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The immune response and fertility of koalas (*Phascolarctos cinereus*) immunised with porcine zonae pellucidae or recombinant brushtail possum ZP3 protein

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Abstract

To evaluate the potential contraceptive effect of immunisation with zona pellucida antigens, 50 free-ranging koalas were immunised with either porcine zonae pellucidae (PZP), recombinant brushtail possum ZP3 (recBP-ZP3) or buffer, in complete Freund's adjuvant. A single booster immunisation in incomplete Freund's adjuvant was administered 3–5 months later. Where possible animals were recaptured, reproductive status was assessed and blood was collected at 1–3-month intervals for the next 33 months. Forty-three koalas were recaptured at least three times allowing reliable assessments of their fertility. Fourteen animals were observed never to have a pouch young. Of the remaining 29 animals the reproductive productivity of PZP treated females was reduced compared with control and recBP-ZP3 treated females, in terms of both total number of young produced, and failure to produce further young in females of proven fertility. One month after the initial immunisation, serum antigen-specific antibody titres were higher in animals immunised with PZP or recBP-ZP3 compared to controls, and reached a plateau by 4 months. Antibody against the relevant immunising antigen was also detected in ovarian follicular fluid, uterine fluid and vaginal secretions. Epitope analysis suggested that immune responses other than antibodies directed against the ZP3 amino acid sequence were responsible for mediating infertility. The results demonstrate that the fertility of female koalas can be compromised by immunisation against zona pellucida antigens. However, unlike in the eastern grey kangaroo and the brushtail possum, immunisation with bacterial recombinant brushtail possum ZP3 did not compromise fertility in the koala.

Keywords: Marsupial; Immunocontraception; Koala; Porcine zona pellucida; Recombinant ZP3

1. Introduction

The koala (*Phascolarctos cinereus*) is an endemic Australian mammal and the sole living member of the marsupial family *Phascolarctidae*. Koala populations have declined in many regions of Australia due to habitat destruction, the impact of the infectious disease *Chlamydia* and earlier hunting for fur. Other popula-

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tions however, on islands and isolated fragments of forest on the southern Australian mainland, have become so locally overabundant that their numbers exceed the carrying capacity of their habitat. In severe cases, over-browsing by koalas leads to widespread tree death, alterations to the tree species composition of the forest (through the killing of favoured food species) and in extreme cases, starvation of koalas (Martin, 1985; Martin and Handasyde, 1999). Since 1923, the Victorian Government has actively managed over-browsing by a program of translocating koalas out of high-density sites. Initial translocations were onto islands considered safe havens. Later, there began an extensive program of re-introduction to mainland habitat left unoccupied following the dramatic population crash that occurred in Victoria in the early 1900s. This intensive management program has continued and translocations have taken place in most years since 1923, although the management objectives have evolved as the conservation status of the koala progressively improved (Menkhorst, 2009). Although translocations achieve an immediate reduction in the breeding population and consequent reduced browsing pressure, it is stressful for the animals involved as well as being time consuming, labour intensive, expensive and unsustainable in the long term (Lee et al., 1990; Menkhorst, 2004).

The present study examined the potential of an anti-fertility vaccine to control breeding in the koala. It follows similar studies in the common brushtail possum (Duckworth et al., 1998, 1999, 2007), tammar wallaby (Kitchener et al., 2002; Kay and Kitchener, 2004; Asquith et al., 2006) and grey kangaroo (Kitchener et al., 2009). These earlier studies used either relatively crude preparations of the entire zona pellucidae isolated from pig ovaries, bacterially produced recombinant zona pellucida (ZP3) protein or homologous whole sperm as the reproductive antigen. The current study used whole porcine zona pellucida (PZP) or bacterial recombinant common brushtail possum ZP3 (recBP-ZP3) as the antigen. The zona pellucida is the acellular coat that surrounds the oocyte and ZP3 is one of the major protein constituents of the zona pellucida. Either PZP, recombinant ZP3 or ZP3 peptides have been commonly used as anti-fertility antigens in a wide range of eutherian mammals (e.g. Millar et al., 1989; Lou et al., 1995; Kirkpatrick et al., 1996; Barber and Fayrer-Hosken, 2000) and the three species of marsupial cited earlier. Although PZP is a highly effective antigen it is a crude natural product posing contamination risks and it is not appropriate for further development of field deliverable vaccines using molecular technology (Mate and Hinds, 2003). Recombinant BP-ZP3 had proven an effective anti-fertility antigen in the brushtail possum and grey kangaroo so it was trialed in the koala. In addition, epitope analysis was carried out using serum from the koalas immunised with PZP and recBP-ZP3 to identify which specific components of the ZP molecule were immunologically dominant and thus potentially comprise a basis for design of more sophisticated zona-based vaccines.

2. Methods

2.1. Animals

The female koalas utilised in this study were captured from a wild population located on Snake Island in the Nooramunga Marine and Coastal Park, southern Victoria. Animal experimentation was approved by the Animal Experimentation Ethics Committee of the Victorian Department of Natural Resources and the Environment. Fifty animals were captured, by professional climbers using established koala handling techniques. The animals were sedated with 0.3–0.4 mL Zoletil 100 (50 mg tiletamine and 50 mg zolazepam per mL; Virbac, Peakhurst, NSW, Australia) and ear-tagged. Radio collars (Titley Pty Ltd., NSW, Australia) specifically designed for koalas were then fitted. All animals were monitored until fully recovered from sedation then released at the base of the tree from which they were captured and their safe progress to the canopy observed.

Initially in June 2000 a pilot group of 18 animals (six in each treatment group—recZP3, PZP or control) were treated as described in detail later, fitted with radio collars and released. In September 2000, using radio signals from the collars, these animals were located, recaptured and boosted. In October 2000 a second group of 32 animals were captured, treated (10 recBP-Zp3, 11 PZP and 11 control), fitted with radio collars and released. Twenty of these 32 animals were recaptured, assessed and boosted in January 2001. The remaining12 animals in the second group not captured in January 2001 were boosted at their first recapture. Recapture of the experimental population was then attempted 12 times (at 1–3-month intervals) between June 2001 and March 2003. At initial capture and subsequent recaptures body weight, general health and reproductive status were assessed for each animal and venous blood collected. Serum was used in the assessment of immune response following treatment.

Frequency of recapture depended on our ability to locate each animal using radio tracking, to visualise them, and to access the animal safely. Of the 50 animals in the study, 48 animals were recaptured at least once and 43 were recaptured at least three times (range 3–12