Virology 511 (2017) 142–151

Contents lists available at ScienceDirect

Virology

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

Virus-like particle vaccine primes immune responses preventing inactivated-virus vaccine-enhanced disease against respiratory syncytial virus

Hye Suk Hwang[a,b], Young-Tae Lee[a], Ki-Hye Kim[a], Eun-Ju Ko[a], Youri Lee[a,b], Young-Man Kwon[a,b], Sang-Moo Kang[a,*]

[a] Center for Inflammation, Immunity & Infection, Institute for Biomedical Sciences, Georgia State University, Atlanta, GA 30303, USA
[b] Department of Microbiology, Chonnam National University Medical School, Gwangju, Republic of Korea

ARTICLE INFO

Keywords:
VLP priming
Vaccine-enhanced disease
Respiratory syncytial virus

ABSTRACT

Formalin inactivated respiratory syncytial virus (FI-RSV) vaccination caused vaccine-enhanced respiratory disease (ERD) upon exposure to RSV in children. Virus-like particles presenting RSV F fusion protein (F VLP) are known to increase T helper type-1 (Th1) immune responses and avoid ERD in animal models. We hypothesized that F VLP would prime immune responses preventing ERD upon subsequent exposure to ERD-prone FI-RSV. Here, we demonstrated that heterologous F VLP priming and FI-RSV boosting of mice prevented FI-RSV vaccine-enhanced lung inflammation and eosinophilia upon RSV challenge. F VLP priming redirected pulmonary T cells toward effector CD8 T cells producing Th1 cytokines and significantly suppressed pulmonary Th2 cytokines. This study suggests that RSV F VLP priming would modulate and shift immune responses to subsequent exposure to ERD-prone FI-RSV vaccine and RSV infection, suppressing Th2 immune-mediated pulmonary histopathology and eosinophilia.

1. Introduction

A formalin inactivated respiratory syncytial virus (FI-RSV) vaccine in the alum adjuvant formulation did not confer protection in children, and rather caused severe disease after subsequent infection with RSV, resulting in 80% hospitalizations and deaths of two children (Kim et al., 1969). Key features of vaccine-enhanced respiratory disease (ERD) by FI-RSV vaccination include T helper type 2 (Th2) immune responses, eosinophil infiltrations into bronchoalveolar lavage fluids, mucus production in the airways, and severe lung histopathology (Graham et al., 1993; Murphy et al., 1988; Olson and Varga, 2007; Polack et al., 2002).

Animal models of RSV vaccination have been informative in understanding the mechanisms of RSV vaccine-ERD. Both RSV fusion (F) and attachment (G) protein vaccines induce RSV neutralizing antibodies and confer protection against lung viral replication (Connors et al., 1992b; Quan et al., 2011). In addition to FI-RSV, other RSV vaccine platforms such as soluble F proteins with postfusion or prefusion conformation, or G proteins, recombinant vaccinia virus expressing RSV G (Vac-G), and G-deleted FI-RSV were reported to cause vaccine-ERD after RSV challenge (Castilow et al., 2007; Connors et al., 1992a; Hancock et al., 2001; Johnson et al., 2004; Murphy et al., 1990; Schneider-Ohrum et al., 2017). Immune responses to RSV G proteins appear to be more responsible for vaccine-enhanced respiratory disease compared to those to RSV F (Lee et al., 2014a). These studies utilizing mouse models suggest that priming of CD4 T cells, particularly Th2 cells, is a main driving factor leading to pulmonary eosinophilia in RSV vaccinated mice (Connors et al., 1992c). However, a vaccine priming protective innate and adaptive immune responses preventing ERD still remains to be developed.

Chimeric virus-like particles (VLP) vaccines composed of Newcastle disease virus (NDV) structural proteins (NP, M) and RSV G or F and G ectodomains were produced by DNA transfection of avian cells (McGinnes et al., 2011; Murawski et al., 2010). Chimeric NDV–RSV VLP vaccines were demonstrated to induce RSV neutralizing activity and IgG2a isotype antibodies indicating Th1 type immune responses (McGinnes et al., 2011; Murawski et al., 2010), which might have contributed to preventing vaccine-ERD. Clinical trials of safety and efficacy tests were reported with VLP vaccines that were produced in insect cells utilizing recombinant baculoviruses expressing influenza hemagglutinin and neuraminidase as well as matrix core proteins (Fries et al., 2013; Khurana et al., 2011; Lopez-Macias et al., 2011).

* Corresponding author.
E-mail address: skang24@gsu.edu (S.-M. Kang).

http://dx.doi.org/10.1016/j.virol.2017.08.022
Received 13 June 2017; Received in revised form 8 August 2017; Accepted 15 August 2017

Influenza virus matrix protein derived VLP vaccines containing RSV F (F VLP) or G (G VLP) produced in insect cells were shown to elicit Th1 type IgG2a isotype antibodies (Quan et al., 2011). Immunization of mice with RSV F VLP, F plus G VLP vaccines, or F and G VLP in combination with F DNA induced protection against RSV without vaccine-ERD (Kim et al., 2015; Lee et al., 2015, 2017). These studies suggest that appropriately balanced T cell immune responses are important for preventing pulmonary eosinophilia and histopathology in mice after vaccination and challenge.

RSV vaccine candidates should prime an immune response that is protective and safe. We hypothesized that F VLP would prime immune responses to avoid ERD by suppressing pulmonary inflammation and eosinophilia even after subsequent ERD-prone FI-RSV vaccination and RSV challenge. This study reports that F VLP priming of mice modulated and shifted immune responses toward Th1 pattern and effector CD8 T cell responses in response to subsequent FI-RSV vaccination, resulting in the prevention of RSV disease by ERD-prone FI-RSV vaccination and RSV infection. Findings in this study provide insight into mechanisms responsible for RSV vaccine-ERD and developing safe RSV vaccination.

2. Materials and methods

2.1. Preparation of FI-RSV and F VLP vaccines

FI-RSV was prepared by a method previously described (Kwon et al., 2014; Quan et al., 2011). Briefly, RSV was inactivated with formalin (1:4000 [vol/vol]) for 72 h at 37 °C. The formalin- treated RSV was pelleted by ultracentrifugation at 4 °C (30,000 rpm, 1 h) and then resuspended in serum-free medium. Inactivation of FI-RSV was confirmed by an immune-plaque assay and then adsorbed to alum adjuvant (4 mg/ml, aluminum hydroxide, Sigma Aldrich) for use in FI-RSV vaccination in this study. F VLP that presents RSV A2 fusion (F) protein was produced in insect cells co-transfected with recombinant baculoviruses expressing influenza virus M1 core protein and RSV F protein, and purified as previously described (Quan et al., 2011). The culture supernatants containing F VLP were collected by centrifugation (30,000 rpm, 1 h). The cleared supernatants containing F VLP were purified by sucrose gradient ultracentrifugation.

2.2. Mouse immunization and RSV infection

BALB/c mice (n = 5; Charles River Laboratories, Inc., Wilmington, MA) aged 6–8 weeks were used for vaccination. Mice were intramuscularly (i.m.) immunized at a 4-week interval; with FI-RSV (2 μg) + alum adjuvant (20 μg) prime – FI-RSV (2 μg) + alum adjuvant (20 μg) boost [FI-RSV/FI-RSV] or F VLP (10 μg) prime – FI-RSV (2 μg) + alum adjuvant (20 μg) boost for the heterologous [F VLP/FI-RSV] group. Blood samples were collected at 3 weeks after prime and boost administrations. Naïve control and vaccine-immunized mice were infected with 10^6 particle forming units (PFU) of RSV A2 under isoflurane anesthesia at 15 weeks after boost immunization to determine the efficacy of protection and to assess ERD. Body weight changes were monitored for 5 days. Bronchoalveolar lavage (BAL) fluids (BALF) from individual mice were collected by infusing 1 ml of PBS into the lungs *via* the trachea using a 25-gauge catheter (Exelint International Co., Los Angeles, CA) day 5 post challenge. Lung tissues were separately obtained for histology and cellular analysis. All animal studies were performed according to the guidelines of Georgia State University Institutional Animal Care and Use Committee (IACUC) and the approved IACUC protocol.

2.3. Lung viral titration and RSV neutralization assays

RSV titers were determined for lung samples collected at day 5 post challenge as previously described (Hwang et al., 2014). The viral titer detection limit is 50 PFU in the lung samples. RSV neutralizing activity of immune sera was measured using RSV expressing the red fluorescent monomeric Katushka 2 protein (A2-K-line19F) as previously described (Hwang et al., 2016). Briefly, 500 PFU of live A2-K-line19F RSV and serially diluted inactivated immune sera were added to the HEp-2 cell monolayer plates, adsorbed for 2 h at 37 °C, and then incubated at 37 °C for 2 days. The plates were fixed with 5% formaldehyde in PBS and fluorescence was measured under a fluorescence ELISA reader with excitation at 588 nm and emission at 635 nm (BioTek, Synergy H1 Hybrid Reader, VT, USA).

2.4. Antibodies, cytokines and chemokine assays

F protein-specific antibody responses were determined in immune sera by enzyme-linked immunosorbent assay (ELISA) using purified RSV F protein (100 ng/ml, BEI resources) as a coating antigen (Hwang et al., 2014). The levels of cytokines and chemokines, such as gamma interferon (IFN-γ), interleukin-4 (IL-4), IL-5, IL-6, IL-13 (eBioscience, San Diego, CA) and eotaxin (R & D Systems, Minneapolis, MN), in BALF or lung homogenates were measured using cytokine ELISA kits according to the manufacturers' instruction.

2.5. Cytokine expressing T cell analysis

BAL cells from individual mice were prepared day 5 post challenge for flow cytometry analysis. To assess IFN-γ, TNF-α, or IL-4 cytokine producing cells, BAL cells were stimulated with 4 μg/ml RSV peptides (Jiang et al., 2002; Olson and Varga, 2008); $F_{92\text{-}106}$ (ELQLLMQSTPATNNR) + $F_{85\text{-}93}$ (KYKNAVTEL) for CD8 T cells and $G_{183\text{-}195}$ (WAICKRIPNKK) for CD4 T cells for 5 h prior to staining of intracellular cytokines. Then, the cells were fixed and permeabilized using a Cytofix/Cytoperm kit according to the manufacturer's instructions (BD Biosciences). Intracellular cytokines and surface markers for T cells were stained with antibodies for IFN-γ, IL-4 (eBioscience), TNF-α (BioLegend), CD45, CD3, CD4, and CD8 (BD Biosciences). Stained cells were acquired on a FACSCanto flow cytometer (BD) and analyzed using FlowJo software (Tree Star, Inc., Ashland, OR).

2.6. Lung histopathology

Lung samples from individual mice were fixed in 10% neutral buffered formalin, transferred to 70% ethanol, embedded paraffin blocks, and sectioned into a thickness of 5 μm. Lung tissue sections were stained with hematoxylin and eosin (H & E), periodic acid-Schiff stain (PAS), and hematoxylin/ Congo red (H & CR) to assess pulmonary histopathologic changes, mucin expression, and eosinophils, respectively as described (Hwang et al., 2014). The magnitudes of histopathology and pneumonia in lung tissues, the bronchioles, blood vessels and interstitial space were scored on a scale of 0–3 by blinded observers using the severity scoring system as previously described (Meyerholz et al., 2009).

2.7. In vivo and in vitro acute stimulation of cytokines

For *in vivo* acute stimulation of cytokines, BALB/c mice (n = 5) received intraperitoneal (IP) injection of PBS (200 μl), FI-RSV (2 μg in 200 μl) with alum (20 μg) or F VLP (10 μg in 200 μl). The peritoneal cavities were harvested at 24 h after IP injection and used to determine cytokines. For *in vitro* stimulation, bone marrow (BM) derived dendritic cells (BMDCs) were generated from BM cells of BALB/c mice by culturing in the presence of granulocytes-monocyte colony stimulating factor (GM-CSF) as described (Ko et al., 2017; Lee et al., 2014b). BMDCs were *in vitro* stimulated with F VLP, FI-RSV + alum adjuvant, cultured for 48 h, and cytokines were determined in the culture supernatants.