FINAL REPORT

GRDC
GRAINS RESEARCH & DEVELOPMENT CORPORATION

CSV16

Biological control of mice - immunocontraception

PROJECT DETAILS

PROJECT CODE:	CSV16
PROJECT TITLE:	BIOLOGICAL CONTROL OF MICE - IMMUNOCONTRACEPTION
START DATE:	01.07.2000
END DATE:	30.06.2005
SUPERVISOR:	CHRISTOPHER HARDY (PRINCIPAL RESEARCH SCIENTIST)
ORGANISATION:	CSIRO ECOSYSTEM SCIENCES
CONTACT NAME:	CHRISTOPHER HARDY

Summary

The European house mouse (*Mus domesticus*) is an introduced pest in Australia that causes significant losses to the grains industry. There is as yet no routine means of controlling infestations of wild mouse populations in Australia apart from chemical rodenticides that pose significant environmental hazards. Over the past five years, considerable progress has been made by GRDC and the Pest Animal Control Cooperative Research Centre (PAC-CRC), now the Invasive Animals CRC, -funded researchers to develop a disseminating immunocontraceptive virus as a viable alternative for mouse control. The work is approaching readiness for trials in simulated and contained environmental settings.

Level 4, East Building | 4 National Circuit, Barton ACT 2600 | PO Box 5367, Kingston ACT 2604 | t. +61 6166 4500 | f. +61 2 6166 4599 | grdc@grdc.com.au | grdc.com.au

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The information contained in these older reports is now several years old, and may have been wholly or partially superseded or built upon in subsequent work funded by GRDC or others. Readers should be aware that more recent research may be more useful for their needs. Findings related to agricultural chemical use are also potentially out of date and are not to be taken as a recommendation for their use.

Conclusions

Results of laboratory studies conducted to date support the feasibility of applying a disseminating immunocontraceptive virus for mouse control. Major achievements have been made in the past year in four key areas of research - contraceptive efficacy, vaccine transmission, risk assessment and modelling. This has ensured continued support for the project within the new Invasive Animals CRC and from GRDC to further develop the vaccine for testing in field trials at the Gatton Campus of the University of Queensland where suitable facilities are available.

In particular, the project has demonstrated the following:

1. Proof of concept that a recombinant murine cytomegalovirus (MCMV) expressing ZP3 (recMCMV-G4-mZP3) can be used to sterilize mice.
2. Fertility of closely related species such as rats remains unaffected by the recMCMV-G4-mZP3.
3. Transmission of recMCMV-G4-mZP3 can occur between mice.
4. Strains of MCMV can be selected that overcome known MCMV resistance mechanisms in certain inbred strains of mice.
5. Mice infected by oral delivery can be made infertile.
6. MCMV appears unable to infect insect cells in tissue cultures.

The project has encountered one set-back - failure to demonstrate that the first intended product recMCMV-eG4-mZP3 can efficiently transmit infertility between mice.

Three independent approaches to address this point are being adopted

The first intended product recMCMV-eG4-mZP3 is being passaged *in vivo* through live mice to select for variants with improved transmission properties.

A second product, recMCMV based on a wild isolate of MCMV from the Murrimbidgee Irrigation Area with naturally higher transmission rates relative to strains such as G4, is being produced

Finally, a third product, recMCMV with the mZP3 gene inserted into a novel location of the virus is being produced. It has recently shown to be dispensable for *in vivo* replication of MCMV in salivary glands (and hence less likely to affect transmission).

Recommendations

Further research is required to determine if a transmissible vaccine can be produced that will fulfil all the criteria for further testing in contained field trials and will specifically address the following points:

1. Construction and selection of a recMCMV-mZP3 that will efficiently transmit between mice AND induce infertility.
2. Production and testing of suitable oral formulations to deliver recMCMV-ZP3 in the field.
3. Determine the extent to which prior infections in wild mice by non-recombinant MCMV affect transmission and fertility

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(and hence efficacy in the field) of recMCMV-mZP3.
4. Collate data for submission of a proposal to the Office of the Gene Technology Regulator (OGTR) to conduct field trials.
5. Assessment of public acceptance of the recombinant technology to control mice.

The research detailed above is expected to be completed within one year (by June 2006). It will be conducted within the Invasive Animals CRC, with support requested from GRDC.

Outcomes

Mouse plagues cause substantial economic and social hardship to graingrowers. The current use of broad acre chemical rodenticides (zinc phosphide[#]) also presents environmental risks, particularly to non-target species. The development of a biocontrol agent to replace chemical rodenticides is expected to cause a reduction in the impact of mouse plagues and provide significant economic, social and environmental benefits.

Economic outcomes:

The annual average loss to the grain industry as a result of mice is estimated at $10-15 million. On this basis, there are significant economic benefits to the Australian grain industry if mouse plagues can be prevented. There can be an expected benefit of $7 million per annum by reducing rodent damage by 20% through the control of the frequency and severity of eruptions of mouse populations. Reference: McLeod R (2004). Counting the cost: Impact of Invasive Animals in Australia 2004. Cooperative Research Centre for Pest Animal Control. Canberra. ISBN 0-9750979-2-X. http://www.pestanimal.Cooperative Research Centre (CRC).org.au/info/Mcleod.pdf

Social outcomes:

The removal of social stress due to mouse plagues experienced by rural communities.

Environmental outcomes:

A reduction in the broad scale application of non-specific chemical pesticides.

Achievements/Benefits

The mouse research program's major goal was "to produce an effective, safe and practical anti-fertility vaccine for mice" by the end of the PAC-CRC. Key findings show that greater than 90% of wild female mice can be rendered infertile using prototype recombinant MCMVs expressing mouse ZP3 protein. However, this effect is dependent on dose, prior exposure to MCMV and route of infection. The research addressed four key questions:

Q1: Can mice be made infertile using a vaccine?

Several recombinant MCMV-mZP3 vaccines cause high levels of long-lasting infertility in wild and laboratory mice.

Q2: Will the vaccine spread in the field?

The first recMCMV-mZP3 vaccines tested transmits between mice, but contact animals remain fertile despite becoming infected. Alternative recMCMV-mZP3 vaccines are being constructed using antigens and viruses selected to increase transmissibility and infertility in contact mice. A second approach is to passage existing recMCMV-eG4-mZP3 virus in mice to select for isolates that replicate more effectively in salivary glands (the source of transmissible virus) to increase transmission rates.

Q3: Is the vaccine mouse-specific and safe?

The fertility of rats is unaffected by a recMCMV-mZP3 vaccine suggesting that the vaccine is mouse-specific.

Q4: Is the vaccine cost effective and acceptable to the public?

A business case and assessment of social attitudes towards acceptability of the mouse immunocontraceptive product were commissioned by the PAC-CRC. Both were favourable for application of a disseminating mouse-specific vaccine based on

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MCMV.

Other research

The Invasive Animals-CRC will continue to develop control methods for vertebrate pest species in the foreseeable future. Amongst these are other rodent pest species, notably rats. The contraceptive vaccine technology developed in the mouse program could be readily developed to address other major rodent pest species. These include rats in Australia as well as rodents overseas, such as voles in China. In particular, the distinct rat cytomegaloviruses from *Rattus norvegicus* and *Rattus rattus* have been identified and isolated. Their growth characteristics have been assessed (Smith et al. J Gen Virol 85:1313-1317 (2004)). These viruses could be used to produce rat-specifc contraceptive vaccines, comparable to the mouse vaccine, by genetically engineering them to express rat ZP3s.

Consideration should also be given to development of alternative, more conventional biocontrol agents for mice, should the mouse contraceptive vaccine prove unsuitable for application for scientific reasons or for reasons such as inability to obtain regulatory or public approval for use of a genetically modified (GM) based technology. The feasibility of using bait-delivered biocontrol or chemical agents for rodents that do not contain genetically modified material may need to be explored. In particular, lethal, orally infectious viruses might be considered for controlling mouse plagues in Australia. Such viruses, delivered through baits before mice reach plague densities could be tested to determine if lethal biocontrol can be applied as a cost-effective alternative to virally vectored immunocontraception or zinc phosphide[#]. Their advantage over zinc phosphide would be species-specificity and reduction in application rate for a disseminating biocontrol.

Finally, considerable expertise has been developed within the mouse program in the areas of diagnostic and risk assessment protocols, as well as predictive modelling. These could be readily applied to manage risks associated with unintentional import and export of mice or other pests of significance to graingrowers and the GRDC.

Intellectual property summary

All research outputs are assessed by the PAC-CRC business director prior to publication to ensure protection of IP. Best practice is followed in maintenance of laboratory records to USA standards for product registration.

Additional information

Species specificity

Pathogenesis and fertility studies for the recombinant virus, recMCMV-G4-mZP3. have been completed in inbred (Lewis) and outbred (Wistar) rats. These studies have now been published. No replicating virus or viral DNA can be found in any rat tissues. In addition and most importantly an early prototype virus recMCMV-K181-mZP3 failed to influence the breeding of female rats.

There was no change to the ovarian pathology in female rats infected with recMCMV-G4-mZP3 compared to uninfected control rats or rats infected with G4 non-recombinant virus. However, there was a significant and increasing serum anti-MCMV and anti-mZP3 antibody response. This was not surprising as viruses are typically good immunogens and an MCMV antibody response was expected. The anti-mZP3 response was more of a surprise. However, the anti-mZP3 antibodies are specific for mouse ZP3 and do not cross-react to rat ZP3. Hence there appears to be an additional level of species specificity provided by the mZP3.

Inoculation of outbred rats with UV-killed (i.e. non-replicating) recMCMV-G4-mZP3 also results in anti-MCMV and anti-mZP3 antibody production, although to a lesser extent.

Transmission

Studies have shown that recMCMV-G4-mZP3 is able to transmit from inoculated adults to naive adult mice, as well as to F1 and F2 generations, within a laboratory setting. The presence of MCMV in the recipient and donor mice was detected by real-time polymerase chain reaction (PCR) and serology. The number of naive mice that seroconverted to MCMV was enhanced when the naive recipient mice were exposed to recMCMV-mZP3 inoculated female mice rather than inoculated male mice (36% more transmission, n=12). In spite of virus transmission, recipient mice are not rendered sterile. In addition, inoculation of male mice with recMCMV-G4-mZP3 had no effect upon the fertility of female mice they were mated with. Normal ovarian morphology was observed in the fertile mice even though they were PCR positive for MCMV virus.

Refer to 'Report Disclaimer'

Transmission experiments have also been conducted in wild mice. Preliminary evidence was obtained that virally-vectored immunocontraception (VVIC) was possible when laboratory bred (pathogen-free) wild mice were directly inoculated with the first product virus constructed using an earlier passage of G4 (recMCMV-eG4-mZP3) and co-housed with uninfected (naive) wild mice in groups of five (two inoculated females, two naive females and one male). Both directly infected and in-contact mice failed to produce pups. However, a subsequent experiment, using more replicates of wild mice housed under similar conditions, showed either that infertility in the in-contact mice might be due to loss of late stage foetuses rather than to prevention of pregnancy (normally seen in directly inoculated mice), or that there was no transmission of infertility.

Resistance studies

A study of resistance to the effects of recMCMV-G4-mZP3 has been undertaken, demonstrating that recMCMV-G4-mZP3 is able to induce infertility in mouse strains that are genetically resistant to the laboratory strain of MCMV, K181.

C57BL/6J mice that are moderately resistant to recMCMV-K181-mZP3 were completely infertile following ip inoculation with MCMV-G4-mZP3. CBA mice, which are highly resistant to recMCMV-K181-mZP3, show reduced fertility following i.p. inoculation with recMCMV-G4-mZP3. The reason for the ability of recMCMV-G4-mZP3 to evade the anti-MCMV immune response in C57BL/6J mice has been identified as having a genetic basis. The effect in CBA mice is not understood at this stage. These data show that a MCMV G4 vector expressing mZP3 delivers effective VVIC.

Product development

It was previously demonstrated that naturally infected wild-caught mice in the Murrumbidgee Irrigation Area (MIA) transmit MCMV to naive mice more rapidly than mice infected with the G4 strain. Over the past 12 months, several isolates of these MIA field viruses have been plaque purified. One isolate in particular has retained the ability to transmit within social groups of mice. This isolate is being used to construct a new product recombinant MIA MCMV expressing mZP3.It is expected to transmit more readily than the first product recMCMV-eG4-mZP3. Experiments to determine whether mice already infected with MCMV are made infertile upon subsequent exposure to recMCMV-mZP3 viruses are ongoing, with early indications that infertility may be less pronounced. However, these experiments need to be repeated using the new recombinant MIA MCMV expressing mZP3.

Risk assessment

A mathematical model of mouse dynamics and epidemiology of immunocontraceptive MCMV (icMCMV) and field strains has been constructed and used to evaluate the risk of inadvertent export of a sterilising icMCMV from Australia to related mouse species overseas. This analysis will provide key documentation determining some safety aspects of an application to conduct field trials.

Modelling

Models for the likely effects and requirements for icMCMV have been significantly advanced. Current models indicate that approximately 70% of mice need to be rendered infertile to prevent substantial damage to crops, provided there is no competitive disadvantage of the icMCMV compared to wild-type viruses. If the icMCMV is less fit than wild-type viruses, or if prior infection with MCMV prevents infertility on subsequent infection with icMCMV, there is a significant reduction in the effectiveness of icMCMV. Simulations also confirm the need to retain high transmission rates of icMCMV.

More recent research may be available at Invasive Animals CRC

www.invasiveanimals.com

Reviews

Hardy CM, Hinds LA, Kerr PJ, Lloyd ML, Redwood AJ, Shellam GR and Strive T. 2006. Biological control of pest animals using virally vectored immunocontraception. *J Reprod Immunol 71: 102-111*

Redwood AJ, Smith L, Lloyd ML, Hinds LA, **Hardy CM** and Shellam GR. 2007. Prospects for virally vectored immunocontraception in the control of wild house mice (*Mus domesticus*). *Wildl Res 34:530-539*