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Mini-Review

Biological control of vertebrate pests using virally vectored immunocontraception

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Abstract

Species-specific viruses are being genetically engineered to produce contraceptive biological controls for pest animals such as mice, rabbits and foxes. The virus vaccines are intended to trigger an autoimmune response in the target animals that interferes with their fertility in a process termed virally vectored immunocontraception. Laboratory experiments have shown that high levels of infertility can be induced in mice infected with recombinant murine cytomegalovirus and ectromelia virus expressing reproductive antigens as well as in rabbits using myxoma virus vectors. The strategies used to produce and deliver species-specific immunocontraceptive vaccines to free-living wildlife are presented in this review. Discussion includes coverage of the likely safety of the proposed vaccines as well as the implications of the approach for fertility control in other species.

Keywords: Infertility; Mouse; Rabbit; Fox; Zona pellucida; Wildlife

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1. Introduction

Vertebrate pests cause widespread environmental and economic damage. Current control measures which rely on poisoning, trapping and shooting (Norris and Low, 2005) can be effective in the short term, but are unsustainable and often lead to losses in non-target species. Reducing fertility rather than increasing mortality is a potentially more humane, species-specific and long-term solution (Tyndale-Biscoe, 1994). Fertility control has been used to manage small populations of captive and wild animals using chemical implants or immunization by injection against reproductive proteins to cause autoimmune infertility or 'immunocontraception' (Kirkpatrick and Frank, 2005; Barfield et al., 2006). Species-specific virus vectors that either spread by natural infection or can be broadcast using baits would further enable fertility control to be applied to widespread wildlife populations (Tyndale-Biscoe, 1994). Research in Australia has focused on providing proof of concept for virally vectored immunocontraception (VVIC; Fig. 1) by genetically engineering species-specific viruses to express contraceptive antigens for three key pest species:

1. Foxes using canine herpesvirus-1 (CHV)
2. Rabbits using myxoma virus
3. Mice using murine cytomegalovirus (MCMV) and ectromelia virus (ECTV)

Apart from the technical and ecological hurdles facing development of VVIC, a clear need to apply caution has been recognised (Angulo and Cooke, 2002). It is vital that all public concerns and legal requirements about the risks of disseminating genetically modified viruses are adequately addressed during the development phase before any recombinant vaccine can be applied for wildlife control.

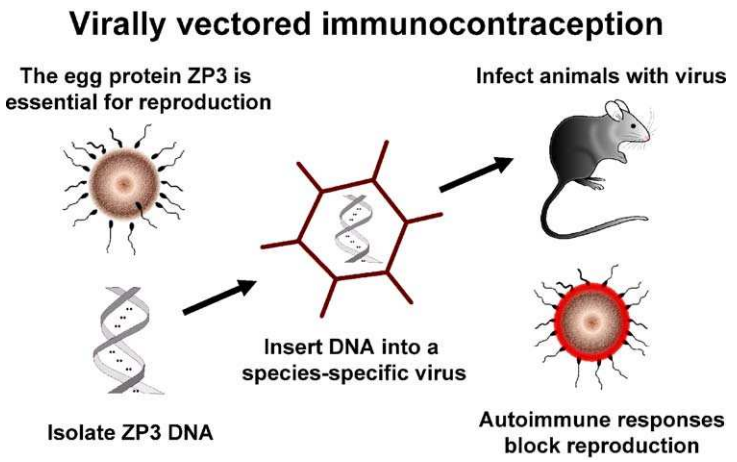

Fig. 1. Virally vectored immunocontraception.

2. Immunocontraceptive antigens

Numerous studies have sought to identify immunocontraceptive antigens and immunization against reproductive proteins has been shown to cause infertility in a variety of species, including humans – for recent reviews, see Ferro and Mordini (2004), Naz et al. (2005) and Suri (2005). Nevertheless, certain antigens that are highly conserved between species have been avoided when developing virally vectored immunocontraceptives that are intended to disseminate. In particular, highly conserved reproductive proteins such as luteinising hormone (LH), luteinising hormone releasing hormone (LHRH), follicle stimulating hormone (FSH) and their receptors present unacceptable risks of inadvertently affecting fertility in humans and other non-target species. Some of these antigens can cause undesirable side effects in the target species that raise ethical issues, such as premature termination of pregnancies or damage to non-reproductive tissues due to suppression of steroidogenesis (Talwar, 1997; Delves, 2004; Ferro and Mordini, 2004). They may also induce alterations in sexual and social behaviour within dominance hierarchies of some species, leading to increased breeding by untreated, subdominant animals and a reduction in immunocontraceptive effectiveness at the population level (Tyndale-Biscoe, 1994; Tuyttens and Macdonald, 1998).

As fertilization is a species-specific process (Hoodbhoy and Dean, 2004; Conner et al., 2005), targeting the proteins involved in the interactions between gametes provides the greatest likelihood of achieving acceptable and effective contraception with least risk to non-target species. Sperm proteins have been suggested as ideal immunocontraceptive antigens as they are able to induce infertility in males and females (Suri, 2005). Nevertheless, consistent immunocontraceptive responses remain to be demonstrated for sperm antigens that have been produced using recombinant DNA technology. On the other hand, high levels of long-term infertility in females have been achieved in many species following immunization with native and recombinant antigens derived from the zona pellucida (ZP), a protein matrix that surrounds the oocyte (Gupta et al., 2004). ZP proteins have therefore been preferentially selected for use in VVIC.

3. Virally vectored immunocontraceptives

3.1. The mouse

Proof of concept for VVIC was first achieved in female mice using a recombinant ECTV (Table 1). This virus (recECTV-mZP3) expressed mouse zona pellucida subunit 3 glycoprotein (mZP3) from a synthetic poxvirus early–late promoter (Jackson et al., 1998). A single inoculation with virus into the footpad of BALB/c mice caused reversible infertility in 70% of mice and sub-fertility in the remaining animals. Subsequently, higher levels of infertility have been achieved using recombinant MCMV to express the mZP3 antigen (recMCMV-mZP3) driven by a constitutive human cytomegalovirus immediate early (*ie1*) gene promoter (Chambers et al., 1999a; Lloyd et al., 2003; Redwood et al., 2005). In long-term trials, all BALB/c mice became infertile within 3 weeks of receiving a single intraperitoneal injection of recMCMV-mZP3 and sterility was observed for up to 250 days (Table 1). Some laboratory strains of mice other than BALB/c are reported

Table 1
Fertility of animals immunized with virally vectored immunocontraceptives

Species	Antigen	Virus vector	Total animals[a]	% infertile	Mean litter size	Contraceptive effect
Mouse	mZP3	MCMV	9	100	0	Sterility[b]
	mZP3	MCMV	6	100	0	Sterility[b]
	mZP3	MCMV	10	90	1.0	Sterility[c]
	mZP3	MCMV	6	100	0	Sterility[d]
	mZP3	MCMV-δ	6	100	0	Sterility[d]
	mZP3	ECTV	13	69	1.8	Sterility/subfertility[e]
	mPH20	MCMV	6	0	6.5	None[f]
	mPH20	MCMV	6♂	0	6.9	None[f]
	None	None	10	10	5.1	None[c]
Rat	mZP3	MCMV	6	0	7.7	None[g]
	None	None	6	0	7.6	None[g]
Rabbit	rZPC	Myxoma	12	75	4.7	Transient infertility[h]
	rZPC	Myxoma	11	73	2.7	Transient infertility[h]
	rZPC	Myxoma	6	67	ND	Transient infertility[h]
	rZPC	Myxoma	6	67	ND	Transient infertility[h]
	rZPC	Myxoma	12	58	4.0	Transient infertility[h]
	rZPB	Myxoma	12	25	7.0	None[i]
	rZPA	Myxoma	6	0	6.3	None[h]
	rZPC-T	Myxoma	6	0	8.3	None[h]
	None	None	84	9	6.0	None[h]

mZP3, mouse zona pellucida subunit 3; mPH20, mouse sperm protein PH20 (SPAM-1); rZPA, B and C, rabbit zona pellucida subunits A, B and C; rZPC-T, rZPC lacking a transmembrane anchor MCMV, murine cytomegalovirus; MCMV-δ, attenuated MCMV lacking open reading frames *m07-m12*; ECTV, ectromelia virus; ND, not determined.

[a] Animals all received a single dose of vaccine and were females unless otherwise indicated.
[b] Lloyd et al. (2003) long-term trials.
[c] Lloyd et al. (2003) short-term trial, one pup born to one female.
[d] Redwood et al. (2005).
[e] Jackson et al. (1998).
[f] Hardy et al. (2004a).
[g] Smith et al. (2005).
[h] Mackenzie et al. (2006).
[i] Kerr et al. (1999).

to be less susceptible to immunocontraception using recMCMV-ZP3 (Chambers et al., 1999a; Lloyd, unpublished results). However, specific pathogen-free outbred wild mice from Australia showed low prevalence of genetic resistance to MCMV infection (Scalzo et al., 2005) and were effectively sterilised following inoculation with recMCMV-mZP3 (Lloyd, unpublished results). These results indicate that wild populations of Australian mice should be susceptible to an immunocontraceptive MCMV.

Infection with recECTV-mZP3 or recMCMV-mZP3 leads to induction of mZP3-specific antibodies and progressive loss of follicles from the ovaries. This loss was attributed to disruption of developing follicles by antibody-dependent mechanisms since overt inflammatory responses in the ovaries were not detected (Jackson et al., 1998; Lloyd et al., 2003).

Antigens other than mZP3 have so far failed to cause infertility in mice when delivered using recombinant MCMV. These include sperm protein PH20 (Hardy et al., 2004a), pig ZPC, germ cell factor, oviduct glycoprotein and four synthetic multi-antigen peptide constructs (A. Redwood, unpublished results).

All the recombinant viruses described above replicated normally in cell culture but were highly attenuated *in vivo*, although some still induced strong immunocontraceptive responses. In ECTV, attenuation was due to inactivation of the thymidine kinase (*tk*) gene (Jackson et al., 1998). In MCMV, attenuation was attributed to either interference with viral replication due to immune responses to the co-expressed ZP3 antigen or to inactivation of the viral immediate early 2 (*ie2*) gene (Redwood et al., 2005), although other studies have shown that the *ie2* gene is not essential for the growth of MCMV *in vitro* or *in vivo* (Manning and Mocarski, 1988; Cardin et al., 1995).

3.2. The rabbit

The three rabbit zona pellucida glycoproteins (rZPA, rZPB and rZPC) have all been evaluated as immunocontraceptive antigens under the control of a late promoter in a naturally attenuated strain (Uriarra) of myxoma virus (Table 1). The fertility of rabbits immunized with recombinant viruses expressing rZPA (Mackenzie et al., 2006) or rZPB (Kerr et al., 1999) was not significantly affected. However, 70% of rabbits immunized with recombinant myxoma viruses expressing rZPC were infertile 30–35 days after immunization, but fertility was restored within 70 days (Mackenzie et al., 2006). Immunization with recombinant myxoma virus was sufficient to stimulate B lymphocytes to the self-antigens, although strong helper T cell responses appeared not to be elicited and antibodies to the ZP antigens were short-lived. In more recent experiments, recombinant myxoma viruses constructed with rZPC under the control of a synthetic combined early/late promoter induced infertility in 90-100% of rabbits mated 35 days after immunization and in 50% of rabbits at subsequent matings (Kerr, Perkins and van Leeuwen, unpublished results).

3.3. The fox

Studies in European red foxes have been conducted to assess the immunocontraceptive potential of fox ZPC (fZPC) and pig ZPC (pZPC) using either vaccinia virus (Reubel et al., 2005) or canine herpesvirus (CHV) vectors (Strive et al., 2006). All recombinant viruses constitutively expressed fZPC or pZPC in cell culture but were highly attenuated in foxes *in vivo*. The viruses failed to induce antibodies to the encoded antigens even after repeated inoculations, although serum antibody levels against the viruses themselves were high. The attenuation was mainly attributed to inactivation of the viral *tk* genes in both vaccines. However, a recombinant CHV with an intact *tk* gene that expressed pZPC from an intergenic region between the UL21 and UL22 genes was also highly attenuated *in vivo* and failed to induce anti-ZPC antibodies or infertility in foxes (Strive and Reubel, unpublished results). Although recombinant vaccinia and CHV appeared unable to replicate sufficiently to induce antibody responses to contraceptive antigens in foxes, they may still prove useful in the dog, the natural host for CHV.

4. Enhancing contraceptive efficacy

The main technical impediments for VVIC are attenuation of recombinant viruses, short-lived contraceptive responses in some species and potential lack of penetrance into populations due to pre-existing immunity or genetic resistance to the virus vectors (Magiafoglou et al., 2003). These concerns are being addressed in a number of ways.

The first approach has been to select wild isolates of the virus vectors that already display competitive advantages. In mice, a wild MCMV isolate from Australia with high natural transmissibility has now been isolated (Hinds, unpublished results) and is being engineered to express the mZP3 gene. Novel insertion sites for expression of mZP3 in MCMV are also being tested to assess whether the *in vivo* attenuation seen with previous recombinants can be avoided.

An alternative approach to reduce attenuation is to co-express immune modulating molecules such as cytokines in the vaccines (Ramsay and Ramshaw, 1997). Co-expression of cytokines such as interleukin 4 (IL-4) with *lacZ* in ECTV has been shown to counteract viral attenuation due to transgene insertion and enable the recombinant viruses to overcome genetic resistance to ECTV (Jackson et al., 2001). In myxoma virus co-expression of rabbit IL-4 with either rZPB or rZPC led to increased levels and duration of infertility in rabbits (Kerr, Perkins, McLaughlin, van Leeuwen, unpublished results). However, as co-expression of IL-4 in both ECTV and myxoma virus led to greatly increased virulence, caution must be advocated when considering the use of cytokines in conjunction with contraceptive antigens.

Another approach has been to fuse antigens to carrier molecules to increase immunogenicity and prolong the contraceptive effects. Fusion of mZP3 to ubiquitin in MCMV caused alterations in the immune responses to mZP3 whilst retaining contraceptive efficacy (Redwood, unpublished results). In other studies, vaccinia virus that expressed hCG to a membrane anchor sequence was considerably more immunogenic in rats than a secreted form, although effects on fertility were not reported (Srinivasan et al., 1995).

Finally, efforts to identify alternative and potentially more species-specific contraceptive antigens are ongoing. Recent experiments indicate that antigens containing repeated immunogenic peptide fragments from multiple ZP genes are promising candidates for inclusion in recombinant immunocontraceptive viruses (Hardy et al., 2004b).

5. Ecological feasibility

Several field and pen trials have been conducted in Australia to assess the feasibility of fertility control using surgical sterilization to mimic immunocontraception. In mice (Chambers et al., 1999b; Singleton et al., 2002), rabbits (Twigg et al., 2000; Williams et al., in press) and foxes (Saunders et al., 2002), results from large scale trials indicated that sterility in at least 70–80% of animals is required to significantly reduce population sizes. In other experiments, introductions of identifiable field strains of MCMV to mice (Farroway et al., 2005) and myxoma virus to rabbits (Merchant et al., 2003) have led to spread and persistence of the viruses. These results, combined with modelling studies (Courchamp and Cornell, 2000; Arthur et al., 2005), demonstrate that it should be possible to introduce recombinant viruses into populations of wild animals, provided they are not at any competitive disadvantage.

6. Species-specificity

The species-specificity for VVIC relies heavily on the virus vectors. Vaccinia virus infects a variety of hosts, including humans (Cliquet and Aubert, 2004), but MCMV, ECTV and myxoma virus are all considered species-specific (Fenner, 2000; Mocarski and Courcelle, 2001). CHV is relatively less species-specific, although only canids appear susceptible to infection (Appel, 1987; Reubel et al., 2001). Whilst immune responses to antigens derived from native porcine zona pellucida interfere with fertility in many mammals (Frank et al., 2005), sufficient differences exist between species in the protein composition of the zona pellucida (Conner et al., 2005) for some degree of immunocontraceptive specificity to be anticipated. In one case, a recombinant zona pellucida protein has indeed been directly shown to provide additional specificity to the virus vector. Female rats infected with recombinant MCMV expressing mZP3 were fully fertile (Table 1) despite the induction of significant and increasing serum anti-MCMV and anti-mZP3 antibody titres (Smith et al., 2005). The anti-mZP3 antibodies were specific for mouse ZP3 and did not cross-react with native rat zona pellucida.

7. Conclusions

Contraception is being successfully applied as a method for reducing the population growth rates in a range of mammals including humans. However, delivery of fertility control to large numbers of animals in the wild remains a daunting task. Immunogenicity and fertility trials with VVIC in foxes and rabbits have illustrated the difficulties in gaining long-term contraceptive effects using current knowledge. This has led to a reassessment of whether a disseminating VVIC approach is appropriate for pest species whose reproductive life-spans outlast the contraceptive effects. The additional requirement for a high percentage of animals in the wild to be made infertile means that VVIC for the rabbit and fox remains a long-term and costly proposition. Nevertheless, the technology has the potential to deliver highly effective ‘one shot’ contraception for animals when administered by injection. Disseminating virally vectored immunocontraception remains feasible for mice provided the vaccines can be made transmissible and demonstrated experimentally not to affect other animals. In this respect, public concerns and regulatory arrangements for genetically modified organisms have increased significantly in Australia since this research commenced. Australian regulations (principally the Gene Technology Act 2000, administered through the Office of the Gene Technology Regulator; http://www.ogtr.gov.au/), with which all VVIC research has conformed, are designed to ensure public accountability and will constrain field-testing until all risks to non-target animals have been thoroughly explored and mitigated.

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