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Immunization with an adjuvanted low-energy electron irradiation inactivated respiratory syncytial virus vaccine shows immunoprotective activity in mice

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ABSTRACT

Respiratory syncytial virus (RSV) is a pathogen causing severe lower respiratory tract disease in infants and the elderly. In spite of the great need for a vaccine against RSV, currently there is no licensed product on the market. A very early vaccine candidate developed in the 1960s based on formaldehyde inactivation (FI) turned out to instead enhance the disease. Our novel inactivation method applied low-energy electron irradiation (LEEI) to produce a killed RSV vaccine. LEEI yielded inactivated virus particles with a reproducible virus antigen conservation above 70%, while FI resulted in highly variable antigen conservation. Immunization of mice with LEEI-RSV elicited a strong immune response, resulting in a drastic reduction in viral load upon challenge in two independent studies. These results have implications for the development of an RSV vaccine and should be validated in further preclinical and clinical studies.

1. Introduction

Human respiratory syncytial virus (RSV) belongs to the Pneumoviridae family causing upper and lower respiratory tract infections in humans. While an infection with RSV might only cause cold-like symptoms in healthy adults, few other viruses impact the health of infants and young children in the same way, making RSV the leading cause of hospitalization [1]. RSV globally causes approx. 33.8 million cases of acute lower respiratory infections in children, resulting in 53,000–199,000 deaths per year [2]. Currently, the infection can only be treated prophylactically with the monoclonal antibody Palivizumab [3], while no other preventive therapies are available. In spite of dozens of RSV vaccine candidates having been tested in clinical trials for the last decades, no vaccine is on the market to date. One very early attempt to develop a protective vaccine against RSV was undertaken more than 50 years ago. In a clinical trial in 1966, the formaldehyde inactivated RSV (FI-RSV) vaccine showed, however, not only to be non-protective, but even led to an enhanced onset of the disease [4]. It is hypothesized that treatment with formaldehyde deforms the virus, resulting in a vaccine that triggers poorly designed antibodies [5] and in an inappropriately T_H2-biased immune response causing lung eosinophilia [6,7]. Formaldehyde has successfully been used for inactivation of many pathogens for vaccine production, for example for the Salk vaccine against polio or the tick-borne encephalitis virus vaccine. However, for viruses such as RSV or measles, where vaccines based on formaldehyde inactivation have been associated with an enhanced or atypical course of the disease after subsequent infection [7–9], it is not an option. As an alternative, different irradiation methods have been employed for pathogen inactivation, circumventing the use of toxic and potentially structure-altering chemicals. Gamma-rays or high-energy electron radiation have the ability to inactivate pathogens by damaging nucleic acids rather than proteins, yielding a suitable method for inactivation for the purposes of vaccine production [10]. The main drawback of these technologies is the emission of secondary radiation and high amounts of X-rays, creating a need for extensive shielding constructions. Ultraviolet light also successfully inactivates pathogens, but causes substantial degradation of viral proteins [11]. Low-energy electron irradiation (LEEI) is an alternative method for irradiation-based inactivation of pathogens. Electrons are emitted from power sources with less than 500 keV, which only generates minimal amounts of X-rays as a byproduct, and makes extensive shielding unnecessary [12]. We have recently shown that viruses and bacteria in liquid solutions can successfully be inactivated with LEEI, and that LEEI-inactivated Influenza A viruses induced protective immune responses [12]. Therefore, we set out to test whether LEEI is a suitable method for inactivation of RSV with the aim of vaccine production.

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Apart from ensuring that the structure of the virus in a killed-vaccine preparation resembles the native virus as much as possible, choosing suitable adjuvants – that appropriately direct the immune response – might also be key for protection. We therefore tested the LEEI-RSV vaccine with four different adjuvants, the alum-based Alhydrogel, squalene-based oil in water nano-emulsion MF59, the saponin QuilA, as well as Poly IC:LC – a double stranded RNA adjuvant.

2. Materials and methods

2.1. Mice

Seven week-old female BALB/c mice were obtained from Charles River (Sulzfeld, Germany). The mice were randomly assigned into groups of six animals, keeping three mice per isolated ventilated cage.

All animal experiments were carried out in accordance with the EU Directive 2010/63/EU for animal experiments and were approved by local authorities (No.: TVV 07/15; DD24-5131/331/9).

2.2. Cells

Human epithelial cells type 2 (HEp2) were used for all RSV in vitro assays. Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) with GlutaMAX (Thermo Fisher Scientific), containing 10% FBS and Penicillin/Streptomycin (Thermo Fisher Scientific) at 37 °C with 5% CO_2.

2.3. Respiratory syncytial virus

RSV laboratory strain A long was obtained from ATCC (VR-26). M. Peeples and P. Collins (NIH, Maryland, USA) kindly provided recombinant RSV expressing GFP (rgRSV). Virus propagation and titer determination was performed as previously described [13,14].

2.4. Quantitative real-time PCR

45 ng of RNA isolated from mouse lungs was reverse transcribed and analyzed with the QIAGEN QuantiTECT RT-qPCR Kit using SYBR green for detection. PCR amplification and quantification was done as described elsewhere [14].

2.5. Virus inactivation

2.5.1. Irradiation

For inactivation of RSV-containing cell culture supernatant, samples were prepared by applying 70 µl or 230 µl of pathogen suspension into the center of a sterile 100 mm petri dish (Primaria™, Corning). The suspension was covered by a round, oriented polypropylene (OPP)-foil resulting in a liquid film of approx. 100 µm thickness below the foil. Petri dishes were put on the sample holder and irradiated with the indicated doses with a 200 keV electron beam using an EB Lab-200 device (COMET AG, Switzerland). The final dose administered to the sample (range 0–20 kilo-gray (kGy)) was calculated from the applied current. The irradiated pathogens were checked for inactivation by adding at least 5% of the respective irradiated solution in duplicates to 10,000 HEp2 cells in a 96-well plate. After 5 days, the cells were stained immunocytochemically for RSV-P to visualize any infected cells. To exclude any residual virus activity not visible after 5 days, the supernatants were passaged once to fresh cells, and immunocytochemistry staining was repeated.

2.5.2. Formaldehyde inactivation

RSV containing cell culture supernatant was incubated for 96 h at 37 °C with a final concentration of 0.025% (v/v) formaldehyde (F1635-25ML, Sigma-Aldrich) according to the protocol for "Lot#100" [15].

2.6. ELISA – virus antigenicity testing

Antigen recognition on virus samples with and without irradiation was determined by direct enzyme-linked immunosorbent assay (ELISA). In short, wells of a NUNC Maxisorp 96-microwell plate were coated with dilutions of RSV in coating buffer (35 mM Na_2HCO_3/15 mM Na_2CO_3, pH 9.6) – a total volume of 100 µl per well – and incubated at 4 °C overnight. Wells were blocked with 5% skim milk in PBS. 18F12, a monoclonal antibody recognizing RSV-F (1:200 dilution), or human polyclonal serum (1:5000 dilution) were added and incubated for 2 h at room temperature. Plates were then washed with PBS containing 0.05% Tween 20, and incubated with a 1:1000 dilution of a peroxidase-conjugated sheep anti-mouse immunoglobulin G antibody or with a 1:5000 dilution of a donkey anti-human immunoglobulin G antibody (both from Dianova) at room temperature for 1 h. The plate was then washed three times, and TMB-ELISA substrate was used for color development (77,248, BioLegend). The reaction was stopped by addition of 1 M H_2SO_4 after 10–30 min, and absorbance was determined with a standard ELISA reader at 450 nm and reference wavelength at 520 nm. Background levels were subtracted and OD values were normalized to the levels measured when untreated virus was used as antigen, as published previously [12].

2.7. Immunization

In both immunization studies groups of 6 mice were vaccinated twice in a prime-boost manner at a 4-week interval by intramuscular injection.

2.8. Vaccine preparation I: LEEI- and FI-RSV

50 µl electron beam (20 kGy)-irradiated RSV containing cell culture supernatant with a titer of approx. 10^8 pfu/ml was mixed with 50 µl Alhydrogel (aluminium hydroxide gel adjuvant, 10 mg/ml aluminium, Invivogen, France) per dose. The same batch of virus-containing cell culture supernatant was used for the preparation of both the LEEI- and formaldehyde-treated viruses used for immunizations. Control mice were immunized with 50 µl cell culture supernatant plus 50 µl Alhydrogel ("mock").

2.9. Vaccine preparation II: LEEI-RSV with different adjuvants

Purified RSV was irradiated with 20 kGy and diluted with 10% sucrose in PBS to yield a concentration of approx. 10^8 pfu/ml. 50 µl of the virus suspension was mixed with 50 µl of Alhydrogel, 50 µl AddaVax (concentration not disclosed; Invivogen, France), 50 µl QuilA (diluted to a concentration of 0.3 mg/ml, Invivogen, France) or 50 µl Poly IC:LC (0.2 µg/µl; kind gift of Andres Salazar, Vladimir Temchura and Klaus Überla), respectively. AddaVax (by Invivogen) has a formulation similar to MF59, which is a registered trademark of Novartis.

Control mice were not immunized.

2.10. Blood collection and challenge

Blood for serum samples was collected from the retrobulbar venous plexus one week before the first immunization and 21 days after each immunization. Mice were challenged intranasally with a dose of 10^6 pfu RSV per animal. On day 3 and 5, respectively, after

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