Anti-fertility immunisation has been applied as a humane alternative to surgical, chemical and lethal methods for preventing breeding or modifying behaviour in livestock, captive zoo animals and small populations of wild animals\textsuperscript{1,2,3}. In this approach, animals are immunised against reproductive proteins to trigger auto-immune responses that block fertility through a process generally referred to as immunocontraception. Current experimental vaccines require repeated injections, are less than 100\% effective and cannot be applied to large populations of free-ranging wild animals. Alternative immunocontraceptive vaccines are therefore being actively developed to improve efficacy, reduce the need for repeated injections and enable large numbers of wild animals to be targeted. Some employ live genetically modified viruses to deliver a contraceptive effect. In this approach, termed virally-vectored immunocontraception (VVIC), the gene for a fertility antigen is inserted into the genome of a virus. The genetically modified virus then expresses the antigen in infected animals and induces a contraceptive autoimmune response. The viral vectors can either be attenuated for use on individuals or be able to transmit (self-disseminate) for application to free-ranging wildlife and pest animals. These vaccines are intended to supplement current control practices of shooting, trapping and poisoning for pest animals such as rabbits, foxes and mice in Australia.

VVIC has been tested under laboratory conditions, with varying degrees of success, by targeting the zona pellucida in foxes using vaccinia virus and canine herpesvirus vectors, in rabbits using myxoma virus and in mice using murine cytomegalovirus\textsuperscript{4}. The approach has been particularly successful in mice, with complete, long-term infertility in animals now readily achieved following a single inoculation. The strategies applied and recent advances made in the development of species-specific immunocontraceptive virus vaccines will be presented. The potential for the technology to be adapted for use in domestic and wild dogs is appealing, provided the efficacy, safety, regulatory requirements and public acceptance of genetically modified vaccines can be established.
References


Immuocontraceptive antigens and strategies for mice and foxes

Overview

- Virally vectored immunocontraception
- Approaches for mice (transmissible agent)
  - Recombinant MCMV
  - Efficacy
  - Transmission studies
- Approaches for foxes (bait-delivered)
  - Recombinant Vaccinia and Canine Herpesvirus
  - Efficacy
- Conclusions and Prospects
Contraceptive vaccines are being developed that prevent fertility by triggering an autoimmune response against molecules essential for reproduction.

1. Autoimmune infertility occurs naturally
2. Immunization against various proteins in sperm, testis, ovary, hormones and their receptor proteins has successfully reduced fertility in a wide range of species
Session III: What’s New in Contraceptive Vaccines?
Immunocontraceptive Antigens and Strategies
By Dr. Christopher Hardy

The zona pellucida

Virally vectored immunocontraception

The egg protein ZP3(C) is essential for reproduction

Infect animal

Insert DNA into a virus vector

Autoimmune responses block reproduction

Isolate ZP3 DNA
Session III: What’s New in Contraceptive Vaccines?
Immunocontraceptive Antigens and Strategies
By Dr. Christopher Hardy

Viral vectors
- Mouse
  - Murine cytomegalovirus
  - Ectromelia virus
- Rabbit
  - Myxoma virus
- Fox
  - Vaccinia virus
  - Canine herpesvirus

Generation of recombinants
- Viral DNA
- Gene
- Plasmid
- Recombinant viral DNA
- Homologous recombination
- Plaque purification
- Infection of new cells
- wt
- Recombinant virus!!
Session III: What’s New in Contraceptive Vaccines?
Imunocontraceptive Antigens and Strategies
By Dr. Christopher Hardy

Mouse Immunocontraception

(Courtesy, Dr Herwig Leirs)
Murine cytomegalovirus (MCMV)

- House mouse specific
- Transmitted by close contact
- Induces long-lasting immune responses
- Establishes latent infections in mice
- Widespread in wild mice
- Multiple re-infections possible
- Usually does not cause disease symptoms
- Readily genetically engineered

Recombinant MCMVs

- recMCMV-βgal
- recMCMV-ZP3
- Murine CMV

\[ \text{MCMV} \rightarrow \text{HindIII} \rightarrow \text{ie1/3} \rightarrow \text{ie2} \rightarrow \text{HindIII} \]

\[ \text{recMCMV-βgal} \]

\[ \text{recMCMV-ZP3} (P \text{Hcmv}) \]
Session III: What’s New in Contraceptive Vaccines?
Imunocontraceptive Antigens and Strategies
By Dr. Christopher Hardy

Number of pups produced by mice inoculated (i.p.) with recMCMVs

RecMCMV-ZP3 infected mice

<table>
<thead>
<tr>
<th>Mouse Strain</th>
<th>% Infertility (K181)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/c, A/J</td>
<td>90-100</td>
</tr>
<tr>
<td>Wild Mouse</td>
<td>90-100</td>
</tr>
<tr>
<td>C57BL/6J</td>
<td>50- (100% G4)</td>
</tr>
<tr>
<td>CBA</td>
<td>0- (90% G4)</td>
</tr>
</tbody>
</table>
RecZP3 and infertility (mice)

Effect of recZP3 on ovaries
Specificity: recMCMV-ZP3

- No evidence for MCMV in other rodent species
- MCMV cannot establish productive infections in non-mouse cell lines
- Fertility of rats unaffected by recMCMV-mZP3

Can transmission of VVIC be achieved for wild mice?
Transmission of recMCMVs

- Examined using groups of wild mice: (3 to 4 females plus a male)
- One animal infected with MCMV; rest are naïve contacts
Session III: What’s New in Contraceptive Vaccines?
Imunocontraceptive Antigens and Strategies
By Dr. Christopher Hardy

Transmission of infertility

Selection for transmission

6 groups of SPF wild mice
(1 naturally infected male + 4 SPF females)
Transmission of recMCMV (seroconversion) in mice

(Wild mice)

<table>
<thead>
<tr>
<th>Day</th>
<th>21</th>
<th>35</th>
<th>42</th>
<th>49</th>
<th>63</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed field strains (natural infection)</td>
<td>77%</td>
<td>89%</td>
<td>86%</td>
<td>93%</td>
<td></td>
</tr>
<tr>
<td>MIA28 (ip infected)</td>
<td>0%</td>
<td>22%</td>
<td>78%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rec MIA28-EGFP (ip infected)</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>11%</td>
<td></td>
</tr>
</tbody>
</table>

Cumulative incidence of infection %. N=8-28
Criteria for infection is >Normal mouse serum +3xSD at 1:20 dilution

Summary of mouse VVIC

- Long-term infertility (>250 days) with recMCMV-mZP3
- Single intraperitoneal injection
- Possible biocontrol for mice?
  - Transmission of infertility not yet achieved
  - Genetic resistance to MCMV in some lab strains or pre-exposure to MCMV reduces contraceptive efficacy
  - Evidence for attenuation of infection and loss of transmission of all recMCMV
  - Bait (oral) delivery of recMCMV not practical
- Nevertheless remains a useful model for other species
Session III: What’s New in Contraceptive Vaccines?
Immuocontraceptive Antigens and Strategies
By Dr. Christopher Hardy

Fox Immunocontraception

Photos: John Bray
Australia’s No.1 Predator

- Introduced in 1871
- Spread over almost entire continent
- Conservation: preys on native animals
- Economy: contributes to lamb loss
- Management: shooting, trapping, poisoning
- Bait delivery of vaccines feasible

Recombinant vaccinia virus (rabies vaccine)
Session III: What’s New in Contraceptive Vaccines?
Immuocontraceptive Antigens and Strategies
By Dr. Christopher Hardy

Texas Department of State Health Services

Recombinant VV-ZPC

(Wild foxes)
N=6 foxes
10^5 pfu/dose

Sera vs. recVV-pZPC
1 preimmune
2 IZPC i.d. (X6)
3 preimmune (dog)
4 anti-VV (rabbit)
5 anti-PZP (rabbit)

Sera vs. Porcine ZPC
1 preimmune
2 IZPC i.d. (X6)
3 preimmune
4 pZPC p.o. (X3)
5 PZP (rabbit)
Canine Herpesvirus (CHV)

- Species restricted
  - Infests dogs, coyotes, foxes
- Can be delivered in baits
- Induces long-lasting immune responses
- Australian wild foxes appear sero-negative
- Readily genetically engineered

CHV Bait Trial – Fox-Off Baits
Session III: What’s New in Contraceptive Vaccines?
Imunocontraceptive Antigens and Strategies
By Dr. Christopher Hardy

**CHV Serology – Bait trial**

- 6 wild foxes were intranasally inoculated with CHV and paired with 6 naïve, seronegative wild foxes (2 foxes / cage)
- All inoculated foxes seroconverted
- After 1 year, inoculated foxes seropositive, but all in-contact foxes remained seronegative
- CHV infection in foxes cannot be reactivated

Likelihood of CHV shedding and transmission from fox to fox in the environment low
Session III: What’s New in Contraceptive Vaccines?
Immuocontraceptive Antigens and Strategies
By Dr. Christopher Hardy

Recombinant CHVs

RecCHV-EGFP
RecCHV-ZPC

RecCHV - Immunostaining

Recombinant CHV (200x)  MDCK cell control (100x)
Recombinant CHV (400x)  Wildtype CHV (400x)
Session III: What’s New in Contraceptive Vaccines?
Imunocontraceptive Antigens and Strategies
By Dr. Christopher Hardy

TK(-) recCHVs

<table>
<thead>
<tr>
<th>9 foxes (♂/♀) recCHV-foxZPC</th>
<th>(12 weeks)</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 foxes (♂/♀) recCHV-EGFP</td>
<td>Oral Swabs</td>
<td>(0/19)</td>
</tr>
<tr>
<td>Inoculation: 10⁴ pfu i.v.</td>
<td>Genital Swabs</td>
<td>(0/19)</td>
</tr>
<tr>
<td>10⁶ pfu i.n. (2-4 wks)</td>
<td>Spleen</td>
<td>(0/19)</td>
</tr>
<tr>
<td>Serology: α foxZPC: (0/9)</td>
<td>Liver</td>
<td>(0/19)</td>
</tr>
<tr>
<td>α EGFP: (1/10)</td>
<td>Lung</td>
<td>(0/19)</td>
</tr>
<tr>
<td>α CHV: (19/19)</td>
<td>Kidney</td>
<td>(0/19)</td>
</tr>
<tr>
<td>• No signs of illness</td>
<td>Heart</td>
<td>(0/19)</td>
</tr>
<tr>
<td>• No virus shedding in swabs</td>
<td>Int. lymphn.</td>
<td>(0/19)</td>
</tr>
<tr>
<td>• Large insertion of foreign DNA</td>
<td>Head lymphn.</td>
<td>(0/19)</td>
</tr>
<tr>
<td>TK(-) vaccine is ineffective</td>
<td>Tonsils</td>
<td>(0/19)</td>
</tr>
<tr>
<td></td>
<td>Spinal ganglia</td>
<td>(0/19)</td>
</tr>
</tbody>
</table>

TK(+) recCHV

<table>
<thead>
<tr>
<th>5 foxes (♂/♀) recCHV-pigZPC</th>
<th>(12 weeks)</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculation: 10⁴ pfu i.v.</td>
<td>Oral Swabs</td>
<td>(0/5)</td>
</tr>
<tr>
<td>Serology: α CHV: (5/5)</td>
<td>Genital Swabs</td>
<td>(0/5)</td>
</tr>
<tr>
<td>α pigZPC: (0/5)</td>
<td>Spleen</td>
<td>(0/5)</td>
</tr>
<tr>
<td>TK(+) vaccine is also attenuated</td>
<td>Liver</td>
<td>(0/5)</td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>(0/5)</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>(0/5)</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>(0/5)</td>
</tr>
<tr>
<td></td>
<td>Intes. lymphn.</td>
<td>(0/5)</td>
</tr>
<tr>
<td></td>
<td>Head lymphn.</td>
<td>(0/5)</td>
</tr>
<tr>
<td></td>
<td>Tonsils</td>
<td>(0/5)</td>
</tr>
<tr>
<td></td>
<td>Spinal ganglia</td>
<td>(0/5)</td>
</tr>
</tbody>
</table>
**Summary of fox VVIC**

- Infected foxes produce antibodies against recombinant CHV and VV
- Poor antibody response against co-expressed antigens
- Recombinant CHV and VV are attenuated in foxes
- Combination of CHV or VV and ZPC is not sufficient to deliver immunocontraception to foxes

**Conclusions**

- Most research in Australia into virally vectored immunocontraception for wildlife has been suspended
- Technical difficulties
  - Attenuation *in vivo* of recombinant vaccines (mice & foxes)
  - Delivery method (natural transmission) not achieved (mice)
  - Non-response to ZPC (fox)
  - Duration of infertility insufficient (rabbit)
- Ecological issues
  - Prior exposure to virus vectors in the field (mice & rabbits)
  - Evidence for genetic / immunological resistance to vaccines
Prospects

- Alternative antigens (LHRH/GnRH?)
- Immune enhancers
  - Cytokines
  - Carriers
- Choice of virus vectors
  - Avoid attenuation (strain selection)
  - No pre-existing immunity (alternative vectors)
- CHV-based vaccines may be effective in dogs
  - Better replication (natural host for CHV)
  - Latent infection possible (cf MCMV)
  - Reproductive cycle different to foxes (multiple vs. 1/yr)
  - Direct injection feasible

Mouse Team

<table>
<thead>
<tr>
<th>CSIRO</th>
<th>UWA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chris Hardy</td>
<td>Geoff Shellam</td>
</tr>
<tr>
<td>Lyn Hinds</td>
<td>Alec Redwood</td>
</tr>
<tr>
<td>Tanja Strive</td>
<td>Megan Lloyd</td>
</tr>
<tr>
<td>Katrina Leslie</td>
<td>Lee Smith</td>
</tr>
<tr>
<td>John Wright</td>
<td>Mal Lawson</td>
</tr>
<tr>
<td>Sandra Beaton</td>
<td>Sonia Nicolovski</td>
</tr>
<tr>
<td>Barbara Skoro</td>
<td>Nicole Harvey</td>
</tr>
<tr>
<td>Juliet Fisher</td>
<td>Paula Cunningham</td>
</tr>
<tr>
<td>Alice Kenney</td>
<td>Shelley Gorman</td>
</tr>
<tr>
<td>Peter Brown</td>
<td>Kathy Williams</td>
</tr>
<tr>
<td>Duncan Sutherland</td>
<td>Ngoc Lai</td>
</tr>
</tbody>
</table>
Session III: What’s New in Contraceptive Vaccines?
Immunocontraceptive Antigens and Strategies
By Dr. Christopher Hardy

Fox Team
Gerhard Reubel
Tanja Strive
Jenny Pekin
Nigel French
Daryl Venables
John Wright
Steven Zabar
Lyn Hinds
Chris Hardy

Thank You!