Vaccine xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

A novel method for strict intranasal delivery of non-replicating RSV vaccines in cotton rats and non-human primates

Michael P. Citron [a,*], Manishkumar Patel [b], Mona Purcell [b], Shu-An Lin [b], Daniel J. Rubins [b], Paul McQuade [b], Cheryl Callahan [a], Alexa Gleason [b], Ioan Petrescu [c], Walter Knapp [c], Chinedu Orekie [d], Sai Chamarthy [d], Zhiyun Wen [a], Sinoeun Touch [a], Matthew Pine [a], Jane Fontenot [e], Cameron Douglas [a], Xiaoping Liang [a], Amy S. Espeseth [a]

[a] Infectious Disease/Vaccines, Merck & Co., Inc., Kenilworth, NJ 07033, United States
[b] Translational Imaging Biomarkers, Merck & Co., Inc., Kenilworth, NJ 07033, United States
[c] Safety Assessment and Laboratory Animal Sciences, Merck & Co., Inc., Kenilworth, NJ 07033, United States
[d] Biopharmaceutics & Specialty DF/Development, Merck & Co., Inc., Kenilworth, NJ 07033, United States
[e] The University of Louisiana New Iberia Research Center, New Iberia, LA 70560, United States

ARTICLE INFO

Article history:
Received 16 November 2017
Received in revised form 26 January 2018
Accepted 26 February 2018
Available online xxxx

Keywords:
Intranasal immunization
Animal models
Respiratory syncytial virus
Vaccine
Cotton rat and non-human primate

ABSTRACT

Respiratory syncytial virus (RSV) is the most common viral cause of bronchiolitis and pneumonia in children twelve months of age or younger and a significant cause of lower respiratory disease in older adults. As various clinical and preclinical candidates advance, cotton rats (*Sigmodon hispidus*) and non-human primates (NHP) continue to play a valuable role in RSV vaccine development, since both animals are semi-permissive to human RSV (HRSV). However, appropriate utilization of the models is critical to avoid mis-interpretation of the preclinical findings. Using a multimodality imaging approach; a fluorescence based optical imaging technique for the cotton rat and a nuclear medicine based positron emission tomography (PET) imaging technique for monkeys, we demonstrate that many common practices for intranasal immunization in both species result in inoculum delivery to the lower respiratory tract, which can result in poor translation of outcomes from the preclinical to the clinical setting. Using these technologies we define a method to limit the distribution of intranasally administered vaccines solely to the upper airway of each species, which includes volume restrictions in combination with injectable anesthesia. We show using our newly defined methods for strict intranasal immunization that these methods impact the immune responses and efficacy observed when compared to vaccination methods resulting in distribution to both the upper and lower respiratory tracts. These data emphasize the importance of well-characterized immunization methods in the preclinical assessment of intranasally delivered vaccine candidates.

1. Introduction

RSV is the most common viral cause of lower respiratory infection and because of a limited number of preventative and treatment options continues to be a major, global public health burden especially in the young and older adult populations, In the United States (U.S.) RSV related illness is responsible for 75,000–125,000 hospitalizations annually, especially in high risk populations including: infants with premature birth, chronic lung disease, or congenital heart disease [1–4]. There are 66,000–199,000 childhood RSV associated deaths per year worldwide, mostly from low and middle income countries as compared to the <500 deaths reported in the US [5,6]. In the elderly, the disease incidence is comparable to that of influenza virus with about 125,000 hospitalizations in adults 65 years old or older [7–10].

Despite the disease burden, there has been a six decade long drought for prophylactic treatment against RSV [11]. The gap is not due to a lack of effort; however. Numerous candidate vaccines have been evaluated preclinically through the years without clinical success. The vaccines tested had various forms, including live attenuated, subunit, particle or nucleic acid based; but, shared either one of two popular routes of administration; intramuscular or intranasal. Until the unmet medical need for a safe and effective vaccine against RSV is achieved it is necessary to consider both

* Corresponding author at: Infectious Disease/Vaccines, Merck & Co. Inc., WP14-3200, 770 Sumneytown Pike, West Point, PA 19486-0004, United States.
E-mail address: michael_citron@merck.com (M.P. Citron).

https://doi.org/10.1016/j.vaccine.2018.02.110
0264-410X/

routes of administration. Intranasal immunization stands out as an attractive route of immunization for several reasons. First because RSV enters the body via the mucosal sites in the upper respiratory tract and can therefore stimulate strong local and systemic antibody responses, including generation of the IgA isotype in the upper airway mucosa, the surrounding nasal-associated lymphoid tissue (NALT) as well as the distal compartments. These responses provide the first barrier to viral entry [12–15]. In addition, the intranasal route may be more appropriate for the seronegative pediatric population; first as a cocooning strategy for vulnerable populations such as the elderly, the immune-compromised and neonates. Furthermore, intranasal immunization may minimize the theoretical risk of priming for enhanced respiratory disease (ERD) due to antigens that become replication defective from a systemic delivery route of administration [16–20]. Lastly, novel mucosal adjuvants are becoming increasingly available, which could offer a solution for providing robust immune responses against natural infection, where reinfection is common [21–25].

Cotton rats and non-human primates (NHPs) in particular have been extensively used in RSV research for the last several decades to evaluate prophylactic monoclonal antibodies, vaccines and antiviral compounds [26–30]. Experiments using cotton rats supported the successful licensing of the only two commercially available anti-RSV specific products; Palivizumab (Synagis®) and RSV immunoglobulin (RSV-IVIG, RespiGam®) [31–35]. Various monkey species and chimpanzees, have derisked candidates entering human trials [36–42]. Yet, the methodology for intranasal immunizations in these systems has been poorly examined, despite evidence that immunizations can elicit markedly diverse immunological responses depending on whether delivered to the upper (URT) or lower (LRT) respiratory tract [43]. Studies in mice aimed at differentiating nasal and pulmonary immunization by varying the volume of inoculum or type of anesthesia used during the administration have been conducted to enable either nose or lung specific deposition [44,45]. However, similar methods have not been established in cotton rats or monkeys, which are considered more permissive to hRSV than many other commonly used laboratory animal species. In order to increase the utility of these species for RSV vaccine discovery, we first examined the biodistribution of commonly applied immunization methods using imaging and then systematically implemented techniques to limit the inoculum to the URT in both animal species. Applying the new, strict intranasal methods we evaluated the immune responses elicited and the protection conferred by intranasal administration of RSV specific vaccine candidates in both animal systems. Defining the methods for true intranasal delivery for these two preclinical species is important for increasing the translational relevance of the models, which has potential to derisk the assessment in the clinic.

2. Materials and methods

2.1. Protein conjugation and radiolabeling

RSV Protein conjugation was performed by combining 1.45 mg (25.9 nmol) DSCav1 RSV F prefusion protein, (RSV-F protein) and 0.22 mg (0.26 μM), 1, 4, 7, 10-tetraazacyclododecane-1, 4, 7, 10-tetraacetic acid mono *N*-hydroxysuccinimidyl ester (DOTA). The mixture was stirred for 18 h at 4 °C in 1 ml of Na_2HPO_4 (0.1 M, pH 8.5). Prefusion DSCav1 protein was generated using mammalian codon-optimized plasmids previously described [46]. Briefly, the DNAs were used to transfect Expi 293F cells (Life Technologies) and the resulting proteins were purified from the supernatants, exactly as described by Flynn et al. [47]. The reaction mixture was transferred to a Centricon YM-10 Centrifugal Filter (Millipore Corp., Milford, MA) and mixed with ammonium citrate (3.0 ml, 0.1 M, pH 5.5). The filter was centrifuged for 20 min. This process was repeated twice with an additional 4 ml of ammonium citrate added and then centrifuged. Purified DOTA-RSV was collected in ammonium citrate (900 μl), with the concentration determined by UV absorbance and stored at −70 °C in 100 μl aliquots. The protein was radiolabeled by adding 3 μl of $^{64}CuCl_2$ in 0.1 M HCl to 100 μl of DOTA-RSV (Conc. 1.37 μg/μL). The reaction mixture was incubated at 37 °C for 30 min and allowed to cool for 5 min before diethylenetriaminepentaacetic acid (DTPA, 3 μl, 10 mM) was added. ^{64}Cu DOTA-RSV (^{64}Cu-RSV) was purified via a Bio-Spin 30 column (Bio-Rad, Hercules CA) that had been pretreated with phosphate buffered saline (PBS). Specific activity and radiochemical purity was determined via a Waters Acquity LC/MS system (Milford, MA, USA) and a β-RAM Model 4 Radio-HPLC detector (IN/US Systems, Brandon, FL, USA).

2.2. Animal Experiments

All experiments involving laboratory animals were approved by the Merck Institutional Animal Care and Use Committee (IACUC) of Merck & Co., Inc., Kenilworth, NJ USA and were conducted in accordance with all the animal care and use laws, regulations and guidelines. The monkey challenge study performed at the New Iberia Research Center was also approved by the UL Lafayette IACUC.

2.3. Cotton rat imaging and biodistribution studies

Cotton rats, on DYET chow, were anesthetized with either inhaled isoflurane/oxygen (1–4%) or a Ketamine Xylazine (50–100 mg/kg/2–5 mg/kg) mixture in PBS and administered intramuscular (IM). Once animals were fully sedated a total of 1 nmol of AngioSense750 EX (PerkinElmer, Waltham MA) in volumes of 5, 10, 20, 40 and 100 μl was administered over both nares to each anesthetized animal (two per treatment group) using a pipette. AngioSense750 EX is a blood pool agent and was chosen due to the low likelihood of absorption into the bloodstream when introduced via the nasal cavity. Animals sedated with isoflurane were kept anesthetized using continuous gas via a nose cone post-administration of AngioSense750. Animals anesthetized using Ketamine/Xylazine were administered AngioSense750 about 15 min prior to dosing. The biodistribution of the dye was imaged using an IVISSpectrum (PerkinElmer, Waltham MA) with an ex/em filter of 745/800 nm. The nose of the animal was covered with black paper during imaging in order to visualize the biodistribution in the trachea and lungs. Analysis was performed using LivingImage 4.3.1 (PerkinElmer, Waltham MA).

2.4. NHP imaging and biodistribution studies

^{64}Cu-RSV was combined with 20% nanoemulsion mucosal adjuvant W805EC (NE), previously described [48] in either phosphate buffered saline (pH7.2) or Thixotropic hydrogel (2% Avicel). Domestically bred and raised African Green monkeys (*Chlorocebus sabaeus*) were dosed intranasally (approximately half into each nostril) for each of the following dosing paradigms: 250 μl or 100 μl of ^{64}Cu-RSV dosed, dropwise in a 1 ml syringe, 250 μl ^{64}Cu-RSV administrated via a VaxInators™ syringe device (Telefelex Inc., Salt Lake City, UT) or 250 μl ^{64}Cu-RSV formulated with Thixotropic hydrogel (2% Avicel). Animals were anesthetized with Ketamine/Xylazine. A 30 min positron emission tomography (PET) scan was initiated with the URT and LRT within the field of view (FOV) immediately before delivery. At the end of the scan, animals were switched to an infusion of Propofol at a rate of

Please cite this article in press as: Citron MP et al. A novel method for strict intranasal delivery of non-replicating RSV vaccines in cotton rats and non-human primates. Vaccine (2018), https://doi.org/10.1016/j.vaccine.2018.02.110