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Experimental inoculation of European red foxes with recombinant vaccinia virus expressing zona pellucida C proteins

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Abstract

The antifertility potential of zona pellucida proteins was tested in European red foxes by immunizing females with recombinant vaccinia viruses that express zona pellucida subunit C proteins. The fox zona pellucida C (fZPC) protein was newly identified and isolated as a cDNA clone from fox ovary RNA. Eighteen European foxes were inoculated with the recombinant vaccinia viruses or with wildtype vaccinia virus (wtVV) and their clinical, virological and immune responses evaluated. Following intradermal inoculation with wtVV or recombinant vaccinia virus expressing fox zona pellucida C (rVV-fZPC), or after peroral administration with recombinant vaccinia virus expressing the porcine zona pellucida C protein (rVV-pZPC) clinical signs of disease were not observed. Five out of six foxes developed antibodies to wtVV proteins. However, none of 12 foxes (six inoculated intradermally with rVV-fZPC, six perorally with rVV-pZPC) reacted in immunoblots with the transgenic fZPC or pZPC, respectively. Infectious wtVV, rVV-fZPC or rVV-pZPC was not isolated from mucosal secretions of any of the foxes whereas viral DNA was present in oral swabs of 3/18 foxes as determined by PCR. Hematological parameters remained mostly unchanged. Histopathological changes were not observed in the ovaries of rVV-fZPC or wtVV inoculated foxes. The data indicate that inoculation of foxes with cell culture infectious wtVV, rVV-fZPC or rVV-pZPC did not result in production of infectious progeny virus in vivo. For this reason transgene expression may have been insufficient to induce adequate immune responses against the transgenic proteins.

Keywords: Fox; Vulpes; Vaccinia virus; Zona pellucida; ZPC; Antifertility; Immunocontraception

1. Introduction

Fertility control of free-ranging animals has gained considerable attention as a potential tool to reduce the impact of unwanted or pest animal species on native fauna and livestock. Antifertility (immunocontraceptive) vaccination is particularly appealing for use in wildlife because of its humane, non-lethal approach and the potential to reduce the size of pest animal populations by reducing recruitment rather than increasing mortality (for review see [1]). However, before antifertility vaccination can be considered for use at an ecological level, substantial challenges have yet to be overcome. Among these are cost-effective production of an efficacious and safe vaccine and suitable methods to deliver it to a large number of animals over a wide area. So far, only one experimental antifertility vaccine has been applied as a management tool in free-ranging or captive wild animals. This vaccine consists of a mixture of native proteins extracted from the pig zona pellucida (pZP) and needs to be injected repeatedly together with a potent adjuvant to achieve the desired contraceptive result [2]. Although numerous reports are published about the successful use of this vaccine in a variety of species (for review see [3,4]), the limitation of this vaccine to individual and selective application is evident. Therefore, more appropriate vaccine production and delivery strategies need to be developed if pest animal problems across continental scales such as the fox problem in Australia are to be

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tackled using antifertility vaccination [5]. In Australia, the overabundance of introduced European red foxes poses not only a major threat to the survival of endangered native fauna, but also impacts on lamb production.

Two different approaches are currently adapted to overcome the above mentioned problems with vaccine production and delivery. One approach involves the use of a transgenic self-disseminating virus which can spread naturally through the target population. Prototypes of such disseminating viral antifertility vaccines have already been successfully used in experimental mouse and rabbit trials [6,7]. The second approach is based on bait-delivered oral vaccines (produced either in bacterial or viral systems) that are not designed to spread within the target population [5]. Antifertility vaccines of this type have not yet been used in the field.

Vaccination of wildlife is generally carried out on a large scale which precludes in most cases individual parenteral application of vaccines. Distribution of the vaccine via oral baits is therefore the most practical and widely applied method. The only product currently in use for wildlife immunisation on a wide geographical scale is a vaccine based on orally delivered vaccinia virus. Over the last two decades, extensive oral immunisation campaigns have been undertaken in Europe and North America to mitigate and prevent the spread of rabies in wildlife, especially of foxes in Europe and coyotes and racoons in North America [8]. Among the various rabies vaccines in use is a recombinant viral vaccine which is presently the only genetically altered vaccine in field use worldwide. It is based on a recombinant vaccinia virus that expresses the glycoprotein G of rabies virus. This vaccine has been designed for aerial baiting and been shown to have an increased safety for numerous target and non-target species in comparison to other rabies vaccines based on attenuated live rabies virus [9].

Vaccinia virus is a commonly used and well suited viral vector for the development of recombinant vaccines (for review see [10]). It was therefore chosen for this study as a model virus to generate transgenic viruses that express proteins with potential antifertility effect in foxes. A variety of antigens originating from male and female reproductive tissues have been intensively studied for their suitability as antifertility antigens, most notably sperm and oocyte antigens [11–13]. Among the female-derived antigens with the most pronounced antifertility effects are immunogens originating from the oocyte zona pellucida [14,15]. The zona pellucida is the extracellular matrix that surrounds growing oocytes, ovulated eggs and pre-implantation embryos and plays a key role in sperm and oocyte interaction during fertilisation [16]. Zona pellucida proteins are well researched targets for immunological disruption of reproduction and were therefore selected for use in this study.

The aims of this study were three-fold. Firstly, to identify and clone the fox zona pellucida C (fZPC) protein, secondly, to engineer transgenic recombinant vaccinia viruses that express European red fox zona pellucida C protein or the zona pellucida C protein of the domestic pig, and thirdly, to use these recombinant vaccinia viruses as model vaccines to evaluate their immunogenicity and, if warranted, their antifertility potential in foxes.

2. Materials and methods

2.1. Identification and cloning of fox zona pellucida C cDNA

A fox ovary cDNA library was constructed using a Stratagene lambda ZAP kit (Stratagene, USA) with polyA$^+$ RNA isolated from the ovaries of six European red foxes, captured in the wild in Australia and aged from one month to adult. The library was screened with a 1.7 kilobase pair (kb) full length cDNA clone of the rabbit zona pellucida B (rZPB) (kindly provided by Bonnie Dunbar, Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, USA) [17] under the following conditions: 50% formamide, 4% SDS, 5× SSPE, 0.5% Blotto at 40 °C. A 1.3 kb full length clone was isolated and further characterized. Single-stranded DNA from this clone was prepared and sequenced with a Pharmacia Autoread sequencing kit (AMRAD, Sydney, Australia), using the dideoxy chain termination method. The reactions were separated on a 6% acrylamide gel and analysed with a Pharmacia LKB Automated Laser FLuorescent DNA sequencer. Nucleic acid and protein searches of the Genbank Database were performed at the National Center for Biotechnology Information (Bethesda, USA), using the BLAST search program [18]. Sequence alignments were conducted using CLUSTAL W [19]. The clone was identified as a homologue of the dog zona pellucida C cDNA and was designated pSK-fZPC. The DNA sequence of fZPC was submitted to GenBank and assigned the GenBank accession number AY598032.

2.2. Construction and in vitro expression of transgenic recombinant vaccinia viruses

Two recombinant vaccinia viruses were constructed that express the marker gene beta-galactosidase (β-gal) under the control of the poxvirus 7.5 kilo Dalton (kDa) early promoter (EP) and either the fox zona pellucida C or the porcine zona pellucida C (pZPC) proteins under the control of a synthetic late poxvirus (SLP) promoter. The recombinant viruses were designated rVV-fZPC and rVV-pZPC, respectively. The shuttle vector for introducing fZPC into vaccinia virus was produced by inserting fZPC cDNA (excised from pSK-fZPC by restriction enzymes *Eco*RI and *Xho*I) into the vaccinia virus shuttle vector pMJ601 (kindly provided by Ian Ramshaw, John Curtin School of Medical Research, The Australian University, Canberra, Australia) [20]. Plasmid pMJ601 is a vaccinia virus recombination plasmid containing the SLP and is designed to allow the insertion of transgenes into the thymidine kinase gene of vaccinia virus. Plasmid pMJ601 also