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Short communication

Vaccinia virus as a vaccine delivery system for marsupial wildlife

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ABSTRACT

Vaccines based on recombinant poxviruses have proved successful in controlling diseases such as rabies and plague in wild eutherian mammals. They have also been trialled experimentally as delivery agents for fertility-control vaccines in rodents and foxes. In some countries, marsupial mammals represent a wildlife disease reservoir or a threat to conservation values but, as yet there has been no bespoke study of efficacy or immunogenicity of a poxvirus-based vaccine delivery system in a marsupial. Here, we report a study of the potential for vaccination using vaccinia virus in the Australian brushtail possum *Trichosurus vulpecula*, an introduced pest species in New Zealand. Parent-strain vaccinia virus (Lister) infected 8/8 possums following delivery of virus to the oral cavity and outer nares surfaces (oronasal immunisation), and persisted in the mucosal epithelium around the palatine tonsils for up to 2 weeks post-exposure. A recombinant vaccinia virus construct (VV399, which expresses the Eg95 antigen of the hydatid disease parasite *Echinococcus granulosus*) was shown to infect 10/15 possums after a single-dose oronasal delivery and to also persist. Both parent vaccinia virus and the VV399 construct virus induced peripheral blood lymphocyte reactivity against viral antigens in possums, first apparent at 4 weeks post-exposure and still detectable at 4 months post-exposure. Serum antibody reactivity to Eg95 was recorded in 7/8 possums which received a single dose of the VV399 construct and 7/7 animals which received triple-dose delivery, with titre end-points in the latter case exceeding 1/4000 dilution. This study demonstrates that vaccinia virus will readily infect possums via a delivery means used to deploy wildlife vaccines, and in doing is capable of generating immune reactivity against viral and heterologous antigens. This highlights the future potential of recombinant vaccinia virus as a vaccine delivery system in marsupial wildlife.

1. Introduction

Vaccination of mammalian wildlife species can be used as a management tool in certain circumstances. Examples include those cases where wildlife species are a reservoir of an infectious disease that threatens humans or livestock; or where the impacts of that species' populations threaten native ecosystems; or in the case of endangered wildlife species themselves, when the species' viability is threatened by endemic disease [1]. In the case of vaccines to combat infectious diseases, one of the most widely used wildlife vaccines has been Raboral™, which has been used to control sylvatic rabies among mesocarnivores in North America and Europe [2]. Raboral™ comprises a recombinant poxvirus (vaccinia, Copenhagen strain) which has been engineered to express the 80 kDa protective glycoprotein G of the rabies virus (termed VRG-vaccine; [3]); over 100 million doses have been deployed since its development in the 1980s (reviewed in [4]). A further example is the prototype vaccine for use against plague in prairie dogs; this uses a recombinant raccoon poxvirus, engineered to express the protective F1 antigen of *Yersinia pestis* [5]. In the case of vaccines to control the reproductive rate of wild animal populations, several prototypal vaccines have been developed that aim to reduce fertility of mammalian pest species by invoking an autoimmune response in the reproductive system [6]. Here again, recombinant poxviruses have been trialled as vaccine-delivery systems [7,8]: Jackson et al. [9] and Kerr et al. [10] showed that ectromelia or myxoma virus, engineered to express homologous egg zona pellucida (ZP) antigens, reduced reproductive success when injected into female mice or rabbits, respectively. However, for a vaccine to ever be considered practicable for large-scale immunisation of wildlife, it will need to be in an oral-delivery format [1].

While eutherian mammals constitute the major wildlife species of concern to disease managers and conservationists, in some

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countries marsupial mammals also pose problems. In New Zealand, the introduced brushtail possum (*Trichosurus vulpecula*) is the primary reservoir of bovine tuberculosis and represents an on-going threat to agriculture (reviewed in [11]); additionally, possums damage native flora and fauna [12]. The drive for more cost-effective and socially acceptable possum control has led to research to develop fertility-control vaccines based on immunisation using possum egg ZP antigens [13–15]. In Australia, native macropod species can serve as intermediate hosts for the hydatid disease parasite *Echinococcus granulosus*, which is a zoonotic and livestock disease risk. Research there has highlighted use of a recombinant vaccine containing the protective oncosphere-stage antigen Eg95 to reduce hydatid disease in the tammar wallaby, *Macropus eugenii* [16]. However, as outlined above for eutherian mammals, a practical-use vaccine for free-ranging marsupial wildlife will need to employ some form of oral-delivery.

Poxviruses infect via mucosal and dermal routes and so are amenable to development as oral vaccine-delivery systems for wildlife. Indeed, both the Raboral^{TM} rabies vaccine (based on VRG) and the prototype plague vaccine (recombinant raccoon poxvirus) are oral-delivery vaccines. Hence, poxviruses may have potential as oral-delivery systems to vaccinate wild marsupials. However, two important questions exist: first, whether a poxvirus which ordinarily infects eutherian mammals is capable of infecting a marsupial animal at all; and secondly, whether sufficient replication of such a poxvirus occurs following oral delivery to a marsupial to invoke immune reactivity in the host. In the present report, we have sought to answer these questions by conducting the first bespoke study of a vaccinia-based vaccine in an Australian marsupial, the brushtail possum. Research has already highlighted the potential for fertility-reduction in this marsupial using parenteral-route vaccines based on possum egg ZP antigens [13,14]. In this study, we have oronasally delivered the Lister strain of the poxvirus vaccinia to possums and measured infection dynamics and immune responses. Further, we have utilised both parent-strain and a recombinant vaccinia in this study; the recombinant construct studied (VV399) was one expressing *E. granulosus* Eg95 antigen, which was chosen so that responses generated in possums could be compared – in very broad terms – to responses reported in another marsupial species to the same model antigen by injection [16].

2. Materials and methods

2.1. Animals

Thirty adult female possums were trapped from the wild and acclimatised to captivity for at least four weeks prior to experimentation. Possums were maintained individually in wire frame cages and supplied with a nesting box; animals were fed daily with a mixed and varying diet of fruit, vegetables and commercial possum pellets (CRT Reliance Mills, Rolleston, New Zealand) and provided with water *ad libitum*. As required, possums were either sedated by intra-muscular injection of 0.75 mg/kg ketamine hydrochloride (for oronasal infection procedures) or were anaesthetised by inhalation of 3–5% isoflurane in oxygen (for drawing blood and undertaking tonsillar swabs). All procedures were undertaken under Landcare Research Animal Ethics Committee approval number 10/06/04 in accordance with the Animal Welfare Act 1999 of New Zealand.

The vaccinia infection experiment was undertaken under Physical Containment level 2 conditions (New Zealand Environmental Risk Management Authority project license number GMD100283). At commencement, mean body weight of the possums was 3.06 ± 0.08 kg (range, 2.39–3.67). Twenty-four possums were assigned randomly to one of three experimental groups of $n = 8$, while 6 non-assigned animals served as non-treated controls (4 controls were held in an adjacent room separately from the virus-exposed possums, and 2 were maintained in the PC2 room but distant from the virus-infected possums as sentinels to monitor for incidental immune reactivity).

2.2. Viral construct and antigens

Vaccinia virus (Lister strain) was maintained by routine passage over 143BTK-cells as described elsewhere [17] and utilised as the parent-strain virus. A recombinant vaccinia Lister construct (termed VV399) was studied as a model heterologous antigen-expressing virus, as described previously [18]. VV399 expresses the Eg95 antigen of the parasitic nematode *E. granulosus* (an immunogenic 16.5 kDa protective antigen used in vaccination of livestock against hydatid disease [19,20]); it also expresses the low immunogenicity marker gene beta-galactosidase (β-gal). The recombinant was constructed using standard methods [21] and employed PUV1 as the transfer vector [22], such that expression of Eg95 was under the regulation of a well-characterised, strong vaccinia virus late promoter P11 [23,24]. The heterologous gene coding regions for Eg95 and β-gal were inserted at the thymidine kinase (TK) gene site of the VV399 construct.

Both the VV399 construct and the parent strain virus were produced to high concentrations by seeding at a high multiplicity of infection onto Vero cells. Supernatants were harvested from heavily infected cells and clarified of cell debris by low-speed centrifugation. For oronasal infection experiments, live viral titre was adjusted to 10^8 pfu/mL using sterile physiological saline. To produce viral antigen for cell culture work, virus-containing supernatant (parent-strain) was heat-inactivated, denatured by sonification in 3.33% SDS (following a published protocol [25]), filter-sterilised and adjusted to an equivalent of 5×10^6 viral particles/mL in tissue culture medium.

The Eg95 molecule was expressed in *Escherichia coli* as a GST-tagged fusion protein. *E. granulosus* Eg95 DNA (comprising a 715 bp sequence) was cloned into the *E. coli* expression vector pGEX-3EX and expressed as described previously [26]. GST-fusion protein was extracted from inclusion bodies of *E. coli* BQ1.1 by solubilisation in 8 M urea; this solution was then diluted further in PBS, filter-sterilised and stored in multiple aliquots as 50 μg/mL protein for use in ELISA.

2.3. Infection procedures

For vaccinia infections, possums were sedated using ketamine, since under the influence of this drug they exhibit notable mastication and swallowing activity, which mimics feeding activity. Sedated possums were held vertically but with the head kept at 45° to the horizontal and the jaws maintained open with soft-edged callipers: 1 mL suspension containing 10^8 pfu virus was added dropwise into the buccal cavity and to the external surface of the nose (but not into the nasal canals) and allowed to infect for 30 s; this procedure was designed to mimic the natural feeding activity of possums. Possums were then returned to their individual cages for recovery. Three treatment groups of $n = 8$ received vaccinia infection; Group 1 received a single inoculum of parent-strain virus, Group 2 received a single inoculum of the VV399 construct, and Group 3 received three doses of the VV399 construct with each dose spaced 7 days apart. Animals were bled at week 0, and again at 4, 8, 12 and 16 weeks post exposure to virus to assess immune reactivity.

One of 24 possums exposed to vaccinia by oronasal droplet, a multi-dose animal, died 4 weeks into the study (post-mortem revealed a gut blockage, unrelated to viral exposure). Its data were omitted from the analyses.