Progress on vaccination against the sheep blowfly

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Summary

This paper describes a large body of research that was conducted over several years between scientists at The Centre for Animal Biotechnology, The University of Melbourne and the CSIRO Division of Tropical Agriculture into the feasibility of developing a recombinant sheep blowfly vaccine. A total of 12 individual antigens were evaluated as potential vaccine candidates for use in a sheep blowfly vaccine. The results indicated that 8 of the larval antigens evaluated could significantly inhibit larval growth (P <0.05) in an in vitro *growth assay using sera from vaccinated sheep. While reductions in biomass of up to 60% were recorded in some* in vivo *trials the variation in the response within groups of vaccinated sheep meant that this protection was not statistically significant. The finding that many of the larval antigens were glycosylated, posed specific problems in the expression of these molecules. The use of alternative expression systems in conjunction with recent improvements in carbohydate synthesis technologies may enable the production of more efficacious antigens and hence improve protection. In addition, the ability to manipulate the immune response through the use of different adjuvants may provide an opportunity for improving vaccine efficacy.*

Keywords

Sheep blowfly, vaccination, antigens, peritrophic membrane, proteases

Introduction

The sheep blowfly, *Lucilia cuprina* is responsible for significant economic loss to both wool and meat producers in Australia, New Zealand and the United Kingdom. In Australia alone the sheep blowfly is estimated to cost the sheep/wool industry in excess of AUD$160 million per annum (McLeod, 1995). Current control strategies rely primarily on the use of broad spectrum insecticides to kill the larvae, with management practices designed to reduce the attractiveness of sheep to the blowfly. The use of broad spectrum chemicals poses a number of very serious problems for the wool producer, operator and processor including the development of resistance of the larvae to many of these chemicals, operator exposure to toxic chemicals and residue contamination in wool, meat and the environment. The search for improved control of flystrike led to investigations into the feasibility of developing a sheep blowfly vaccine (Tellam and Bowles, 1997).

Previous vaccination trials conducted at the Centre for Animal Biotechnology (CAB), The University of Melbourne identified 4 native larval antigens, referred to as Lc1-Lc4 which together were able to significantly protect sheep against flystrike (Bowles *et al*., 1996). At the same time, CSIRO Long Pocket was investigating a number of antigens mainly associated with the peritrophic membrane as vaccine candidates. It was found that when larvae were fed on the sera of sheep immunised with these proteins in an *in vitro* assay their growth could be significantly inhibited (Casu *et al.,* 1997). These results indicated that vaccination could significantly influence larval growth and survival both *in vitro* and *in vivo*.

In view of the encouraging results obtained by both groups the project Blowfly Vaccine Technology 1 (BVT1) was initiated in July, 1996. This project involved a collaboration between The University of Melbourne, The CSIRO Division of Animal Production, the Bett Trust and The Woolmark Company. The aim of BVT1 was to harness the considerable knowledge and resources held by the different members to evaluate the feasibility of developing a sheep blowfly vaccine based on the available antigens. This paper will describe the progress made from December, 1996 through to October, 1999, when the project was terminated.

Methods

The CAB approach to antigen identification involved using Antibody Secreting Cell (ASC) probes to identify potential vaccine antigens (Bowles *et. al*, 1996). These antigens were recognised by the sheep immune system during natural blowfly infections. At CSIRO antigens were selected on the basis of their ability to produce antibodies which were able to inhibit larval growth *in vitro*. Most of these antigens were gut associated and poorly immunogenic during natural infection. A major focus of BVT1 was to (1) standardise and optimise *in vitro* and *in vivo* protection assays and (2) to compare the efficacy of native versus recombinant larval antigens for their ability to protect sheep against flystrike using both *in vitro* and *in vivo* assays. Studies were undertaken to purify, characterise and evaluate the larval antigens. In total, 17 vaccination trials were conducted during the BVT1 funding period using a total of 489 sheep. The majority of the trials undertaken as part of BVTI used a standard adjuvant (Montanide ISA-25 and recombinant IL-1β), route of vaccination (subcutaneous) and infection protocol. The challenge involved placing 300 *L. cuprina* eggs at two separate sites. Protection was assessed through examining larval survival and larval weights *in vivo* and determining biomass. Sera from these trials were also examined pre- and post-vaccination using an *in vitro* feeding assay.

Results

Results for the vaccine trials conducted under BVT1 are presented in Table 1.

Table 1. Results of BVT1 vaccine trials.

Antigen	Glycosylation status	Expressed as recombinant protein in bacteria	Expressed as recombinant protein in eukaryote	*In vitro* larval growth inhibition native (N)/ recombinant proteins (R) (%)	*In vivo* protection[3] with native (N) or recombinant (R) proteins (%)	*Future development*
PM44	High mannose	Yes	Yes (Baculovirus)	24-42# (N) 15-34(R)	n.d.[2]	No
PM48	High mannose	Yes	No	31#(N) 27(R)	n.d.	No
PM30	High mannose	No	No	31# (N)/ n.d.	n.d.	No
Ag55	Glycosylated	Yes	No	61-66# (N) 9(R)	9 (R: GST-fusion protein)	Test new recombinant
Cut-1[4]	No	Yes	No	55#(N) 14(R)	n.d.	No
L-protein	N and O-linked; WGL^+	Yes	No	48# (N) 10(R)	n.d.	No
PM95= LC1	N and O-linked; WGL^+	Yes	Yes (Baculovirus)	61-66#(N) 22-27(R)	18-26 (N) 0 (R)	New expression systems
LC2	WGL^+	No	No	27(N) -31(N)#	+13 to –36 (N)	Express and test
PM35	Native n.d.	Yes	Yes (Pichia)	n.d.	0 (R)	No
LC3	Native n.d.	No	No	n.d.	42*(N)	Sequence, clone, express, test
LC4 *variants* (~LCTb)	No	Yes	Yes (Pichia)	0 / 0 (LC4)(N) 0 LCTb) (N)	0-60* (R-LC4)	In combination
AgX	Native n.d. Recombinant glycosylated	Yes	Yes (Pichia)	0 (R)	22 (R pichia AgX), -18 (R bact. AgX)	In combination

[1] # $P < 0.05$ [2]n.d.; not determined

[3]Reduction in larval weight, biomass* or number of strikes after implantation of blowfly eggs/larvae on sheep

[4]GST-CUT1 was incorporated into a multiple antigen combination trial, which showed no significant effects (*in vivo* assessment).

A total of 12 individual antigens were evaluated as potential vaccine candidates for use in a sheep blowfly vaccine. The native larval antigens were firstly purified and antibodies raised to these antigens in sheep were tested in the *in vitro* larval growth assay. Table 1 indicated that 8 of the 12 larval antigens evaluated in this manner could significantly inhibit larval growth ($P<0.05$). During the purification and subsequent characterisation of these antigens it was also noted that a number of these molecules were heavily glycosylated as determined by lectin binding studies. These studies indicated the presence of both N and O-linked sugars. A number of these antigens were subsequently expressed in both prokaryotic and eukaryotic expression systems and then evaluated. Testing was undertaken using a reproducible protocol that was established under BVT1 for evaluating protection levels on sheep after *in vivo* challenge with blowfly larvae or eggs. The results indicated that in contrast to the native antigens there was no significant inhibition of larval growth when larvae were fed on the sera from sheep vaccinated with recombinant forms of the larval antigens. Limited protection studies were undertaken using both native and recombinant antigens *in vivo*. While reductions in biomass of up to 60% were recorded in certain trials the variation in the response within groups of vaccinated sheep meant that this protection was not statistically significant.

Discussion

In the majority of flystrike cases newly hatched larvae migrate rapidly from the wool onto the skin and initiate a wound. These larvae are then vulnerable to serum and soluble host factors providing an opportunity for immune-mediated attack. Successful vaccination of sheep against flystrike will require appropriate immune responses to be activated upon contact of the larvae on the skin of the host. This immune response must be both rapid and highly effective in order to produce an environment in which the larvae are unable to survive. Such a response is likely to involve both antibodies and cellular components of the immune system.

In the course of studies described under BVT1, it was found that all of the proteins isolated and tested from the peritrophic membrane were able to elicit significant growth-inhibitory activity against *Lucilia* larvae in *in vitro* assays when tested in their native forms. However, *in vivo* the reduced inhibitory effects are believed to be due to the larvae ingesting significantly less antibody (Eisemann *et al*., 1993). This problem may be overcome by the production of significantly higher antibody titres following vaccination however this would require a substantial advance in vaccination technology. The promising *in vitro* inhibition results obtained with native PM antigens could not be reproduced with the recombinant forms of these molecules. This result may be explained by the importance of the antibody response to both the polypeptide and oligosaccharide components of the larval molecules (Tellam *et al*., 2001). There is therefore a need to produce the recombinant molecules with an appropriate polypeptide structure containing the correct oligosaccharides. Use of alternative expression systems together with major advances in carbohydrate chemistry offers exciting potential for advancing this area in a manner that was not possible just a few years ago. In addition recent reports in other parasite systems showing the importance of immune responses to carbohydrate molecules will see rapid progress in this area.

Of the other antigens examined interesting data was obtained for Lc2 and Lc3. Sheep vaccinated with semipurified native Lc3 resulted in a 42% reduction in total biomass compared to controls. In addition, the sera from sheep vaccinated with native was able to significantly inhibit larval growth *in vitro* ($P<0.05$). In contrast, similar growth inhibition was not observed *in vivo*, in fact there was an exacerbation of disease. While this result was unexpected, it raises the possibility that adjuvants might play a role in directing the immune response resulting in exacerbation rather than protection. There are an increasing number of reports in the scientific literature where the outcome of vaccination is directly linked to the type of immune response induced which in turn is determined by the adjuvant. Further studies using different adjuvant systems may assist in the identification of adjuvants that can induce a protective response in vaccinated sheep.

Conclusion

Many of the blowfly antigens are rich in disulphide bonds and heavily glycosylated. Correct folding and glycosylation is going to be critical for inducing effective immune responses as has been shown for PM-95. It is suggested that future expression in more analogous systems may be worth investigating. For example, the Drosophila Expression System (DES) could be used for expressing blowfly vaccine antigens.

The mechanism(s) by which the natural antigens can induce protection are not known. The use of different adjuvants as well as more thorough biochemical characterisation and analysis of Lc2 and Lc3 would permit a more comprehensive evaluation of the potential of these two molecules in a blowfly vaccine.

To overcome current limitations in vaccination, defined molecules playing a key role in larval development could be targeted in novel vaccination strategies, eg. hormones or proteases involved in moulting, larval growth and development. New developments in vaccine formulation and delivery have resulted in significant progress in this area.

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