TESTING CANDIDATE GENES AS MARKERS FOR DISEASE RESISTANCE IN MERINO SHEEP

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SUMMARY

Genetic markers offer potentially greater rewards in areas where traditional selection is difficult and/or costly, such as resistance to disease. A wide range of candidate genes can now be examined in sheep for their association with resistance to disease. We have initiated an investigation to examine genes associated with immune function for possible association with resistance to footrot. Our preliminary results indicate that genes associated with complement warrant further investigation. A proposed study outlining a more comprehensive evaluation of genetic markers for disease resistance is presented.

INTRODUCTION

Scope for genetic improvement in resistance to all major production diseases in Merino sheep has been demonstrated for resistance to internal parasites (Woolaston et al., 1991), fleece rot and flystrike (Raadsma, 1992), footrot (Raadsma et al., 1994), and dermatophilosis (Raadsma unpublished). Exploitation of genetic variation in resistance to these diseases is often complicated by the low and variable prevalence of the disease, which limits the effectiveness of direct selection. Furthermore, in the case of serious disease outbreaks, management will aim to prevent the expression of disease in animals offered as replacement breeding stock to the industry. In short, selection for resistance to the diseases listed above is possible, but is unlikely to be effective if selection is limited because of environmental constraints. Indirect selection using indirect selection traits with a moderate co-heritability for resistance to disease, or using specific gene markers, has long been thought to be a desirable and complementary selection option. For resistance to the major diseases in sheep, the search for genetic markers has been limited . For successful application of disease resistance markers at the population level (i.e. where they can be used in the industry) they must be very close to the genes which affect resistance.

Despite enormous advances in the development of a genetic linkage map for sheep (Crawford et al., 1994, Broad et al., 1995, these proceedings) the number of markers across the genome is currently insufficient to evaluate their use for selection at the population level. Chromosomal regions need to be identified which contain potential quantitative trait loci (QTL) for disease resistance, to allow fine-interval mapping to identify markers within close (<1 centimorgan) proximity of QTL for disease resistance.

An alternative means to identify potential markers for disease resistance is to exploit the candidate gene approach. Here we are capitalising on information, largely derived from other species, on known genes which have a role in disease resistance. The advantage of a candidate gene approach is that allelic variants of targeted genes can usually be identified within or adjacent to the "causative" exons, thereby providing

markers that are immediately useful at the population level. Rapid developments over the last 10 years have identified a number of candidate genes that are implicated in disease resistance and can be screened in sheep. In this paper we report on the preliminary evaluation of a number of candidate genes for disease resistance and their possible involvement in resistance to footrot.

MATERIALS and METHODS

Animals

Animals for this experiment were a subset of all animals used in a major study on genetic variation in resistance to footrot in Merino sheep (Raadsma et al., 1994). In brief, four groups of approximately 400 animals were exposed to footrot (*Dichelobacter nodosus*) infection on two separate occasions at 10 and 18 months of age over a four year period (as described by Raadsma et al., 1994). The sheep were monitored for the development and remission of footrot on 6 occasions in the first challenge and 5 occasions in the second challenge. From the first group of 400 animals, all animals were ranked on the basis of their total combined OVERALL footrot score. The most extreme phenotypes (n=56) were identified and classed as Resistant (n=28) or Susceptible (n=28). In addition, for the evaluation of MHC Class I microsatellites, 15 Resistant and 18 Susceptible sheep were included from a second group of 420 animals which had been treated and selected in a similar manner.

Genotyping

DNA was digested with the restriction enzymes *Pvu*II or *Taq*I. Southern transfer membranes were prepared from DNA of the Resistant and Susceptible animals as described by Litchfield et al. (1993). The source of the probes used in this investigation is shown in Table 1. All probes were labelled with $^{\alpha\text{-}32}$P-dCTP using a random priming method. For the Class I MHC microsatellite, primer sequences, PCR conditions and detection of PCR product were identical to those described by Groth and Wetherall (1994). All PCR samples were amplified in a Corbett micro-capillary machine.

RESULTS AND DISCUSSION

To our knowledge, this represents the first investigation where these immune response genes have been targeted in relation to resistance to footrot. With the exception of the Interleukin 1ß probe, all probes described in Table 1 were polymorphic for at least one enzyme. Only the 2.07kb band generated with the C4 and *Taq*I probe-enzyme combination showed a significant difference in frequency between footrot Resistant (21/24) and Susceptible (17/27) phenotypes (Table 1). It is clear that more animals will need to be screened to confirm this observation.

Table 1 Candidate genes examined in phenotypes Resistant and Susceptible to footrot

		Number of RFLP bands			
Candidate gene	Source	Total	Polymorphic	Significant	P
Interleukin 1ß	Wood	5 *Taq*I	0	-	-
		3 *Pvu*II	0	-	-
Interleukin 4	Sandeman	7 *Taq*I	3	0	-
		3 *Pvu*II	0	-	-
GM.CSF	Wood	10 *Taq*I	0	-	-
		3 *Pvu*II	3	0	-
γ Interferon	Wood	4 *Taq*I	2	0	-
		3 *Pvu*II	0	-	-
T cell receptorα	Hein et al. (1991)	11 *Taq*I	5	0	-
		7 *Pvu*II	0	-	-
T cell receptorß	Grossberger et al.(1993)	4 *Taq*I	2	0	-
		2 *Pvu*II	0	-	-
IgE- Constant	Sandeman	3 *Taq*I	2	0	-
		3 *Pvu*II	2	0	-
Complement C4	Wetherall	7 *Taq*I	3	1	0.035
		7 *Pvu*II	1	0	-

The MHC Class I microsatellite is designated as the MHC 4.1.2 locus within the ovine Class I region (Groth and Wetherall, 1994). We observed a total of 12 alleles. The frequency of each allele in the Resistant and Susceptible phenotypes is presented in Table 2. No significant differences were found in any of the 12 alleles between the two groups of sheep sampled.

This preliminary investigation has been extended to include Interleukin 2, Immunoglobulin genes, and markers for the ovine MHC Class II region. In due course it will be possible to extend the search for genes which have a regulatory function in immune response, these may include differential regulation of cytokine expression, cytokine receptor gene expression, expression of adhesion molecules in lymphocyte circulation and recruitment. Similarly within the MHC, numerous genes have now been identified in humans in addition to the Class I, II and III genes which may have an important role in disease resistance.

The screening of extreme phenotypes from a larger population offers a rapid and relatively simple means to conduct preliminary evaluations on a wide range of candidate genes. The next step we have taken is to extend this approach to genotype the 162 sires which were used in our resource flock. The evaluation of candidate genes will consist of analysing the relationship between each allele and the Estimated Breeding Value (EBV) for a wide range of traits. This means that only one, relatively small, set of animals has to be genotyped for each marker which can be evaluated for all major disease traits and immune response traits. In addition multiple candidate genes can be analysed simultaneously.

Table 2 Frequency of MHC class I micro-satellite alleles in footrot Resistant (n=40) and Susceptible (n=46) phenotypes.

Band size (bp)	Frequency Resistant	Frequency Susceptible	Significance
182	0.13	0.06	n.s.
184	0.01	0.00	n.s.
190	0.03	0.05	n.s.
192	0.11	0.09	n.s.
194	0.06	0.13	n.s.
196	0.19	0.26	n.s.
198	0.08	0.12	n.s.
200	0.08	0.06	n.s.
202	0.01	0.00	n.s.
204	0.05	0.01	n.s.
206	0.04	0.06	n.s.
212	0.21	0.15	n.s.

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