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The attenuated NYCBH vaccinia virus deleted for the immune evasion gene, E3L, completely protects mice against heterologous challenge with ectromelia virus

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ABSTRACT

The New York City Board of Health (NYCBH) vaccinia virus (VACV) vaccine strain was deleted for the immune evasion gene, E3L, and tested for its pathogenicity and ability to protect mice from heterologous challenge with ectromelia virus (ECTV). NYCBHΔE3L was found to be highly attenuated for pathogenicity in a newborn mouse model and showed a similar attenuated phenotype as the NYVAC strain of vaccinia virus. Scarification with one or two doses of the attenuated NYCBHΔE3L was able to protect mice equally as well as NYCBH from death, weight loss, and viral spread to visceral organs. A single dose of NYCBHΔE3L resulted in low poxvirus-specific antibodies, and a second dose increased levels of poxvirus-specific antibodies to a level similar to that seen in animals vaccinated with a single dose of NYCBH. However, similar neutralizing antibody titers were observed following one or two doses of NYCBHΔE3L or NYCBH. Thus, NYCBHΔE3L shows potential as a candidate for a safer human smallpox vaccine since it protects mice from challenge with a heterologous poxvirus.

1. Introduction

The New York City Board of Health (NYCBH) vaccine strain which was manufactured by Wyeth and designated as Dryvax® is a heterologous mixture of VACVs that was historically used to vaccinate populations in the Americas and West Africa during the smallpox eradication program [1]. A single plaque isolate of Dryvax®, named Acambis 2000™, was purified in tissue culture conditions in order to increase safety from contaminants and was tested clinically in comparison to Dryvax® [2]. Vaccination with either Dryvax® or Acambis 2000™ results in equivalent neutralizing antibody and T cell responses, however similar adverse reactions, specifically myocarditis/myopericarditis, are observed [2]. Due to the potential for other known serious side effects such as eczema vaccinatum, progressive vaccinia, postvaccinial encephalitis, and generalized vaccinia, we sought to attenuate further the NYCBH VACV strain (Acambis 2000™) and test its ability to protect against challenge in established animal models of poxvirus infection.

The NYCBH VACV was attenuated by deletion of the immunomodulatory gene, E3L (NYCBHΔE3L). The E3L gene is expressed early during VACV infection and codes for proteins that contain an N-terminal Z nucleic acid binding domain (Zα) and a C-terminal dsRNA-binding domain [3–5]. The dsRNA-binding domain inhibits the activation of type I interferon (IFN)-induced proteins such as protein kinase R (PKR) and oligoadenylate synthetase (OAS) by binding and sequestering activator dsRNA, a byproduct of viral transcription [6,7]. The ability to bind dsRNA is also responsible for inhibition of proinflammatory signaling in VACV-infected cells [8]. Both the dsRNA-binding and Zα domains are required for optimal pathogenicity in mice, so deletion of the entire E3L gene results in a nonpathogenic virus that replicates to levels in skin 3 logs lower than wild type VACV [3,9,10].

NYCBHΔE3L has been previously tested as a vaccine in a mouse model. Tail scarification using NYCBHΔE3L successfully protected mice from a homologous challenge with VACV WR [10]. This study has extended these observations by testing the NYCBHΔE3L vaccine in a heterologous poxvirus challenge model using ectromelia virus (ECTV). ECTV is the causative agent of mousepox, a disease that mimics smallpox in humans due to its infection of the respiratory tract, ensuing blood viral load, and characteristic skin rash [11,12]. This paper shows that mice immunized with one dose of NYCBHΔE3L were protected from death and weight loss when intranasally challenged with a lethal dose of ECTV. In addition, viral spread to visceral organs was substantially inhibited.

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2. Materials and methods

2.1. Cell lines and virus stocks

Baby hamster kidney (BHK-21) cells and rabbit kidney-E3L (RK-E3L) cells stably expressing the VACV E3L gene (Wong et al., unpublished results) were grown in minimal essential media (MEM, Cellgro) containing 5% fetal bovine serum (FBS, HyClone) and 50 μg/ml gentamycin. Murine fibroblasts (L929) and African green monkey kidney (BSC-1) cells were grown in Dulbecco's-MEM (D-MEM, Lonza) containing 10% heat-inactivated fetal calf serum (FCII, HyClone).

NYCBH (ACAM2000™, kindly provided by Acambis), NYCBHΔE3L [10], MVA (kindly provided by Sanofi Pasteur), and NYVAC (kindly provided by Sanofi Pasteur) were propagated in BHK cells as previously described [9]. Infected cells were freeze-thawed three times followed by sonication and pelleting. Supernatant was partially purified by centrifugation through a 36% sucrose pad. Viral titers were determined by plaque assay using BHK cells for pathogenesis studies or on RK-E3L cells for vaccination studies. ECTV-Moscow was propagated in L929 cells, processed and partially purified through a sucrose pad as described above, and titrated using BSC-1 cells [13]. VACV WR was similarly propagated on HeLa-S3 cells and titrated on BSC-1 cells.

2.2. Pathogenesis and vaccination/challenge of mice

For pathogenesis studies, pregnant CD1 mice were obtained from Charles River Laboratories at two weeks gestation and were housed one mouse per cage for delivery of pups. Newborns at 48–72 h of age were given intracranial injections with the indicated doses of each virus resuspended in Tris-buffered saline (TBS) using a 27-gauge needle to deliver 5 μl of virus preparation. Mice were monitored daily for 2 weeks for morbidity and mortality. All procedures were approved by the Institutional Animal Care and Use Committee at Arizona State University.

Female A/Ncr mice at 5–6 weeks of age were purchased from The Jackson Laboratory (Bar Harbor, Maine). Animals were allowed to acclimate for 1 week prior to vaccination and were distributed into groups with <20% variation in weights. Vaccinations were performed at day 0 or days 0 and 28 by tail scarification using 1×10^6 pfu in a 2.5 μl volume of virus diluted in phosphate buffered saline (PBS) without Ca^{2+} and Mg^{2+} which was distributed into 15 punctures on the tail skin using a bifurcated needle. Mice were anesthetised with a ketamine HCl/xylazine mix (9 mg/ml/1 mg/ml) by intraperitoneal injection using 0.1 ml per 10 g body weight. Mice were challenged on day 56 by intranasal infection using 5 μl virus diluted in PBS without Ca^{2+} and Mg^{2+} by pipetting the diluted virus into each naris. Animal procedures were approved by the St. Louis University School of Medicine Institutional Animal Care and Use Committee.

2.3. Tissue titers

Mice were euthanized on day 7 post challenge followed by resection of tissues (kidney, liver, lung, spleen). Processing of tissues was performed using glass grinders by pulverizing the tissue at 10% wt/vol in PBS/1% serum, performing freeze/thaw for three cycles, and sonicating in an ice water bath. Virus infectivity levels were assayed using BSC-1 cells. The horizontal dashed line represents the limit of detection of the assay.

2.4. Antibody titers

Blood was obtained from mice at various times during the vaccination schedule via the submandibular vein into microtainer serum separator tubes. Individual samples of serum were separated by centrifugation and stored at −20 °C. A direct anti-vaccinia virus ELISA was performed using a lysate from BSC-1 cells infected with VACV-WR. The clarified cell lysate was diluted in 50 mM carbonate–bicarbonate buffer pH 9.6 and used to coat 96-well microtiter ELISA plates (Immulon-2 HB) at 4 °C overnight. Plates were blocked with PBS pH 7.2, 0.05% Tween-20, 2% normal goat serum (Vector) at room temperature for 30 min. Mouse serum was serially diluted in PBS pH 7.2, 0.05% Tween-20 (PBST) and incubated for 1 h at room temperature. Wells were washed with PBST and bound antibody was detected using biotin-conjugated goat anti-mouse IgG (Caltag) incubated 1 h followed by a wash with PBST and the application of streptavidin-HRP (Zymed) for 30 min. Wells were washed and O-phenylenediamine dihydrochloride (0.4 mg/ml) in 50 mM citrate buffer (pH 5.0) and 0.05% hydrogen peroxide was added for 15 min, then the reaction was stopped with 3 N HCl. Optical density was measured at 490 nm. Titers were determined by calculating the inverse of the serum dilution at which the optical density exceeded the background value of 0.1 as measured with PBST. For measurement of neutralizing antibody titers, serum was thawed and serially diluted in D-MEM/2% serum. A known amount (50 plaque forming units) of ECTV was added to each dilution and incubated at 37 °C for 2 h followed by titration in BSC-1 cells. The neutralizing antibody titer of each sample was calculated as the inverse of the serum dilution at which a 50% reduction in plaquing efficiency occurred.

2.5. Statistical analysis

Statistical analysis was performed using GraphPad Prism 5, GraphPad Software, Inc. Weights were analyzed by two-way ANOVA followed by Bonferroni posttests to determine differences between vaccination groups. Virus titers in tissues and antibody titers were analyzed by one-way ANOVA followed by Tukey's multiple comparison test.

3. Results

3.1. NYCBHΔE3L is highly attenuated for pathogencity in newborn mice

The pathogenicity of NYCBHΔE3L was tested in the highly sensitive newborn CD1 mouse model and compared to NYVAC and MVA [14]. Increasing doses of each VACV strain were injected intracranially and the LD_{50} was assessed. The wild type NYCBH vaccine was pathogenic at low doses with an LD_{50} of 20 pfu (Fig. 1). Deletion of the E3L gene from NYCBH resulted in a virus (NYCBHΔE3L) that was attenuated by nearly 6 logs and had an LD_{50} of 1×10^7 pfu. NYCBHΔE3L was similar in pathogenicity to NYVAC and MVA, two highly attenuated VACV strains [15,16].

3.2. Vaccination with NYCBHΔE3L fully protects mice from challenge with ECTV

Six groups of 9–10 mice per group were vaccinated by scarification with a single dose of 1×10^6 pfu NYCBH or NYCBHΔE3L at day 0, two doses of NYCBH or NYCBHΔE3L at days 0 and 28, or were mock vaccinated with PBS. Four weeks after vaccination (day 56) mice were challenged intranasally with 5.8 pfu ($15 \times LD_{50}$) ECTV. All the mock-vaccinated mice died at 8–9 days post challenge while all vaccinated mice, whether singly or doubly vaccinated with NYCBH or NYCBHΔE3L survived challenge (data not shown). The weights of the mice were recorded for 28 days following challenge with ECTV. Mock-vaccinated ECTV-challenged mice lost