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Vaccinia viruses with mutations in the E3L gene as potential replication-competent, attenuated vaccines: Scarification vaccination

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ABSTRACT

In this study, we evaluated the efficacy of vaccinia virus (VACV) containing mutations in the E3L virulence gene to protect mice against a lethal poxvirus challenge after vaccination by scarification. VACV strains with mutations in the E3L gene had significantly decreased pathogenicity, even in immune deficient mice, yet retained the ability to produce a potent Th1-dominated immune response in mice after vaccination by scarification, while protecting against challenge with wild type, pathogenic VACV. Initial experiments were done using the mouse-adapted, neurovirulent Western Reserve (WR) strain of vaccinia virus. Testing of the full E3L deletion mutation in the Copenhagen and NYCBH strains of VACV, which are more appropriate for use in humans, produced similar results. These results suggest that highly attenuated strains of VACV containing mutations in E3L have the potential for use as scarification administered vaccines.

Introduction

Poxviruses are a large and diverse group of viruses that can infect a wide range of organisms, including birds, insects, and many mammals, including humans. They are double-stranded DNA viruses with linear genomes generally ranging from 130 to 300 kbp. Poxviruses replicate in the cytoplasm of infected cells using both proteins that they encode and resources from the infected cell. Variola virus is the causative agent of smallpox, a devastating human disease [1]. Vaccination against smallpox has thus far relied upon cross-protective antigens from other orthopoxviruses such as vaccinia virus (VACV), which itself causes relatively mild symptoms in humans during natural infections [1]. The use of VACV as a vaccine has resulted in successful eradication of smallpox, but recent concerns about the potential use of variola virus as a bioweapon has renewed interest in developing an improved vaccine. Indeed, the current smallpox vaccine has resulted in a significant number of severe side effects, including encephalitis, eczema vaccinatum, generalized and progressive vaccinia infections, as well as many other less severe adverse reactions. There have also been recent reports of cardiac complications following vaccination [2]. In future vaccination campaigns, these complications are likely to be compounded by the growing numbers of individuals with compromised immune functions due to HIV infection, organ transplants, and cancer treatments [3]. In addition to its use as a smallpox vaccine, VACV is being developed as a vector for vaccination against heterologous antigens.

The risks associated with currently available strains of VACV have prompted generation of a variety of attenuated strains of VACV. These include modified Ankara strain (MVA) [4], a mutant of the Lister strain of VACV deleted for the essential viral UDG gene [5], NYVAC [6] and LC16m8 [7]. MVA and LC16M8 were generated by passaging through alternative hosts, while NYVAC and the Lister mutant were engineered to contain attenuating deletions. All of these attenuated strains, except LC16m8 have very limited capacity for replication in human cells [6,8–10]. Although non-replicating vectors have the advantage of increased safety, high doses of these vectors (10^7 to 10^8 pfu) are often required to induce a strong immune response [9,11,12]. Most of these vaccinations also

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require multiple immunizations [13] often defeating the purpose of using live virus vaccines, which usually induce potent immune response with a single dose.

The vaccinia virus E3L gene products are potent inhibitors of the innate immune response [14–16]. The proteins encoded by the VACV E3L gene contain a C-terminal consensus dsRNA-binding domain that can bind to and sequester viral dsRNA [17] produced in infected cells, probably as a result of convergent transcription of the viral genome [18]. Free dsRNA can act as a pathogen associated molecular pattern (PAMP), leading to pro-inflammatory signal transduction, expression of interferon and other pro-inflammatory genes, and activation of antiviral proteins already induced by interferon [19,20]. The E3L encoded proteins also contain an N-terminal consensus Z-nucleic acid-binding domain [21]. This domain is necessary for virulence in the mouse model.

In an effort to develop replication-competent but attenuated strains of VACV we have constructed and tested a variety of VACV mutants with deletions in the E3L interferon resistance gene. These mutant virus strains are highly attenuated in both immune deficient and immune competent mice, after either intra-nasal or intra-cranial infection. Despite attenuation these viruses can induce a protective immune response after intra-nasal vaccination [22]. In the present study we demonstrate that these viruses can also induce protective takes after administration by the more standard route of immunization by scarification. We also demonstrate that vaccination by scarification induces potent cell mediated immunity to VACV and primes for production of neutralizing and comet inhibition antibodies.

Materials and methods

Recombinant virus construction

Viruses used in these studies were constructed in the Western Reserve strain of vaccinia virus (a mouse-adapted neurovirulent strain developed by serial passage of vaccinia virus strain in the brains of mice [23]), in the Copenhagen strain (VC2, kindly provided by Virogenetics) or in the New York City Board of Health strain (ACAM2000, kindly provided by Acambis) [24]. VACVΔE3L was constructed by *in vivo* recombination resulting in the replacement of the E3L gene with the *E. coli lacZ* gene [25]. Vaccinia virus mutants with N or C terminal sections of the E3L gene deleted or a foreign gene inserted were generated by *in vivo* recombination of E3L mutant genes into VACVΔE3L. VACVE3LΔ7C, which is deleted of the last 7C terminal amino acids, was constructed as previously described [26]. VACVE3LΔ54N, which is deleted of the first 54N terminal amino acids, was constructed as previously described [27]. The wtVACV used in this study is a revertant of VACVΔE3L, wtVACV03. Unless otherwise noted, the WR strain of VACV was used in all experiments.

Cell culture

BHK-21 (Baby Hamster Kidney) and RK-13 (Rabbit Kidney) cells were maintained in Eagle's minimum essential medium (MEM-Gibco, BRL) supplemented with 10% fetal bovine serum (FBS-Hyclone), 50 μg/ml of gentamycin, and 0.1 mM non-essential amino acid solution (Gibco-BRL). Both BHK and RK-13 cells were incubated at 37 °C in 5% CO_2.

Preparation of virus stocks

All virus stocks were prepared in BHK cells, as previously described [27]. Viruses were partially purified by centrifugation through a 36% sucrose pad.

Mice

Severe combined immune deficient (SCID) mice were obtained from either the Jackson Laboratory (CBySmn.CB17-Prkdcscid/J) or Charles River Laboratories (Fox Chase SCID mouse, CB17/lcr-PrKdc^{SCID}/CrL). BALB/c and C57BL/6 mice were obtained from either The Jackson Laboratory or Charles River Laboratories. *mu*MT B cell deficient mice were obtained from the Jackson Laboratory. All mice were housed in the Arizona State University Animal Resource Center according to the university's Institutional Animal Care and Use regulations. Mice were 4–5 weeks of age when used in experiments unless otherwise indicated. Mice were either of both sexes or were females only as indicated in individual experiments.

Scarification infection of mice

BALB/c, C57BL/6 or SCID mice were anesthetized using a intraperitoneally (IP) injected cocktail containing 7.5 mg/ml xylazine, 2.5 mg/ml acepromazine maleate and 37.5 mg/ml ketamine at approximately 1.5 μl/gm of body weight. Hair on a 1-in. strip of the dorsal surface of the base of the tail was then removed with Nair hair remover and the skin rinsed thoroughly with sterile water. Fifteen scratches were then made horizontally across the tail skin with a sterile 26-gauge syringe needle. The skin was then infected by placing 10 μl of virus suspended in 1 mM Tris pH 8.8 on the skin using a gel loading tip and then using the side of the tip to rub the virus into the scratches. Mock-infected animals received 1 mM Tris pH 8.8 containing no virus. Animals were monitored for weight loss, signs of illness and severity of skin pocks. Animals that received vaccination boosts were scarified a second time 1 month after the initial infection using the same procedure.

Infection of mice

BALB/c mice were manually restrained and injected IP in the lower right abdominal quadrant with 100 μl of virus suspended in 1 mM Tris pH 8.8. Mock-infected animals received 100 μl 1 mM Tris pH 8.8 containing no virus.

Recovery of virus from tissues

Tissues were harvested from chemically euthanized animals at 5 days post-infection, as previously described [27]. Briefly, tissues were removed, placed in cryogenic vials, and quick-frozen in liquid nitrogen. Each animal's ovaries were combined upon removal. Harvested tail tissue consisted of the entire scarified skin area down to the level of the connective tissues. Tissues were homogenized either by using a liquid nitrogen cooled mortar and pestle or by using a mechanical tissue homogenizer. The samples were freeze-thawed three times. Samples were then centrifuged at 4 °C for 1 min at 1000 × *g* to remove cell debris. Virus content in the supernatants was assessed by titration in RK-13 cells and the amount of virus per gram of tissue was calculated.

Challenge experiments

Either C57BL/6 or BALB/c mice were vaccinated by scarification and then challenged 1 month later intranasally (IN) with 10^6 pfu of Western Reserve strain wild type vaccinia virus (wtVACV). Mice were monitored for weight loss every other day and signs of illness every day for 2 weeks after challenge. Data points shown on graphs are the average percent weight loss for the mice remaining in each experimental group.