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Intranasal immunization with a replication-deficient adenoviral vector expressing the fusion glycoprotein of respiratory syncytial virus elicits protective immunity in BALB/c mice

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ABSTRACT

Human respiratory syncytial virus (RSV) is a serious pediatric pathogen of the lower respiratory tract worldwide. There is currently no clinically approved vaccine against RSV infection. Recently, it has been shown that a replication-deficient first generation adenoviral vector (FGAd), which encodes modified RSV attachment glycoprotein (G), elicits long-term protective immunity against RSV infection in mice. The major problem in developing such a vaccine is that G protein lacks MHC-I-restricted epitopes. However, RSV fusion glycoprotein (F) is a major cytotoxic T-lymphocyte epitope in humans and mice, therefore, an FGAd-encoding F (FGAd-F) was constructed and evaluated for its potential as an RSV vaccine in a murine model. Intranasal (i.n.) immunization with FGAd-F generated serum IgG, bronchoalveolar lavage secretory IgA, and RSV-specific CD8+ T-cell responses in BALB/c mice, with characteristic balanced or mixed Th1/Th2 CD4+ T-cell responses. Serum IgG was significantly elevated after boosting with i.n. FGAd-F. Upon challenge, i.n. immunization with FGAd-F displayed an effective protective role against RSV infection. These results demonstrate FGAd-F is able to induce effective protective immunity and is a promising vaccine regimen against RSV infection.

Introduction

Human respiratory syncytial virus (RSV) is the major viral agent of severe lower respiratory tract illnesses in infants and young children worldwide [1], and causes an important illness in the elderly and adults with underlying risk factors such as immunodeficiency [2]. WHO-estimated global annual morbidity and mortality for RSV is 64 million and 160,000, respectively [3]. The economic impact of RSV-related disease is greater than influenza in relation to days lost from work [4]. Several approaches have been used to develop vaccines against RSV, but none of these has been approved for use in humans.

The replication-deficient first generation adenoviral vector (FGAd) is readily grown and purified in large quantities, and is able to express high levels of the transgene in dividing and non-dividing cells, therefore, it is considered to be an attractive vaccine vector. Many studies have shown that FGAd-based vaccines can elicit robust protective immune responses against live pathogens [5,6]. For RSV vaccine, this strategy has been used recently to express modified RSV attachment glycoprotein (G) which has successfully elicited long-term protective immunity against RSV infection in mice [7]. This provides evidence that an FGAd-based RSV vaccine is a promising candidate.

RSV-specific T-cell response plays a major role in the clearance of virus and in the clinical outcome of RSV infection [8]. A major problem in developing G-protein-associated vaccine is that G protein lacks MHC- I-restricted epitopes [9] and cannot elicit a CTL response [10]. Meanwhile, it has been shown that full-length G and secreted G are involved in pulmonary cell infiltration [11,12]. In contrast to G, RSV fusion glycoprotein (F) is a major target antigen of CTL in humans and mice. Additionally, F is sufficiently conserved between two RSV antigenic subgroups, A and B, and is capable of evoking cross-protective antibodies against both subgroups [13,14]. Moreover, F has been confirmed as an initiator of epithelial cell shedding, airway obstruction, secondary

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necrosis, and consequent inflammation [15]. Thus, F is one of the most important target antigens for RSV genetic engineering vaccine projects. However, as far as we are aware, no FGAd-F vaccine has yet been reported.

Here, we report that an FGAd-F vaccine induced not only systemic and mucosal antibody responses, but also a RSV-specific CTL response. The CD4+ T-cell immune response had a characteristic balance of Th1 and Th2 cell. Moreover, improved lung histopathology and reduced lung virus load were observed in the vaccinated mice following RSV challenge.

Materials and methods

Preparation of RSV stock. Subgroup A RSV Long strain (kindly provided by Y. Qian, Capital Institute of Pediatrics, Beijing, China) was propagated in HEp-2 cells in Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA) supplemented with 2% fetal calf serum, L-glutamine (2 mol/l), penicillin G (40 U/ml), streptomycin (100 μg/ml) and 0.2% sodium bicarbonate, and titrated for infectivity by 50% tissue culture infective doses (TCID50) per milliliter.

Construction of a replication-deficient adenovirus vector encoding RSV F protein. The recombinant adenovirus FGAd-F was generated in HEK293 cells using the AdEasy adenoviral vector system (a gift from Professor Bert Vogelstein, The Johns Hopkins Oncology Center, Baltimore, MD) according to the manufacturer's protocol (Stratagene, Cedar Creek, TX). The resulting FGAd-F contained standard DNA expression cassettes with the open reading frame (ORF) of wild-type RSV-F under the control of human cytomegalovirus (CMV) immediate early gene promoter. The viral particle number of purified adenoviral vector was determined by the nucleic acid content [16]. Control adenoviral vector (FGAdv) was generated by the same method using an empty adenoviral shuttle plasmid of pShuttle-CMV.

Animals. Specific pathogen-free female BALB/c mice, aged 6–8 weeks, were purchased from Shanghai SLAC Laboratory Animal Co and kept under specific pathogen-free conditions.

Immunization and challenge. BALB/c mice were lightly anesthetized by ether inhalation, and inoculated intranasally with 1×10^{11} viral particles (in 50 μl) of FGAd-F or FGAdv on weeks 0 and 3. Three weeks after the final immunization, mice were challenged intranasally with 100 μl of subgroup A RSV strain Long ($\log_{10}$ TCID50, 10^6/ml).

Collection of splenocytes. Spleens were harvested from vaccinated and control mice at day 7 after boosting, and placed in mouse lymphocyte separation medium. The spleens were triturated and ground gently through cell strainers (Becton–Dickinson, San Jose, CA) to obtain single-cell suspensions. The single-cell suspensions were centrifuged at 800*g* for 30 min. Then, splenocytes were collected and washed with complete 1640 medium.

ELISA. Blood was obtained from the retro-orbital plexus with a capillary tube, and collected in an Eppendorf tube. After centrifugation (5000*g* for 15 min), serum was stored at −20 °C. The bronchoalveolar lavage (BAL) fluid was obtained by lavage with three successive 1 ml volumes of PBS from cannulation of the trachea. RSV F-specific antibody response in immunized mice was measured by ELISA. Briefly, 80 ng purified RSV (Hytest, Turku, Finland) was adsorbed onto ELISA plates overnight in carbonate buffer (pH 9.8) at 4 °C. The plates were blocked with 1% BSA in PBS for 2 h at 37 °C. After thorough washing with PBST–1% BSA, the serum or BAL fluid samples were added to the plate and allowed to incubate for 1 h at 37 °C. The plates were washed again, and HRP-conjugated anti-mouse IgA (1:500 dilution), IgG (1:5000 dilution), IgG1 (1:5000 dilution), or IgG2a (1:5000 dilution) antibodies were added (Santa Cruz Biotechnology, Santa Cruz, CA) and allowed to incubate for another 1 h. Finally, the plates were washed and developed with 100 μl of 3,3,5,5-tetramethylbenzidine (Sigma, St. Louis, MO) substrate solution. The reaction was stopped with 50 μl 2 M H_2SO_4 and analyzed at 450 nm with a Thermo ELISA plate reader (BioRad 550, Hercules, CA).

For the analysis of IgG1 and IgG2a, the standard curves were determined firstly by using purified IgG1 or IgG2a (Sigma, St. Louis, MO) standard preparations of known concentration. The amounts of IgG1 and IgG2a were calculated by OD450 readings onto standard curves, and then the ratio of IgG2a/IgG1 isotype response was calculated according to the amounts of IgG2a and IgG1.

CTL assay. Peptide-specific cytotoxicity of splenocytes was determined by the CytoTox 96 non-radioactive assay (Promega, Madison, WI) as described previously [17]. In summary, BCH4 cells (kindly provided by Professor B.S. Graham, National Institutes of Health, Bethesda, MD) were used as targets in the CTL assays. Spleen cells from BALB/c mice were removed at 1 week after the final immunization, stimulated with peptide F_{85-93}, which corresponded to a known H-2K^d-restricted RSV F protein epitope (KYKNAVTEL, purity of the peptide ⩾95%), at 5 μg/ml for 5 days, and then added to assay plates at the specified effector-to-target ratios (*E:T* ratios). Assay plates were incubated for 4 h at 37 °C in a humidified atmosphere containing 5% CO_2. Fifty-microliter samples of supernatant were transferred to an enzymatic assay plate, reconstituted substrate mix was added at 50 μl/well, and the plate was incubated for 30 min at room temperature, protected from light. Fifty microliters of stop solution was then added to each well. Absorbance was recorded at 490 nm. Specific cytotoxic lysis was calculated as [(experimental − effector spontaneous − target spontaneous)/(target maximum − target spontaneous)] × 100.

RSV titer in lungs. Mice were sacrificed on day 5 after challenge. The left lungs from mice in each group were harvested, weighed, placed in sterile stabilizing buffer (1 ml/g lung), and homogenized with a glass tissue grinder. The homogenates were centrifuged (10,000*g* for 1 min) and RSV titers in the supernatants were measured by TCID50 on subconfluent HEp-2 monolayers. Results were expressed as $\log_{10}$ titer per 0.1 g lung tissue.

Lung histopathology study. Mice were sacrificed on day 5 after challenge. The right lungs were harvested and fixed in PBS-buffered formalin. Four-micrometer-thick sections were stained with hematoxylin and eosin. Lung pathology was scored on the basis of three parameters: peribronchiolitis, alveolitis, and interstitial pneumonitis. Scoring was performed as described by Prince et al. [18]. Briefly, all of the slides were examined to determine the range of pathology for each of the three parameters using a scale of 0 (no inflammation) to 100 (maximum inflammation for each lesion). Infiltrations of inflammatory cells were counted and pulmonary alveolar wall thicknesses were measured in randomly selected visual fields of each slide at a magnification of 400×. Images were captured with a Nikon DXM1200F (Nikon Instruments, Melville, NY) with ACT-1 software and analyzed with Image-Pro Plus 6.0 (MediaCybernetics, Silver Spring, MD).

Statistical analysis. Statistical analysis was performed with SPSS 11.5 software (SPSS, Chicago, IL). $p < 0.05$ was considered significant.

Results

CTL activity

To investigate CD8+ CTL response induced by FGAd-F vaccination, we measured the percentage specific cytotoxic lysis of target cells at various *E:T* ratios in the vaccinated mice. As shown in Fig. 1, spleen cells from the group immunized with FGAd-F demonstrated efficient target cell lysis at *E:T* ratios of 100:1 and 50:1 ($p < 0.05$).