Vaccine 32 (2014) 3050–3057

Contents lists available at ScienceDirect

Vaccine

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

A respiratory syncytial virus (RSV) vaccine based on parainfluenza virus 5 (PIV5)

Shannon I. Phan[a], Zhenhai Chen[a], Pei Xu[a,b], Zhuo Li[a], Xiudan Gao[a], Stephanie L. Foster[a], Michael N. Teng[c], Ralph A. Tripp[a], Kaori Sakamoto[d], Biao He[a,*]

[a] Department of Infectious Diseases, University of Georgia, Athens, GA 30602, United States
[b] Intercollege Graduate Program in Cell and Developmental Biology, Pennsylvania State University, University Park, PA 16802, United States
[c] Division of Allergy and Immunology, Department, Department of Internal Medicine, University of South Florida, Tampa FL 33612, United States
[d] Department of Pathology, University of Georgia, Athens, GA 30602, United States

ARTICLE INFO

Article history:
Received 3 December 2013
Received in revised form 19 February 2014
Accepted 13 March 2014
Available online 8 April 2014

Keywords:
Vaccine
Respiratory syncytial virus
Parainfluenza virus 5

ABSTRACT

Human respiratory syncytial virus (RSV) is a leading cause of severe respiratory disease and hospitalizations in infants and young children. It also causes significant morbidity and mortality in elderly and immune compromised individuals. No licensed vaccine currently exists. Parainfluenza virus 5 (PIV5) is a paramyxovirus that causes no known human illness and has been used as a platform for vector-based vaccine development. To evaluate the efficacy of PIV5 as a RSV vaccine vector, we generated two recombinant PIV5 viruses – one expressing the fusion (F) protein and the other expressing the attachment glycoprotein (G) of RSV strain A2 (RSV A2). The vaccine strains were used separately for single-dose vaccinations in BALB/c mice. The results showed that both vaccines induced RSV antigen-specific antibody responses, with IgG2a/IgG1 ratios similar to those seen in wild-type RSV A2 infection. After challenging the vaccinated mice with RSV A2, histopathology of lung sections showed that the vaccines did not exacerbate lung lesions relative to RSV A2-immunized mice. Importantly, both F and G vaccines induced protective immunity. Therefore, PIV5 presents an attractive platform for vector-based vaccines against RSV infection.

1. Introduction

Respiratory syncytial virus is the most important cause of pediatric respiratory virus infection, and is a major cause of morbidity and mortality among infants, immune compromised individuals, and the elderly [1]. In the early 1960s, vaccination of infants with a formalin-inactivated RSV vaccine not only failed to protect against RSV disease during the following RSV season, but some vaccinees developed enhanced disease upon natural infection, resulting in increased rates of severe pneumonia and two deaths [2]. In the intervening years, a number of different approaches have been evaluated, including subunit vaccines, vectored vaccines, and live attenuated vaccines. However, there remains no licensed RSV vaccine. Therefore, there is a pressing need for a safe and effective vaccine for RSV.

Parainfluenza virus 5 (PIV5), a negative-sense, non-segmented, single-stranded RNA virus, is a good viral vector for vaccine development. PIV5 is safe, as it infects a large number of mammals without being associated with any disease except canine kennel cough [3–7]. Humans have been exposed to PIV5 [8–10], likely due to the wide use of kennel cough vaccines containing live PIV5, which dogs can shed after vaccination [11]. Given anti-PIV5 immunity in humans, anti-vector immunity may be a problem. Our recent studies indicate that pre-existing immunity to PIV5 does not negatively affect immunogenicity of a PIV5-based vaccine in dogs, demonstrating that pre-existing immunity is not a concern for using PIV5 as a vector. This result is consistent with the report that neutralizing antibodies against PIV5 do not prevent PIV5 infection in mice [13].

PIV5 has been used as a platform for developing vector-based vaccines against other viruses. A single-dose immunization of PIV5 expressing the rabies virus glycoprotein G protects mice against lethal rabies virus challenge [14]. Additionally, a single-dose inoculation of PIV5 expressing hemagglutinin (HA) or the NP protein of influenza virus protects against lethal H5N1 challenge in mice [15,16]. Importantly, intranasal administration of PIV5 is effective for eliciting robust mucosal immune responses [17], and is therefore ideal for vaccinating against respiratory pathogens.

* Corresponding author. Tel.: +1 706 542 2855.
E-mail address: bhe@uga.edu (B. He).

http://dx.doi.org/10.1016/j.vaccine.2014.03.049

Since an anti-RSV-F monoclonal antibody has been used to control RSV infection, it may be possible to develop an RSV vaccine by targeting RSV-F. Although several studies have implicated the G protein in RSV disease pathogenesis [18–21], prophylactic or therapeutic treatment with a monoclonal antibody (mAb 131-2G) specific to RSV-G mediates virus clearance and decreases leukocyte trafficking and IFN-γ production in the lungs of RSV-infected mice [22–26]. In this study, we have tested the efficacies of recombinant PIV5 expressing RSV-F (rPIV5-RSV-F) or RSV-G (rPIV5-RSV-G) as potential vaccines in mice.

2. Materials and methods

2.1. Cells and viruses

BSR-T7 cells were maintained in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS), 10% tryptose phosphate broth (TPB), 100 IU/mL penicillin, 100 μg/mL streptomycin (1% P/S; Mediatech Inc., Manassas, VA, USA), and 400 μg/mL G418 sulfate (Mediatech, Inc.). MDBK, BHK21, and Vero cells were maintained in the same media without TPB or G418.

To construct the plasmids for rescuing rPIV5-RSV-F or rPIV5-RSV-G, the coding sequence of the green fluorescent protein (GFP) gene in the BH311 plasmid [27], containing GFP between HN and L of the full-length PIV5 genome, was replaced with the RSV-F or RSV-G gene, respectively. rPIV5-RSV-F and rPIV5-RSV-G were rescued as described previously [27]. PIV5, rPIV5-RSV-F and rPIV5-RSV-G were grown in MDBK cells as described previously [27]. RSV A2 and rA2-Luc (RSV A2 expressing *Renilla* luciferase) were grown in Vero cells as previously described [21].

2.2. Immunoprecipitation and Western blots

Immunoprecipitation (IP) was performed as previously described [27]. A549 cells were infected with rPIV5-RSV-F or RSV A2 in 6-cm dishes. After 18–20 h, the cells were starved and metabolically labeled with ^{35}S-Met and ^{35}S-Cys for 3 h. The cells were lysed with whole-cell extraction buffer (WCEB; 50 mM Tris–HCl [pH 8], 280 mM NaCl, 0.5% NP-40, 0.2 mM EDTA, 2 mM EGTA, 10% glycerol) [28] and immunoprecipitated with anti-RSV-F antibody. The IP products were resolved on a 10% SDS-PAGE gel and visualized using a Typhoon 9700 Phosphorimager (GE Healthcare Life Sciences, Piscataway, NJ, USA).

To examine RSV-G protein expression, rPIV5-RSV-G-infected MDBK cells and RSV A2-infected A549 cells were lysed with WCEB. The lysates were processed and resolved by SDS-PAGE as described before. The proteins were transferred onto a polyvinylidene difluoride (PVDF) membrane and detected using mouse anti-RSV-G antibody (1:2000 dilution) as previously described [14].

2.3. Recombinant PIV5 growth curves and plaque assays

6-Well plates of Vero cells were infected with rPIV5-RSV-F, rPIV5-RSV-G, or PIV5 at a MOI = 5 or 0.01. 100 μL samples of supernatant were collected at 0, 24, 48, 72, 96, and 120 h post-infection. Virus was quantified by plaque assay as described in Chen et al. [14].

2.4. Immunization of mice

All animal experiments were performed according to the protocols approved by the Institutional Animal Care and Use Committee at the University of Georgia. Six-to-eight week-old female BALB/c mice (Harlan Laboratories, Indianapolis, IN, USA) were anesthetized by intraperitoneal injection of 200 μL of 2, 2, 2-tribromoethanol in tert-amyl alcohol (Avertin). Immunization was performed by intranasal administration of 10^6 PFU of rPIV5-RSV-F, rPIV5-RSV-G, or RSV A2 in a 50 μL volume. Negative controls were treated intranasally with 50 μL of PBS.

Three weeks post-immunization, blood was collected *via* the tail vein for serological analysis. Four weeks post-immunization, all mice were challenged intranasally with 10^6 PFU of RSV A2 in a 50 μL volume. Four days later, lungs were collected from 5 mice per group to assess viral burden. The lungs of the other 5 mice in each group were perfused with 10% formalin solution and sent for histology. To detect neutralizing antibody titers, mice were immunized as described above and terminally bled 4 weeks post-immunization.

2.5. ELISAs measuring total RSV antigen-specific IgG, IgG1 and IgG2a

RSV-F and RSV-G-specific serum antibody titers were measured by ELISA. Immulon® 2HB 96-well microtiter plates were coated with 100 μL of purified RSV-F or G protein at 1 μg/mL in PBS [21] and incubated overnight at 4 °C. Two-fold serial dilutions of serum were made in blocking buffer (5% nonfat dry milk, 0.5% BSA in wash buffer; KPL, Inc., Gaithersburg, MD, USA). 100 μL of each dilution was transferred to the plates and incubated for one hour at room temperature. After aspirating the samples, the plates were washed three times with wash buffer. Secondary antibody was diluted 1:1000 [alkaline phosphatase-labeled goat anti-mouse IgG (KPL, Inc.) or horseradish-peroxidase-labeled goat anti-IgG1 or IgG2a (SouthernBiotech, Birmingham, AL, USA)] in blocking buffer. 100 μL of diluted secondary antibody was added to each well, and the plates were incubated for one hour at room temperature. After aspiration, the plates were washed and developed with 100 μL of pNpp substrate or SureBlue Reserve TMB substrate (KPL, Inc.) at room temperature. The OD was read at 405 nm or 450 nm using a BioTek Epoch microplate reader. The endpoint antibody titer was defined as the highest serum dilution at which the OD was greater than two standard deviations above the mean OD of the naïve serum.

2.6. Neutralizing antibody assay

Two-fold serial dilutions of serum were made starting at a 1:10 dilution with Opti-MEM supplemented with 1% BSA and 5% guinea pig complement (Sigma–Aldrich, St. Louis, MO, USA). The diluted serum was incubated with 100 $TCID_{50}$ of RSV A2 expressing *Renilla* luciferase (rA2-Rluc) for one hour at 37 °C, 5% CO_2 [29]. The serum and virus mixture was transferred to confluent monolayers of Vero cells in 96-well plates and incubated for 18 h at 37 °C, 5% CO_2. The cells were then lysed with 70 μL/well of *Renilla* lysis buffer for 20 min while shaking on an orbital shaker. The lysates were transferred to V-bottom plates and clarified by centrifugation at 2000 × *g* for 5 min 40 μL of clarified lysate was transferred to Costar® white 96-well assay plates (Corning, Inc., Corning, NY, USA) and read using a GloMax® 96 microplate luminometer (Promega). Neutralizing antibody titers were reported as the highest serum dilution at which the luminescence measurement was lower than that of 50 $TCID_{50}$ of rA2-Rluc based on a standard curve. Cells treated with 100 $TCID_{50}$ of UV-inactivated rA2-Luc were the negative control.

2.7. Titration of RSV from mouse lungs

Mouse lungs were harvested aseptically into gentleMACS M tubes (Miltenyi Biotec Inc., Auburn, CA, USA) containing 3 mL of Opti-MEM with 1% BSA and stored on ice. Lungs were homogenized at 4 °C using the Protein_01 program of a gentleMACS Dissociator (Miltenyi Biotec Inc.) and then centrifuged at 3000 × *g* for 10 min. RSV titers in the supernatants were determined using plaque assay