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Maternal immunization with chimpanzee adenovirus expressing RSV fusion protein protects against neonatal RSV pulmonary infection

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ABSTRACT

Respiratory syncytial virus (RSV) is a leading cause of lower respiratory tract disease with high morbidity and mortality in young infants and children. Despite numerous efforts, a licensed vaccine against RSV remains elusive. Since young infants form the primary target group of RSV disease, maternal immunization to boost the protection in neonates is an attractive strategy. In this study we tested the efficacy of maternal immunization with a chimpanzee adenovirus expressing codon-optimized RSV fusion protein (AdC7-Fsyn) to protect infants against RSV infection. Single intranasal immunization of mice by AdC7-Fsyn induced robust anti-RSV systemic and mucosal immunity that protected against RSV without causing vaccine-enhanced RSV disease. RSV humoral immunity was transferred to pups born to immunized mothers that provided protection against RSV. Immunization with AdC7-Fsyn was effective even in the presence of Ad5 preimmunity. The maternally derived immunity was durable with the half-life of 14.63 days that reduced the viral replication up to 15 weeks of age. Notably, the passively immunized mice could be actively re-immunized with AdC7-Fsyn to boost and extend the protection. This substantiates maternal immunization with an AdC7-based vaccine expressing RSV F as feasible approach to protect against RSV early in life.

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1. Introduction

Lower respiratory tract infections with respiratory syncytial virus (RSV) are the most common cause for hospitalization of infants and children [1]. It is estimated that globally, RSV causes more than 3 million hospitalizations and up to 200,000 deaths in children under the age of 5 years [2]. More than 70% of the children in the first year of life, and by the age of 2 years almost all children have been infected at least once with RSV and continue to be re-infected throughout life [3]. Premature infants, especially those with chronic lung disease or congenital heart disease, as well as the elderly are most susceptible to severe disease [4]. Early RSV infections are also associated with the later development of asthma [5]. No safe and effective RSV vaccine has yet been licensed. Only immunoprophylaxis with the neutralizing monoclonal anti-RSV-F antibody palivizumab can reduce hospitalization rates due to RSV [6]. The high costs associated with monthly doses of palivizumab remains a challenge for its universal use and for developing countries.

The hurdles for the development of a safe RSV vaccine for vulnerable infants include (1) the immature immune system of infants may not respond well to vaccination; (2) the presence of variable levels of maternal anti-RSV antibodies in infants; (3) the inability of wild-type RSV infection to induce protective immunity; and (4) most importantly, the risk of vaccine-enhanced RSV disease that initially occurred following vaccination with a formalin-inactivated vaccine [7]. A strong mucosal inflammatory and Th2-biased immune response seem to be critical components of this complex pulmonary response triggered by RSV [8]. Adenovirus (Ad)-vectored vaccines are known to favor Th1-biased

Abbreviations: RSV, respiratory syncytial virus; Ad, adenovirus; Ad5, human adenovirus serotype 5; AdC7, chimpanzee adenovirus serotype 7; F protein, fusion protein; Fsyn, codon optimized fusion protein; qRT-PCR, quantitative reverse-transcriptase polymerase chain reaction; FI-RSV, formalin-inactivated respiratory syncytial virus.

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transgene-specific immune response and do not result in vaccine-enhanced disease [9–13], are not adversely affected by maternal anti-RSV antibodies and can be used for mucosal immunization [14]. Although the human Ad serotype 5 (Ad5) has been an effective vaccine platform against a variety of pathogens [15], use of Ad5-based vaccines is limited by widespread pre-existing immunity [16]. Non-human primate-derived Ad vaccine vectors such as chimpanzee Ad serotype 7 (AdC7) have been developed to overcome pre-existing immunity to common human Ad serotypes and to broaden the repertoire of Ad vaccines for booster immunizations. We have previously shown that AdC7 vector induces superior mucosal and protective immunity compared to human Ad5-based vectors that is further enhanced when AdC7 is administered directly to the respiratory tract [17,18].

Neutralizing antibody responses correlate with protection against RSV infections [19,20] and maternally transferred neutralizing antibodies effectively protect RSV-naïve infants against RSV-associated illness [21–23]. Considering that the infants constitute the primary risk group, maternal immunization could be a feasible approach to protect infants during their first months of life [24].

In this study we investigated the efficacy of maternal immunization with AdC7 vector expressing codon-optimized RSV Fusion protein (AdC7-Fsyn) to protect the infants against RSV infection. Immunization of mice with AdC7-Fsyn effectively induced protective neutralizing antibodies that were maternally transferred, even in the presence of Ad5-preimmunity, to protect infant mice from RSV.

2. Materials and methods

2.1. Ad vectors

The recombinant Ad vectors used in this study are replication-defective E1-, E3-deleted Ad vectors based on the chimpanzee AdC7 or human Ad5 genome [25,26]. An expression cassette carrying codon-optimized RSV Fusion gene (Fsyn) under the CMV promoter with tetracyclin operator 2 (TetO2) sequences (CMV/TetO2) and SV40 poly (A) signal was inserted into the E1 region of AdC7 or Ad5 [11] backbone. The AdC7-Fsyn was propagated in T-REx HEK293 cells (Invitrogen) that constitutively express a tetracycline repressor protein. Tet-Off system was used to control expression and potential inhibitory effects of Fsyn during propagation. AdC7-Null and Ad5-Null, vectors with no transgene, were used as controls. The vectors were used on the basis of equal number of particle units (pu) and were propagated and purified as described previously [27].

2.2. RSV

The RSV strain A2 (VR-1540; ATCC) used for protection experiments was propagated, purified and quantified as described previously [9]. Formalin-inactivated RSV (FI-RSV) was prepared [9] as positive control for vaccine-enhanced RSV disease.

2.3. Mice

Female BALB/c mice were obtained from Taconic Farms (Hudson, NY). The animals were housed under specific pathogen-free conditions and were used at 6–8 weeks of age. Mice were immunized by intranasal inoculation of 50 μl of the Ad vectors AdC7-Fsyn, Ad5-Fsyn or AdC7-Null at a dose of 10^{10} pu/animal or FI-RSV (10^5 pfu equivalent) diluted in PBS. To evaluate maternal immunization, mice were immunized 2 weeks prior to breeding. All animal studies were conducted in accordance to the protocols reviewed and approved by the institutional Animal Care and Use Committee.

2.4. Western analysis

To evaluate the expression of the RSV Fsyn, lysate of HEK293 cells infected with AdC7-Fsyn was separated by SDS-PAGE under non-reducing conditions. Following transfer to a PVDF membrane, it was probed with RSV Fusion protein monoclonal antibody (18F12) (1:500) and detected using peroxidase-conjugated goat anti-mouse antibody (1:1000) and chemiluminescent peroxidase substrate-1 (Sigma–Aldrich).

2.5. Anti-RSV humoral immune response

Mice were immunized intranasally with AdC7-Fsyn, Ad5-Fsyn, AdC7-Null or FI-RSV and serum was collected at 2, 4 and 8 weeks following administration. Lung bronchoalveolar lavage (BAL) fluid was collected by intratracheal instillation and aspiration with 0.5 ml PBS. Anti-RSV IgGs were assessed by ELISA as previously described [9]. Pulmonary anti-RSV IgA titers were analyzed using mouse typer isotyping panel (Bio-Rad Laboratories).

To induce anti-Ad5 preexisting immunity, mice were inoculated intranasally with Ad5-Null (10^{11} pu/mouse) 4 weeks prior to immunization. For maternal immunization, female BALB/c mice were intranasally inoculated with AdC7-Fsyn, Ad5-Fsyn or AdC7-Null, 2 weeks prior to breeding. Serum was collected from newborn mice at 2 weeks intervals starting from 3 weeks till 15 weeks of age. Anti-RSV IgG titers were evaluated as described above. Half-life ($t_{1/2}$) of the maternal-derived anti-RSV antibodies transferred to the pups was calculated by the formula – $t_{1/2} = t^* \ln(2)/\ln(N_0/N_t)$, where t = time elapsed, N_0 = antibody titer at 3 weeks of age, and N_t = antibody titer at 15 weeks of age.

Serum anti-RSV neutralizing antibody titers were evaluated as previously described [9].

2.6. Protection of mice from intranasal challenge with RSV

To evaluate protection against RSV pulmonary infection, immunized mice were challenged with RSV (10^6 pfu) by intranasal inoculation. Four days later the mice were sacrificed and total RNA from lung homogenates was isolated with TRIzol reagent (Invitrogen). RNA was converted to cDNA using random hexamers and the Taqman Reverse Transcription Reagents (Applied Biosystems). Hundred nanogram of cDNA was processed for quantitative RT-PCR (qRT-PCR) using RSV G-specific FAM-labeled taqman MGB probe (5′-AAGCCCACCACAAAAC-3′) and primers (Forward: 5′-AAGACCAAAAACACAACAACAACTC-3′, Reverse: 5′-AGTGAAAATCATTATTGGGTTTGCTTGGTG-3′). Endogenous 18S rRNA was used for normalization. The qRT-PCR was performed using the ABI PRISM 7000 Sequence detection system (Applied Biosystems) under the conditions – 45 cycles of denaturation (95 °C for 15 s) and annealing/extension (60 °C for 1 min). The relative quantity of RSV genomes was calculated by $\Delta\Delta C_t$ method and expressed in relation to RSV genomes detected in mock-vaccinated mice.

2.7. Vaccine-enhanced RSV disease

Mice were intranasally immunized by with AdC7-Fsyn, Ad5-Fsyn or AdC7-Null or FI-RSV and challenged with RSV (10^6 pfu) at 5 week post-immunization. Lung histopathology and BAL differential cell counts were evaluated as previously described [9].

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