conditions as specified by the Association for Assessment and Accreditation for Laboratory Animal Care (AAALAC) guidelines. Prior to sample collections, vaccinations or challenges, animals were anesthetized with ketamine hydrochloride (Putney Inc., Portland, ME). Clinical assessments, sample collections, vaccinations and necropsies were conducted at Bioqual. Blood chemistries were conducted at the Idexx Research Laboratory (Totowa, NJ). The RSV challenge stock was pre-tested in two additional AGM that were feral caught, pre-screened to ensure sero-negativity, and housed at the Tulane National Primate Research Center (TNPRC). All animal housing, care and research was performed in compliance with the National Research Council Guide for the Care and use of Laboratory Animals, guidelines at TNPRC (accredited by AAALAC)

and in accordance with the Animal Welfare Act guidelines. Protocols were IACUC approved.

Vaccines

The previously described SeV Enders isolate was grown in the allantoic fluid of hen's eggs [7;8]. The SeVRSV vaccine was produced by reverse genetics by cloning the full-length RSV-F gene from the A2 strain (ATCC, VR-1302) into a T7-driven, full-length, modified SeV genome, between F and HN genes [12;13;16–18]. To rescue virus, 293T cells were infected with a UV-inactivated T7 RNA polymerase-expressing recombinant vaccinia virus (vTF7.3) for 1 h at 37°C. Cells were then co-transfected with the cDNA plasmid containing the recombinant genome described above, and three supporting plasmids expressing the NP, P and L genes of SeV. Cells were cultured for two days in trypsin-supplemented growth medium. Virus in cell supernatants was harvested and expanded in the allantoic fluid of hens' eggs. After stocks of SeV and SeVRSV were prepared, the viruses were purified on sucrose gradients and stored in 0.1% human serum albumin at –80°C.

Immunizations and challenge

On day –3 relative to vaccination, sera, nasal washes, throat swabs and bronchoalveolar lavages (BAL) were collected. To collect nasal washes, a 6 ml syringe and 18 G plastic needle were used to administer 4 ml saline into nasal cavities. Samples were collected into a sterile 15 ml tube. To collect BAL, a #14 French red rubber tube was placed in the trachea and advanced to the bronchi. A 12 ml syringe was used to administer 4 ml saline which was then retracted. Nasal washes, throat swabs and BAL samples were aliquoted immediately after sampling, and quick frozen.

On day 0, four animals received PBS by intranasal (i.n.) and intratracheal (i.t.) routes (1 ml by each route). Four additional animals received SeV, 1×10^6 EID₅₀ by the intranasal (i.n.) route and 1×10^6 EID₅₀ by the intratracheal (i.t.) route. A third group of four animals received SeVRSV, at a 1×10^6 EID₅₀ dose by the intranasal (i.n.) route and 1×10^6 EID₅₀ by the intratracheal (i.t.) route. Additional collections of sera, nasal washes, throat swabs and BAL were on days 3, 5, 7, 10, 14 and 25. On day 28, all animals were challenged with 1.4×10^6 pfu RSV A2 from infected Hep2 supernatants, in 1 ml by the i.n. route. This was considered day 0 for challenge. Samples were then taken on days 3, 5, 7, 10, and 14 and necropsy was performed on Day 20. The RSV A2 challenge stock virus (ATCC, Rockville, MD) had been confirmed infectious by prior i.n. inoculations of two dedicated AGM at the Tulane National Primate Research Center.

SeV and SeVRSV measurements

For SeV or SeVRSV detection, samples were serially diluted in DMEM, 0.5 mg/ml gentamicin, 2mM L-glutamine and 5 µg/ml acetylated trypsin, and added to 96-well plates of LLC-MK₂ cell monolayers in 100 µl. After an overnight incubation, cultures were supplemented with 100 µl DMEM, 0.5 mg/ml gentamicin, 2mM L-glutamine, and 0.15% BSA. After an additional 4 day incubation, 50–100 µl supernatants from wells were used for standard hemagglutination (HA) assays with an equal volume of chicken red blood cells for 30–60 min. TCID₅₀ were calculated using the Reed-Muench formula.

Immune responses

ELISAs were conducted to compare antibody levels at prescreen and on days 10, 14 and 25. Tests for SeV- and RSV-specific antibodies were each performed. For the SeV ELISA, purified virus was first disrupted using 10× disruption buffer (0.05 M Tris, pH 7.8, 0.6 M KCL and 0.5% Triton X-100), brought to a concentration of 10 µg/ml in PBS and added to

ELISA plates at 50 μ l/well. After overnight incubation at 4°C, plates were washed once with PBS and blocked with 3% BSA in PBS for 1–2 hr or overnight. Test samples were diluted in 3% BSA/0.1% Tween in PBS and added to washed plates for 1 hr at 37°C. Plates were washed 7X with 0.1% Tween in PBS. Plates were developed with Goat anti Monkey IgG conjugated to Alkaline Phosphatase (Rockland) in 3% BSA and 0.1% Tween in PBS for 1 hr at 37°C followed by another 7X wash and p-nitrophenyl phosphate (Sigma) used at 1.0 mg/ml in diethanolamine buffer. Assays were read at O.D. 405 nm after 15–30 minutes on a microplate reader (Model 3550, BioRad). For RSV antibody measurements, ELISA plates were coated with purified RSV F. This antigen was prepared by collecting allantoic fluid from eggs infected with a SeV recombinant that expressed secreted RSV F, followed by purification of F protein on an anti-RSV F (Synagis®/Palivizumab) affinity column. RSV ELISAs were conducted with diluted antibodies and developing reagents as described above.

SeV-specific microneutralization titers

One day prior to SeV microneutralization assays, LLC-MK₂ cells were plated at 2×10^5 cells/ml in 96-well flat-bottomed tissue culture plates. On the following day, diluted serum samples were plated in replicate at 100 μ l/well in a separate 96-well plate. Virus was then added at ~15 TCID₅₀/well. After a 1 hour incubation at 37°C, 5% CO₂, media were removed from LLC-MK₂ cell culture plates and replaced with the antibody-virus mixtures. Cells were again incubated overnight. Supernatants were then removed and replaced with DMEM medium with 0.1% BSA, glutamine, gentamicin and 2–4 μ g acetylated trypsin. After an additional three days of culture, plates were developed by ELISA. To conduct the ELISA, supernatants were aspirated and cells were fixed for one minute with 100 μ l acetone/DPBS (80:20 volume ratio, DPBS from LONZA#17-513F). Wells were washed with DPBS. A mixture of two purified murine monoclonal anti-Sendai virus antibodies (S/2 and M57/1) conjugated to horse radish peroxidase was next added to wells in 1% BSA. After one hour at room temperature, wells were washed 6X with DPBS. Assays were developed with TMB Peroxidase and the color reaction was stopped by adding 4N H₃PO₄. Assays were read at OD 450 nm. ‘Max’ controls for the microneutralization assays were wells with virus only and negative controls were wells with medium only. For test wells, an OD reading of $\leq 50\%$ the average ‘Max’ reading was considered positive for neutralization. The neutralization titer for a given test sample was scored as the highest dilution that supported neutralization in $\geq 50\%$ wells.

RSV-specific microneutralization titers

RSV microneutralization assays were the same as described for the neutralization assay above, with a few exceptions. Hep2 cells served as targets for virus growth and were grown in EMEM with 10% FBS, glutamine and gentamicin (no trypsin was used). The RSV A2 virus was plated at 50–100 plaques per well. The developing antibody for the ELISA was horse radish peroxidase-conjugated anti-RSV (Synagis®) antibody.

RSV measurements

For RSV measurements, serially diluted samples in EMEM medium supplemented with glutamine and gentamicin were added to Hep-2 cell monolayers in 12-well plates; after 1 h at 37°C and 5% CO₂, the virus was removed and wells were overlaid with EMEM medium supplemented with glutamine, antibiotics, 10% fetal calf serum and 0.75% methylcellulose. After incubation for 5 to 6 days at 37°C and 5% CO₂, the methylcellulose was removed, cells were washed with PBS and fixed with formalin phosphate, and the plates were stained with hematoxylin and eosin for enumeration of plaques.

Histopathology analyses

Tissues were collected in 10% neutral buffered formalin (with fixative volume used at 15–20X tissue volume). Tissues were processed at St. Jude on an automated tissue processor (ThermoFisher Excelsior Processor). This involved six changes of graded alcohol, three changes of xylene and three changes of paraffin. Tissue was then embedded in paraffin (Richard-Allen Type 9) and sectioned on a rotary microtome (4 microns). Sections were mounted on positively charged glass slides (Superfrost Plus, Fisher Scientific, Pittsburgh, PA) and stained using an automated stainer (ThermoFisher Gemini Stainer) with hematoxylin and eosin for evaluation by P. Vogel, an ACVP board-pathologist.

RESULTS

Vaccination and clearance

Twelve African green monkeys (AGM) were used in this study. Four animals were vaccinated with PBS as controls. Four additional control animals were vaccinated with SeV (1×10^6 EID₅₀ i.n. and 1×10^6 EID₅₀ i.t.). The four test animals were vaccinated with SeVRSV (1×10^6 EID₅₀ i.n. and 1×10^6 EID₅₀ i.t.; Figure 1A). The schedule for evaluations and challenges (with RSV A2 on day 28) is shown in Figure 1B.

A first test was to determine the persistence of vaccines in the URT and LRT. For virus measurement, samples of nasal wash (NW), throat swab (TS) and bronchoalveolar lavage (BAL) were collected on days 0, 3, 5, 7, 10 and 14 relative to the day of vaccination. Virus measurements were on LLC-MK2 cell monolayers. Figure 2 shows the titers of SeV and SeVRSV in NW (top panels) and TS (bottom panels), with data from individual animals in panels A and C and group averages in panels B and D. The SeV and SeVRSV vaccines had similar characteristics in that peak titers were observed on day 3 or 5 and virus was cleared from all animals by day 14. Replication-competent virus could be detected in all animals that received SeV or SeVRSV, but there was no virus in animals that received PBS. To view SeV and SeVRSV titers in comparison with a previously reported candidate RSV vaccine, data were compared to the mean nasopharyngeal peak viral titer of a PIV-3-based RSV vaccine (b/h PIV-3 RSV F2). That type of vaccine has already advanced to an international pediatric clinical trial [19–21]. Its mean peak viral titer was $10^{5.6}$ pfu after a dose of $2\text{--}3 \times 10^5$ pfu was administered to AGM by i.n. and i.t. routes (Figure 2B asterisk [22]). The SeV and SeVRSV vaccines appeared to be more attenuated than b/h PIV-3 RSV F2 in that mean peak viral titers were $<10^3$ in the NW following higher vaccine doses of 2×10^6 EID₅₀. These comparisons illustrate SeV and SeVRSV attenuation relative to another candidate RSV vaccine. Results should be viewed with some caution, because assays for the two viruses were not conducted side by side. When TS were examined, mean peak viral titers for SeV and SeVRSV were between 10^3 and 10^4 (Figures 2C, D). In Figure 3 are shown results for BAL. Here, the mean peak viral titer was approximately 10^4 for SeVRSV (Figures 3A and 3B show plots of individual viral titers and mean titers, respectively). Again, titers were considerably less than the 10^7 pfu value described for b/h PIV-3 RSV F2 (Figure 3B asterisk)[22]. Results again indicate a relative non-permissiveness for SeV growth in non-human primates. The high attenuation and host-range restriction of SeV-based vaccines are characteristics that have also been described in previous publications [7;9].

Development of binding and neutralizing antibodies after vaccination with SeV or SeVRSV

SeV vaccines are attractive respiratory virus vaccine candidates in part because they induce immune responses after one week that persist for many months after a single i.n. vaccine, in small animal models [12]. To test the immunogenicity of SeVRSV in the non-human primate model, we examined anti-SeV and anti-RSV antibodies in blood and NW. Serum antibody binding responses are shown in Figure 4. SeV-specific antibodies are shown in

panels A, B and C for animals that received PBS, SeV and SeVRSV, respectively. RSV-specific antibodies are shown in panels D, E and F. As demonstrated, SeV specific antibodies were evident in all animals that received SeV (panel 4B) or SeVRSV (panel 4C), but not PBS (panel 4A). For RSV F specific antibodies, only the four SeVRSV-primed animals were positive (panel 4F).

The neutralizing capacity of serum antibodies against SeV and RSV was tested on day 25 as shown in Table 1. All animals that received SeV or SeVRSV exhibited positive neutralizing antibody responses toward SeV and all animals that received SeVRSV exhibited positive neutralizing antibody responses toward RSV.

NW samples were also tested for binding antibody responses (Figure 5). IgG antibodies specific for both SeV (panels A, B, C) and RSV (panels D, E, F) were identified in the NW. IgA responses were also present. Like IgG, IgA antibodies were generally detected at sample dilutions up to 1:50 (data not shown). The antibody content in NW was weaker and more variable than in serum. Results nonetheless demonstrated that SeV and SeVRSV vaccines could induce antibodies in both blood and mucosa. The latter is particularly attractive as antibodies in the airway can act as a constitutive first line of defense against an invading respiratory pathogen.

Protection against challenge in the URT

A next question concerned the potential of the SeVRSV vaccine to protect against a challenge with RSV. RSV was administered i.n. at 1.4×10^6 pfu per animal on day 28 after vaccination (Figure 1B). A time course for challenge virus titers in NW and TS samples is shown in Figure 6. NW RSV titers in control animals that received PBS or SeV vaccinations are shown respectively with blue and red lines in Figure 6A. NW results for animals that received SeVRSV are shown in Figure 6B (green lines). For TS samples, results are shown for control animals that received PBS or SeV with blue and red lines in Figure 6C while test animal results are shown in Figure 6D (green lines). As demonstrated, virus was much reduced in the SeVRSV animals in URT samples compared to controls. Seven of eight control animals shed replication-competent virus into the nasal wash, but only one of four test animals shed virus (in this case on day 5). This animal also exhibited virus in the TS on day 5 (panel 6D). One other SeVRSV-vaccinated animal (Z035) exhibited virus in the TS sample on days 3 and 5. In contrast, all eight control animals exhibited virus in TS on days 3 and 5 and in some cases also on days 7 and 10.

Complete protection against RSV growth in the LRT

A most important analysis concerned the growth of RSV in the LRT, as determined by BAL measurements. In Figure 7, RSV challenge virus is shown for PBS and SeV controls in Panel A (blue and red lines respectively). Three of the PBS and four of the SeV controls shed virus at some time during a 10 day time course. In contrast, there was no virus on any test day in any animal that received SeVRSV (panel B, green lines). Differences between control and test animals were statistically significant ($p=0.01$, Fishers Exact Test). Results demonstrated that a single vaccination with SeVRSV conferred complete protection against RSV in the LRT.

Throughout the experiment, animals were monitored for safety. Analyses included physical exams, eye exams, body weights, body temperatures, food consumption, blood chemistries, coagulation including fibrinogen, CBCs and lung X-rays. Twenty days after challenge, all animals underwent necropsy. A full list of tissues were weighed and examined for gross pathology, and a fraction of tissues (brain, olfactory bulbs, trachea, tonsils/pharynx, larynx, lung and lymph nodes) were sectioned for histopathology measurements. Veterinarians

deemed animals to be healthy throughout the study based on physical exams, ophthalmologic exams, radiographical exams, weight gain, lack of elevated body temperature, good appetite, normal stool and normal behavior. Minor deviations in hematology, clotting or chemistry values were random among animals and did not correlate with vaccinations. Upon histopathology examinations, the only findings were minimal to mild spots of lymphohistiocytic inflammation around terminal and respiratory bronchioles in all animals. Representative examples of these lesions are shown in Figure 8 for PBS (panel A), SeV (panel B) and SeVRSV (panel C) primed animals. The number of bronchioles observed with detectable spots were 3, 1, 4 and 1 for the individual PBS controls, 1, 2, 6 and 8 for individual SeV vaccinated animals, and 35, 11, 21 and 11 for SeVRSV-vaccinated animals. Spots were associated with no respiratory ailments or X-ray abnormalities. In total, there were no vaccine-related clinically-relevant signs of toxicity or adverse events after vaccination or after challenge throughout the study.

DISCUSSION

The clinical development of RSV vaccines began approximately ½ century ago with the study of a formalin-treated RSV (FI-RSV) product. Unfortunately, vaccinated children were not protected from RSV infection and were hospitalized at a significantly higher rate than placebo controls after a subsequent natural exposure to RSV. Two deaths were associated with vaccination [23–27]. Results were perhaps due to the absence of robust RSV-specific neutralizing antibodies in the face of robust CD4+ T cell activity [23;28;29].

Immunogenicity and protection with recombinant SeV

This report describes a promising vaccine candidate for RSV, termed SeVRSV. Results showed that antibody responses toward SeVRSV could be detected within 10 days of vaccination. Antibody responses were associated with neutralizing activities toward SeV and RSV. Neutralizing titers toward RSV were comparable to those previously defined as protective, in pre-clinical and clinical studies [28;30–32]. Upon RSV challenge, SeVRSV-vaccinated animals exhibited reduced RSV in the URT and complete protection against virus in the BAL. There were no clinically relevant adverse events associated with the vaccine throughout the vaccination or challenge process, or at necropsy. There were minimal to mild spots of lymphohistiocytic inflammation around terminal and respiratory bronchioles in all animals upon necropsy. The spots were most frequent in vaccinated animals. This might be expected, because viral antigens are known to persist for many weeks after an infection [33], and because the SeVRSV vaccinated animals exhibited an RSV F-specific adaptive immune response.

Our finding that LRT protection was superior to URT protection was reminiscent of other findings in the field. After a first exposure to RSV, an individual is often protected from LRT disease due to the combined beneficial effects of adaptive and innate immune responses [1;2;34]. However, even at the adult stage, individuals are susceptible to RSV infections of the URT [35]. Possibly, natural URT infections without LRT involvement are beneficial in that they may boost immune responses in the absence of serious clinical complications.

Pre-clinical and clinical studies with anti-RSV antibodies (e.g. polyclonal antibodies or Synagis®) have also shown differences in the prevention of URT and LRT infections. Specifically, it has been demonstrated that a ~10X greater titer of serum antibody is necessary to mediate a two-log reduction of RSV in URT versus LRT tissues [30;31]. Because the reduction of lung infection and consequent disease is most desired, clinicians prescribe Synagis® to achieve trough levels sufficient to prevent LRT, not URT infection [36;37]. A vaccine such as SeVRSV may ultimately prove superior to Synagis® as a

prophylactic measure, because LRT protection may be: (i) less expensive to achieve, (ii) easier to achieve logistically, and (iii) more durable.

Attenuated phenotype of SeV-based vaccines

Previous studies by our laboratory and others have shown that SeV can protect AGM from infection with hPIV-1. SeV was associated with no adverse events in both AGM and chimpanzees [7;38]. To gain some sense of SeV attenuation, one may compare its growth in non-human primates with growth of another clinically tested Jennerian vaccine, bovine PIV-3 (bPIV-3). A previous study of bPIV-3 showed that an i.t. dose of 10^4 TCID₅₀ was followed by a mean peak tracheal lavage fluid viral titer of 10^3 . In contrast, a 10-fold higher i.n./i.t. dose of 10^5 TCID₅₀ SeV was followed by a mean peak viral titer of $<10^3$ [38;39]. These data suggested a relatively high attenuation of SeV in non-human primates compared to bPIV-3 (with the caveat that tests were not conducted at the same time).

The current study has allowed an additional comparison between SeV-based and bPIV-3-based vaccines. In this case our RSV vaccine on the SeV backbone (SeVRSV) was compared to a RSV vaccine on the chimeric bovine/human PIV-3 backbone (b/h PIV-3-RSV F2). The latter vaccine concept is currently being tested in a large, international pediatric clinical trial [19–21]. This chimeric virus combines membrane proteins HN and F from the human PIV-3 source with internal proteins from the bovine PIV-3 source. When b/h PIV-3-RSV F2 was tested in AGM, mean peak viral titers reached $10^{5.6}$ in the URT and 10^7 in the BAL following i.n./i.t. doses of $2-3 \times 10^5$. These reported peak titers were orders of magnitude greater than the mean peak viral titers of SeV and SeVRSV despite the administration of SeV or SeVRSV with 10-fold higher i.n./i.t. doses of 2×10^6 (see Figures 2B and 3B). Again, one must consider that tests were not conducted in parallel. Nonetheless, results reinforce the notion that SeV-based products are highly attenuated in non-human primate models. The indication of SeV attenuation is consistent with the finding that there have been no clinically relevant symptoms in any primate experiments with SeV or SeVRSV. The indication of low permissiveness for SeV in the primate respiratory tract is also consistent with the finding that SeV is sensitive to the IFN-associated innate immune responses of humans [9].

SeVRSV as a RSV vaccine candidate

As described above, SeVRSV is an attractive vaccine in that it may provide protection against both RSV and human PIV-1, two serious pathogens of children [40]. SeV is a pathogen of mice and not of humans [6;9] and despite ample opportunity for human exposure to SeV in the laboratory or by contact with pet or wild mice, there has never been a confirmed case of SeV-related human disease. SeV has also been shown to elicit protective immune responses within seven days of vaccination and for many months thereafter in the absence of a booster, a feature typical of replication-competent vaccines [10;11;41–45]. The present demonstration that SeVRSV is attenuated and protective against RSV in primates, coupled with SeV's positive safety profile in a current Phase I clinical trial [8], encourages rapid development of SeVRSV as a candidate pediatric vaccine. Clinical trials may ultimately provide a true test of vaccine safety and efficacy in humans.

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HIGHLIGHTS

- SeVRSV, a recombinant Sendai virus expressing RSV F, was tested in AGM
- SeVRSV elicited antibody responses against SeV and RSV
- SeVRSV fully protected AGM from lower respiratory tract RSV infection
- SeVRSV caused no clinically relevant adverse events
- SeVRSV is an attractive RSV vaccine candidate

A.

Animal #	Vaccine (i.n. + i.t.)	Challenge (i.n.)
R305	PBS	RSV
R368	PBS	RSV
Z033	PBS	RSV
Z034	PBS	RSV
X793	SeV	RSV
Z031	SeV	RSV
Z032	SeV	RSV
Z036	SeV	RSV
R178	SeVRSV	RSV
Y774	SeVRSV	RSV
Z008	SeVRSV	RSV
Z035	SeVRSV	RSV

B.

Figure 1. Experimental design for SeVRSV vaccine analysis

Animals were grouped to receive PBS, SeV or SEVRSV as vaccines by the i.n. and i.t. routes, and were later challenged with RSV (Panel A). Time points for collections, vaccinations and challenges are diagrammed in Panel B.

Figure 2. SeVRSV presence in the URT

Animals were sampled on days 3, 5, 7, 10 and 14 for vaccine virus in the NW or TS. NW results from individual animals are shown in panel A. Averages are in Panel B. Results for animals that received PBS, SeV or SeVRSV are indicated by blue, red and green lines, respectively. The asterisk and solid line in Panel B indicate the mean peak viral titer in nasopharyngeal swab samples for a PIV-3-based RSV vaccine described in previous literature [22]. TS results from individual animals are shown in Panel C. Averages for TS are shown in Panel D. ND=Not Detected.

Figure 3. SeVRSV presence in the LRT

Animals were sampled on days 3, 5, 7, 10 and 14 for vaccine virus in the BAL. Results from individual animals are shown in Panel A. Averages for BAL are shown in Panel B. Results for animals that received PBS, SeV or SeVRSV are indicated by blue, red and green lines, respectively. The asterisk and solid line in Panel B indicate the mean peak viral titer in BAL samples for a PIV-3-based RSV vaccine described in previous literature [22]. ND=Not Detected.

Figure 4. Serum antibodies in primed animals

Animals were sampled prior to vaccination and on days 10, 14 and 25 for serum antibodies. Panels A, B, and C show responses to SeV by animals vaccinated with PBS, SeV and SeVRSV, respectively. Panels D, E, and F show responses to RSV by animals vaccinated with PBS, SeV and SeVRSV, respectively.

Figure 5. Antibodies in the nasal wash of primed animals

Animals were sampled prior to vaccination and on days 10, 14 and 25 for nasal wash antibodies. Panels A, B, and C show responses to SeV by animals vaccinated with PBS, SeV and SeVRSV, respectively. Panels D, E, and F show responses to RSV by animals vaccinated with PBS, SeV and SeVRSV, respectively.

Figure 6. Protection against RSV challenge in the URT

Animals were sampled after challenge on days 3, 5, 7, and 10 for RSV in the NW (A,B) and TS (C,D). Results for PBS and SeV control animals are shown in left panels as blue and red lines, respectively. Results for SeVRSV-primed animals are shown in right panels as green lines. ND=Not Detected.

Figure 7. Protection against RSV challenge in the LRT

Animals were sampled after challenge on days 3, 5, 7, and 10 for RSV in the BAL. Results for PBS and SeV control animals are shown in left panels as blue and red lines, respectively. Results for SeVRSV-primed animals are shown in right panels as green lines. ND=Not Detected.

Figure 8. Spots of lymphohistiocytic inflammation around terminal and respiratory bronchioles
Upon histopathology examinations, there were minimal to mild spots of lymphohistiocytic inflammation around terminal and respiratory bronchioles in all animals. Illustrations of spots in animals that received A. PBS, B. SeV, and C. SeVRSV are shown.

Table 1

Animal #	Vaccine	SeV Neutralization Titer ^a (Log base 2)	RSV Neutralization Titer ^b (Log base 2)
R305	PBS	ND	ND
R368	PBS	ND	ND
Z033	PBS	ND	ND
Z034	PBS	ND	ND
	Group mean±SD	ND	ND
X793	SeV	320 (8.3)	ND
Z031	SeV	2560 (11.3)	ND
Z032	SeV	1280 (10.3)	ND
Z036	SeV	320 (8.3)	ND
	Group mean±SD	1120±531 (10.1)	ND
R178	SeVRSV	1280 (10.3)	2560 (11.3)
Y774	SeVRSV	1280 (10.3)	320 (8.3)
Z008	SeVRSV	640 (9.3)	320 (8.3)
Z035	SeVRSV	640 (9.3)	160 (7.3)
	Group mean±SD	960±185 (9.9)	840±575 (9.7)

^a Serum neutralization assays were conducted on day 25 after vaccination. Titers are shown for both (a) SeV and (b) RSV. ND=Not detected (titer<80).

Mean titers and standard errors are shown. Values are also converted to Log base 2 to facilitate titer comparisons with previous literature.