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Using immunology and resistant sheep to beat the fly

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Abstract

New methods for controlling blowfly strike will be needed when mulesing is phased out and the availability or efficacy of insecticides for control of fly strike decreases. The Australian Sheep Industry CRC has pursued two approaches for the development of new methods to help control blowfly strike. In the first, genetic resistance of sheep to survival and growth of blowfly larvae was examined. Resistance to growth of larvae was heritable (0.29 ± 0.22). The trait was not associated with resistance to internal parasites, nor was it influenced by wool characteristics such as fibre diameter or coefficient of variation of fibre diameter. This new trait differs from resistance to fly strike associated with resistance to fleece rot. Because measurement of the trait is labour intensive, gene markers or correlated measures are needed before it will be suitable for industry adoption. The second approach examined the impact of larval products on the immune system of the sheep. Larvae suppress the sheep immune system and thereby limit the ability of the sheep to reject the larvae. The immunosuppressive agent is being purified and strategies to abolish its activity are being explored. Abolition of immunosuppression would create opportunities for the development of new vaccines against blowfly strike.

Introduction

Fly strike is an important problem for Australian sheep producers. With the general acceptance that eradication of *Lucilia cuprina*, the principal cause of blowfly strike in Australia, is not feasible, there is an ongoing need to reduce the risk of animals being struck. Strategies to reduce the risk of blowfly strike include shearing and crutching, flytraps, the use of insecticides that inhibit the survival of blowfly larvae in wool, mulesing, control of the causes of dags, selection for reduced susceptibility to fleece rot, selection for resistance to blowfly strike and vaccination against blowfly larvae.

As is the case with most parasites that are subject to chemical control, blowflies have developed resistance to insecticides. Countries that import Australian wool are tightening regulations concerned with chemical residues in wool. Mulesing in its current form will be phased out in 2010. These changes have created a demand for new ways to control blowfly strike. This paper describes two CRC projects that are examining resistance of sheep to blowfly strike and how the defence system of the sheep responds to attack by blowfly maggots. These long-term projects will not provide alternatives before mulesing is phased out in 2010 but demonstrate how sheep that are less susceptible to blowfly strike could be bred and how vaccines for controlling the disease could be developed.

Resistance to blowfly strike

The two main types of blowfly strike are body strike and breech strike. Both types of strike are usually preceded by bacterial dermatitis that develops when skin is wet by rain for extended periods or when there is soiling of the breech. Bacteria growing on wet skin break down the normal defence barrier of the skin and provide a site that is attractive to female flies when they are ready to lay eggs. The moist bacteria-laden skin also provides conditions favourable for the survival and growth of blowfly larvae (maggots).

When blowfly strike became a widespread problem in Australia early in the 20th Century, it was recognised that body conformation, wrinkle score and wool characteristics all influence susceptibility to blowfly strike and that these traits are heritable. In the 1970s, the New South Wales Department of Primary Industries established a breeding program at Trangie to examine the possibility of breeding sheep for resistance to blowfly strike. Sheep were scored for fleece rot and fly strike under natural environmental conditions and in the weeks following artificial wetting in a shed. Lines were bred for resistance and susceptibility using an index that included the scores for natural and induced fleece rot and fly strike. These lines have been used in many studies of the underlying mechanisms of resistance and susceptibility (reviewed by (Colditz et al*.,* 2001). The important findings from this research were that selection resulted in changes in wool attributes. Resistant sheep had bright, white wool with an even, blocky tip that dried quickly after wetting. Resistant sheep also had higher levels of antibodies to one of the important bacteria associated with fleece rot, *Pseudomonas aeruginosa* (Chin and Watts, 1991). Other work has shown that sheep that have been vaccinated against *Pseudomonas aeruginosa* are more resistant to fleece rot and fly strike (Burrell, 1990), but a commercial vaccine is not currently available.

In one experiment with the Trangie sheep, we placed freshly hatched larvae on the skin surface and measured their growth after 50 hours. Larval growth on sheep from the two lines was similar (Colditz et al*.,* 1996). This result suggests that the Trangie sheep were resistant to body strike because of their resistance to fleece rot.

Sheep and cattle can be selected for resistance to many parasites, including internal parasites, ticks and lice. In each of these cases, the body's defence mechanisms play an important role in controlling the parasite. Infected animals develop antibodies and strong cellular reactions against the parasite. These facts led us to examine whether there is genetic variation amongst sheep in their propensity to restrict the growth of larvae that is independent of the effect of wool characteristics on larval growth.

Heritable resistance to growth of blowfly larvae

Genetic variation was examined in 246 progeny from 27 sires of medium and fine wool Merinos. The sires were obtained from 10 bloodlines used in the CSIRO fine wool project. The sheep were challenged by the same method described in the previous section for the Trangie selection lines. Fifty freshly hatched larvae were placed on skin sites on the flank of each sheep from which the wool had been removed using clippers. The sites were lightly abraded with a Heath punch to provide some blood serum for the larvae and were kept moist by addition of 2.5 mL water after 24 hours. Each sheep bore four replicated sites. After 50 hours, the larvae were recovered, counted and weighed. The total larval weight per site and mean larval weight per site were calculated.

Measurements made on the 246 progeny revealed that there was a significant difference between bloodlines in total larval weight and between sire groups in total larval weight and mean larval weight. Heritability was 0.21 ± 0.17 for total larval weight and 0.29 ± 0.22 for mean larval weight. Heritability of larval survival was not different from zero. These heritabilities are similar to those reported for resistance to internal parasites in sheep (Albers *et al.,* 1987). It was estimated that annual progress of 5.3% would be made in a single-trait breeding program selecting for reduced mean larval weight, assuming normal industry practices. This also compares favourably with progress made when breeding for resistance to internal parasites (Albers et al*.,* 1987). The large variance (standard errors) associated with the heritability estimates in this study were probably caused by the relatively small number of animals used.

Relationship with other traits

The associations between susceptibility to fleece rot and wool traits that were observed in the Trangie selection lines raise the question of whether the resistance to larval growth described in the previous section is related to wool traits. Animal numbers were too few to estimate genetic correlations. There were no significant phenotypic correlations between larval growth and a range of wool traits, including mean fibre diameter, standard deviation of fibre diameter, coefficient of variation of fibre diameter, clean scoured yield, staple length, staple strength or percent mid-point breaks. These findings indicate that the resistance trait identified in this study is different to that observed in the Trangie selection lines.

The sheep were exposed to wetting for five days in a wetting shed to induce fleece rot. There was a negative phenotypic association between fleece rot scores and larval growth ($R^2 = -0.22 \pm 0.09$), which is in contrast to the results from the Trangie flock in which larval growth did not differ between lines. Worm egg counts in faeces were measured after field infection with internal parasites. There was no significant association between resistance to internal parasites and larval growth.

Industry application of genetic resistance to growth of blowfly larvae

The resistance trait identified in this study is very labour-intensive to measure and is therefore not suitable for use by sheep farmers. It will therefore be necessary to identify gene markers or correlated traits before selection of sheep for this trait can be applied by the sheep industry. Genetic progress for resistance to growth of blowfly larvae is predicted to be similar to that for resistance to internal parasites. The benefits of selection for resistance to growth of blowfly larvae are also likely to be similar to those of selection for resistance to worms. The first few generations of resistant animals will not reduce the necessity of using other methods for controlling flies, just as the first few generations of sheep breed for resistance to worms have had little impact on use of drenches. It is estimated that about 15 years of selection for resistance to worms is needed before sheep will no longer need drenching. However, when this has been achieved, resistance to worms or flies should be permanent and free of ongoing costs. It is important to note that in vaccination studies, a reduction in larval growth of more than 88% is needed to affect the progression of larvae to adult flies. Accumulated genetic progress over a number of years would be required before resistant sheep would have an impact on blowfly population dynamics. It is anticipated that reduced severity of disease in sheep bred for resistance would occur before an impact on blowfly population dynamics would be achieved.

Effect of blowfly larvae on sheep defence mechanisms

The identification of genetic resistance to blowfly strike in sheep will ensure long-term and responsible management of blowfly strike for the Australian sheep industry. Non-genetic solutions can complement genetic approaches and may be quicker to implement. One such option is to develop a vaccine.

Why do we not have a blowfly vaccine?

The Australian sheep industry has made significant investments over the past three decades towards the development of an effective blowfly strike vaccine. Despite this investment, there is still no vaccine available. In the past, blowfly vaccine development studies demonstrated that the sheep's immune system is able to recognise components of the maggots as foreign and consequently generate immune responses. In some laboratory experiments, it was demonstrated that when antibodies produced by sheep were fed to maggots, there was excellent inhibition of maggot growth. However, when sheep were subsequently challenged with live maggots, poor protection was recorded.

The most promising demonstration of protection came from the finding that resistant sheep produced larger inflammatory skin reactions than susceptible sheep (Sandeman et al., 1986; MacDiarmid et al., 1995). In similar trials, sheep were repeatedly immunised in such a way as to induce hypersensitivity reactions to the maggots. Despite exhibiting good levels of protection to the first larval challenge, the initial level of protection did not persist after repeated challenges (Bowles et al., 1987). We have also noted that the immune response of sheep that have been repeatedly challenged with maggots declines over time, which is in contrast to strong increases observed for other pathogens such as viruses and bacteria. This led us to conclude that the maggots are able to modulate the sheep immune system, thus preventing it from recognising the maggots as foreign invaders.

At about the same time that we recognised this phenomenon, similar observations were published by Kerlin and East (1992) of CSIRO. These observations help explain why previous blowfly research has been unsuccessful at developing a vaccine. In essence, when the sheep is vaccinated, certain cells of the immune system retain the ability to recognise these infectious components for long periods of time. When sheep are challenged with the whole pathogen, these cells are responsible for recognising the pathogen and inducing a strong immune response via a cascade of pathways that mobilise the whole immune system into a state of protection. What others and we have found is that if the individual components of the blowfly maggots are injected into sheep they are recognised as foreign, and specific immune responses are generated. However, when vaccinated sheep are challenged with live blowfly maggots, the maggots secrete a molecule that inhibits a key pathway in the mobilisation of the immune system, thus enabling the establishment of blowfly strike. On this evidence, it became very clear to us that for the successful development of a blowfly vaccine it was essential that the molecule responsible for suppressing the immune system be identified and fully characterised.

Characterising the interaction of maggots and sheep

Our research detailing the immune response to fly strike has revolved around characterisation and examination of the ability of cells isolated from the blood of sheep, called lymphocytes, to proliferate in the presence of maggot extracts and to initiate an immune response cascade. These experiments have demonstrated that the secretory/excretory products from first-stage maggots of the blowfly contain a molecule that inhibits lymphocyte growth. During a process of systematically analysing the secretory/excretory products of the maggot, one secretory/excretory component was isolated and found to have a negative effect on the lymphocytes grown in laboratory assays. This inhibition mimicked the inhibition induced by blowfly secretory/excretory products during the challenge experiments with sheep. As the secretory/excretory component protein prevents the induction of a typical immune response, we propose that it plays a critical role in allowing blowfly maggots to colonise sheep by suppressing the immune system.

Recently, we determined that the secretory/excretory component is a protein that belongs to a family of proteins that are involved in regulation of cellular pathways, including those of the immune system. In addition, there are a number of examples in the scientific literature in which proteins of this type were identified as playing roles as immune modulators in the lifecycles of other parasites. Importantly, in some of these cases, the work has progressed to the point where it has been demonstrated that vaccination is possible.

Current efforts are focused on the isolation of the gene that encodes the secretory/excretory component protein. Interestingly, this gene product appears to be rare compared to other proteins found in secretory/excretory material prepared from blowfly maggots. Our data indicate that the secretory/excretory component protein is more closely related to sheep proteins than to blowfly proteins. This may explain why the secretory/excretory component protein is able to interfere with the sheep's immune system. We have also demonstrated that the secretory/excretory component protein is produced by the cells that line the gut of the maggot. These cells also produce other proteins such as proteases that digest the skin of sheep and enable the maggots to feed, which are essential for the establishment of blowfly strike.

We hope to isolate the gene encoding the secretory/excretory component protein in the near future. This will enable us to synthetically reproduce the secretory/excretory component protein and unequivocally confirm its immune modulating activity. This will be the first step in devising a method for circumventing the ability of the secretory/excretory component protein to prevent an immunological response to blowfly strike.

Ultimately, this information will be utilised to develop an immunisation strategy for the shortto medium-term control of blowfly strike in the Australian sheep industry while appropriate genetic strategies are identified and incorporated into the national flock. It is quite likely that the vaccine components developed through previous research in this field will be important in assisting the immune system of sheep to recognise and successfully prevent blowfly strike.

Conclusion

We believe that both genetics and vaccination will play crucial roles in the control of blowfly strike once mulesing is phased out in 2010. Currently, the only readily available method for control of blowfly strike is the use of chemicals. However, in the long-term, it is likely that the use of chemicals will come under increasing pressure from important markets because of consumer preferences.

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