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Raccoonpoxvirus safety in immunocompromised and pregnant mouse models

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ABSTRACT

Numerous poxviruses infect humans and animal hosts, and a poxvirus vaccine with an improved safety profile is needed as the current vaccinia virus vaccine is contraindicated in individuals that have a history of eczema or heart disease, or are immunocompromised or pregnant. In addition, poxviruses make excellent vaccine vectors for other infectious diseases and cancer. Raccoonpoxvirus is a naturally occurring attenuated North American poxvirus, and thus it is of interest as a vaccine vector platform. This study explores the effects of raccoonpoxvirus in SCID and Nude immunocompromised and pregnant mouse models to assess its virulence and probable safety for human and animal populations. We also analyzed the safety of recombinant raccoonpox carrying a gene expressing a foreign antigen, rabies virus glycoprotein, designed for heterologous vaccine protection. Our data show that recombinant raccoonpoxviruses are avirulent in many cases and are much safer than vaccinia virus (strain WR). Raccoonpoxviruses also have the advantage of being able to replicate in mammalian cells. This allows increased immunogenicity and production efficiency, giving an advantage over non replicating vectors such as Modified Vaccinia Ankara MVA or canarypoxvirus.

1. Introduction

Smallpox is estimated to have killed 500 million people during the 20th century, but it was eradicated from nature in 1980 by a robust vaccination program headed by the World Health Organization [1]. It is currently known to exist in only 2 laboratories in the world, the Center for Disease Control in Atlanta, and VECTOR (State Research Center of Virology and Biotechnology), the former soviet biowarfare unit in Novosibirsk, Russia. Most extant poxviruses are zoonotic, transmitted mostly via rodents, and include monkeypox that caused an outbreak in the US Midwest in 2003 with more than 80 cases [2,3]. Monekypox is endemic in Africa and has a case fatality rate of approximately 10% [2,4]. Cowpox, orf virus and molluscum contagiousum virus also infect humans, but these infections are rarely fatal [5–7]. New poxviruses are identified each year in animal populations, and several zoonotic poxviruses appear to be emerging worldwide in humans, Cantagalo in South America [8], Tanapox, in Africa, Europe and the US [9,10], and buffalopox in India [11].

Poxviruses are often used as recombinant vaccine vectors for infectious diseases and cancer [12–15]. The only HIV vaccine with efficacy in humans thus far uses a canarypox vector [16]. Poxviruses such as vaccinia virus (VV) are suitable as vectors because they are easily grown to high titer, grow in a wide variety of animals and cell types, can accommodate insertions of large pieces of DNA into their genomes, are very stable even when dried, and induce robust T cell immunity [17]. VV is the most used poxvirus vector, but its utility as a vaccine is limited by its virulence [18,19]. Vaccination is contraindicated for individuals with any history of eczema or immunodeficiency disorders because fatalities may occur, and otherwise-healthy individuals can spread the virus to contacts, acquire a disseminated poxvirus rash, myopericarditis, and rarely fatal encephalitis from vaccination [20–22]. Modified Vaccinia Ankara strain is much more attenuated, but its replicative ability and immunogenicity are limiting [17,23–25].

Raccoonpoxvirus (RCNV) is a naturally occurring attenuated North American poxvirus, and thus it is of interest as a vaccine vector platform. The raccoonpox strain Herman was originally isolated from a healthy raccoon [26], and it has demonstrated safety in numerous animals [27–32]. Recombinant RCNV vaccines have been administered to raccoons, skunks, foxes, bobcats, rabbits, domestic cats, piglets, sheep and non-human primates [27–29]. None of the immunized animals showed clinical side effects [27], and the

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recombinant RCNV induced protective immune responses against rabies antigen in raccoons, dogs, cotton rats, rabbits, bobcats and foxes [27,28,30]. In addition, recombinant RCNV have been shown to induce protective immune responses in domestic cats against feline panleukopenia virus, feline caliciviruses and feline infectious peritonitis [29,31]. RCNV vectored rabies vaccine was also administered via oral, intranasal, and conjunctival routes (10^8 plaque forming units or PFU) as a mucosal vaccine in cats and was safe [32]. A rabies-RCNV vaccine has been approved by the USDA. These studies suggest that RCNV would be an excellent and safe vaccine vector for use in humans and animals. However, the safety of RCNV in compromised or pregnant mammals had not been tested.

In this study, we assessed the safety of RCNV viruses in two immunocompromised mouse models; the Nude, athymic T lymphocyte deficient mouse, and the severe combined immunodeficient (SCID) mouse, which is lacking in both B and T lymphocyte responses. Both lymphocyte subsets contribute to protection from VV infections [17,33,34]. In addition, we have measured RCNV safety in a pregnant mouse model because VV can infect and kill human fetuses [35]. We compare RCNV to VV Western Reserve, a commonly used lab strain that is virulent in mice [36]. We also assessed the safety of adding the rabies glycoprotein to vaccinia, as this could potentially enhance virulence. The results show that RCNV is much safer than VV, and attenuated RCNV are in most cases avirulent in mammals (similar to canarypox).

2. Materials and methods

2.1. Viruses

Canarypox virus (CNPX) vaccine expressing the rabies glycoprotein was purchased from Merial and reconstituted immediately prior to infection as directed in the manufacturer's instructions. Vaccinia virus (VV) Western Reserve (WR) strain was obtained from the NIH Laboratory of Viral Disease. Raccoonpoxviruses (RCNV) strains Herman, Herman thymidine kinase knock out (TK-), and Herman RCNV-expressing the rabies glycoprotein (G2, which also has the TK and hemagglutinin [HA] genes knocked out) were obtained from Boehringer Ingelheim Vetmedica, Inc. VV WR was grown on BS-C-1 cells and the 3 RCNV were grown in VERO cells. VV and RCNV viruses were purified on sucrose gradients, aliquoted, and titered 3 times on BS-C-1 cells [37]. The titer of canarypox was determined on chick embryo fibroblasts 4 times by staining infectious foci with 0.1% crystal violet.

2.2. Nude mice

All experiments involving animals were approved by the East Carolina University Animal Care and Use Committee and performed in AALAC accredited facilities. Groups ($N = 6$) of 3–5-week-old Nude mice (Charles River, stock number 088) were anesthetized using isoflurane and infected intranasally (i.n.) on Day 0 with purified virus in 18 μl or were mock infected with phosphate-buffered saline (PBS). VV WR was used at 1×10^3 pfu/mouse [37], and CNPX and three RCNV were used 7×10^4 pfu/mouse. Titers were confirmed for each experiment by titering the dilution used to infect the mice that day. Mice were weighed and monitored daily for 94 days and euthanized if 20% weight loss occurred.

2.3. SCID mice

Groups ($N = 6$) of 3–5-week-old SCID mice (Jackson Labs, cbysmn.cb17-prkdc scid/J, stock number 001803) were anesthetized and infected i.n. with purified virus or mock infected with PBS. VV WR and RCNV strains were used at 1×10^3 pfu/mouse, and CNPX at 7×10^4 pfu/mouse. Titers were confirmed on the day of the experiment. Mice were weighed and monitored daily for 174 days and euthanized if 20% weight loss occurred. On day 112, one G2 mouse was sacrificed due to apparent fighting wounds: organs were titered and there was no virus in any of 6 organs.

2.4. SCID mice organ titer infection

To determine virus titers in organs, groups ($N = 4$) of 3–5-week old SCID mice were infected i.n. with VV WR at 1×10^3 pfu/mouse, or Herman, TK-, and rRCNV-Rabies-G2 at 4×10^3 pfu/ms in 18 μl. Mice were weighed daily for ten days and sacrificed. Organs were placed in 1 ml ice-cold RPMI medium, frozen and thawed three times, homogenized (Omni Tissue Master 125) and sonicated as previously described [37]. Viral replication was evaluated by titration of the organ on BS-C-1 monolayers and staining with 0.1% crystal violet in 20% ethanol 40–48 h later.

2.5. Pregnant mice

Groups of pregnant BALB/c mice (5–8 per group) were injected intraperitoneally with 8×10^6 pfu of VV-WR, RCNV (Herman, TK-, or rabies G2), CNPX virus at 4×10^6 pfu, or PBS on the 12th day of pregnancy, similar to published models [35]. Mice were monitored daily and live pups counted. Births occurred over several days around day 20. Data published are from day 26 after conception. Pups counted on that day were normal size and survived up to day 31 when sacrificed, (approximately day 11 post-delivery). Statistical differences were measured by the 2 tailed Student's t test.

3. Results

3.1. Nude mice

In order to assess and compare virulence of these poxviruses in immunocompromised mammals, athymic nude mice deficient in T lymphocytes were infected intranasally (i.n.) with Canarypox virus (CNPX), which is replication deficient in mammalian cells, virulent wild type Vaccinia virus strain Western Reserve (WR), 3 raccoonpoxvirus (RCNV) constructs, or PBS control. Morbidity (weight loss) and mortality were monitored for 94 days. As shown in Fig. 1A, the PBS mice continued to gain weight throughout the experiment. Mice infected with VV WR began losing weight on day 7 and VV WR killed 5/6 mice by day 14 (the remaining mouse died at day 25, Fig. 1B). However the wild type Herman RCNV strain-infected mice (7×10^4 pfu/mouse) gained weight up to day 30 when some mice began losing weight. Five of six RCNV Herman mice died between days 33–87 (1 remained alive to day 94 when the experiment was terminated). These data indicate that wild type RCNV (Herman, which is not attenuated by genetic engineering) is naturally highly attenuated compared to VV WR, but that it can eventually kill T cell deficient mice. All mice infected with RCNV mutants in which the thymidine kinase virulence gene has been deleted (TK- and G2 mutants) were healthy to the end of trial (day 94) with no signs of infection, similar to CNPX infected mice. Wild type RCNV demonstrated delayed virulence in immunocompromised mice, but deletion of the TK gene rendered both the TK- and the G2 mutants avirulent in this model. Additionally, there is no evidence that a RCNV expressing rabies glycoprotein (G2) is more virulent than other RCNV.

3.2. SCID mice

Since TK knockout RCNVs were avirulent in Nude mice, we next assessed their virulence in SCID mice, which are deficient in both B and T lymphocytes and thus severely immunocompromised. SCID

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