A PRELIMINARY SEARCH FOR RFLP MARKERS FOR RESISTANCE TO ECTO-PATHOGENS IN SHEEP, USING HUMAN CLASS II MHC PROBES

H.W.RAADSMA*, A.M.LITCHFIELD#, J.R.EGERTON*, F.W.NICHOLAS#, and S.C.BROWN#

* Department of Animal Health, The University of Sydney, Camden, N.S.W.
# Department of Animal Science, The University of Sydney, Sydney, N.S.W.

SUMMARY

Extensive restriction fragment length polymorphism (RFLP) was observed in DNA from 79 sheep, following digestion with two endonuclease enzymes (TaqI and PvuII) and probing with human cDNA DRB and DQA clones. Disease data were available on fleece rot, body strike, footrot, and antibody response to D.nodosus antigens. Although the role of Class II MHC genes on resistance could not be excluded, initial analyses of possible associations between RFLP bands and the expression of disease, highlighted the need to adopt a conservative approach. From the 76 tests which were potentially of interest ($P<0.05$), only 39 remained significant after adjusting for differences between sires, 11 after taking account of the number of statistical comparisons which were made, and 3 bands after adjusting for both differences between sires and the number of comparisons which were made. No one band is associated with all three diseases examined here. Preliminary results suggest that 2 bands (TaqI/DRB2.6 and PvuII/DRB1.9) are associated with resistance to footrot, and one band (PvuII/DRB1.4) is associated with increased susceptibility to body strike.

INTRODUCTION

Scope for genetic improvement in resistance to a number of potentially serious external diseases has recently been reviewed for footrot (Egerton and Raadsma 1991), fleece rot and flystrike (Raadsma 1991). Exploitation of genetic variation in resistance to these diseases is often complicated by the low and variable prevalence of the diseases, which limits the effectiveness of direct selection. Furthermore, in the case of flystrike and footrot, management will aim to prevent the expression of these diseases in animals offered as replacement breeding stock to the industry. In short, selection for resistance to the diseases listed above may be possible, but is unlikely to be effective if selection is limited because of environmental constraints. The scope for indirect selection using indirect selection traits with a moderate co-heritability for resistance to disease, or on the basis of specific gene markers has long been thought to be a desirable and perhaps complementary selection option. Some fleece and physiological indicators have the potential to be used as indicator traits for selection against fleece rot and body strike, but too little work has been conducted in the case of footrot (Egerton and Raadsma 1991). The search for genetic markers aimed directly at the level of the gene or the specific gene product, has been extremely limited for resistance to the major ecto-pathogens in sheep.

The study of variation in DNA sequences between individuals is now possible through the use of the restriction fragment length polymorphism technique. The targeting of potential candidate gene(s) is also possible when genetic variation in disease resistance is considered. From the human field it is accepted that genes within the Major Histocompatibility Complex (MHC) play an essential role in most aspects of the immune response. In particular, the highly polymorphic Class II MHC genes (also known as immune response, Ir, genes) are essential in many aspects of the immune response. Although most of the study of the MHC Class II region has focussed on the human (HLA) field, similarities at the DNA level between HLA and the MHC from other species have

resulted in human Class II probes being successfully used in other species. Thus it is now possible to target candidate genes within the ovine MHC. The cloning of parts of the ovine MHC, as described by Scott et al. (1987) and Deverson et al. (in press), will increase the specificity of genes to be targeted. Eventually sequence information on isolated OLA genes is likely to lead to locus- and allele-specific synthetic oligonucleotide probes, as developed in the human field (Robinson et al., 1989).

This paper describes a pilot experiment in which we have combined sheep RFLP information on the MHC, derived from the use of human Class II cDNA probes, and information on disease resistance for a number of important diseases in sheep.

MATERIALS and METHODS

This experiment was designed as a pilot experiment for a major study of genetic variation in resistance to footrot, fleece rot and flystrike in Merino sheep (Raadsma et al. 1990). For the pilot experiment, 84 ram and wether weaners 10 months of age were exposed to an experimental footrot (Dichelobacter nodosus) infection, as described by Raadsma et al.(1990) The sheep were monitored for the development and remission of footrot on 3, 6, 9, 12, 15 and 26 weeks after challenge. All sheep were vaccinated with an homologous rDNA pilus vaccine at 9 and 12 weeks after challenge. Circulating K-agglutinating antibodies were measured prior to challenge and at each time the feet were inspected. The sheep were shorn at 10 and 16 months of age. Fleece rot was scored in all sheep 1 week prior to shearing. Flystrike was recorded as the sheep were treated; no preventive jetting was undertaken.

DNA was extracted from the white cell fraction that had been harvested from 30 ml of blood. Full details of DNA extraction and subsequent generation of RFLP data will be presented elsewhere (Litchfield et al., in preparation). In brief, the DNA was digested separately with 2 restriction enzymes, TaqI and PvuII and separated by agarose gel electrophoresis. Southern blots were prepared and probed (on separate membranes) with two human Class II cDNA probes, namely a DRbeta (Gustafsson et al. 1984) and a DQalpha (Schenning et al. 1984) probe. The probes were labelled with ^{32}P and radiographs developed on X-ray film. The size of all bands present in each sheep was recorded for each probe/enzyme combination.

Bands with a low(<10%) or high (>90%) frequency were excluded from further analyses. In order to minimise the possibility of spurious associations, the effect of each band was fitted as a fixed effect in a mixed model, allowing sire effects to be taken into account. To make allowances for the number of comparisons which were being performed on all the available RFLP bands, the P value for bands which showed a significant effect on the traits of interest, was multiplied by the number of comparisons made on the relevant probe/enzyme RFLPs. Least squares means were calculated for bands which remained significant, thus indicating the performance of animals which were positive for certain bands compared to those which were negative. RFLP bands were subjected to cluster analysis (Pickbourne et al 1978) to identify bands which could be grouped together.

RESULTS and DISCUSSION

Detailed results of the RFLP patterns generated using the above probe/enzyme combinations are being presented elsewhere (Litchfield et al., in preparation). In brief, extensive polymorphism was detected for the four probe/enzyme combinations. The TaqI/DRB, TaqI/DQA, PvuII/DRB and PvuII/DQA Southerns revealed 27, 13, 22 and 12 bands respectively, including 2 monomorphic bands.

The frequency of polymorphic bands ranged from 0.04 to 0.98. Information on 17 bands was not analysed due to monomorphism or extremes in frequency. Of the 57 bands remaining, extensive cross-hybridisation between the DQ and DR probes was observed (Litchfield et al., in preparation) and preliminary cluster analysis revealed a strong clustering of bands generated with TaqI and PvuII.

A brief summary of the footrot results has been described by Raadsma et al. (1990), and full results will be presented elsewhere (Raadsma et al., in preparation). Immediately prior to shearing at 16 months, 40.5% of sheep had developed fleece rot with an average severity of 2.1 ± 0.2. During this period 18.9% of sheep were treated for body strike, which followed a period of prolonged rainfall in spring.

Table 1 Number of RFLP bands with a potential effect (P<0.05) on disease and immune response traits.

Trait	Unadjusted (Band effect alone)	Adjusted for number of comparisons made	Adjusted for sire effects	Adjusted for sires and number of comparisons made
Fleece rot severity	6	0	5	0
Fleece rot %	6	0	2	0
Body strike %	7	0	4	1
Foot rot scores				
Challenge week 3	7	3	1	0
Challenge week 6	3	0	2	0
Challenge week 9	1	0	2	0
Vaccination V1	3	0	0	0
Vaccination V2	0	0	2	0
Post vaccination	5	1	9	2
Antibody titres				
During challenge	19	6	8	0
Post vaccination	19	1	2	0

From the 627 comparisons made, 76 indicated significant (P<0.05) correlation with resistance to disease (Table 1). Taking into account the number of comparisons which were made, only 11 bands were significantly (P<0.0009 x 57 comparisons= P<0.05) associated with footrot (Table 1). For 56 of the animals examined, the effect of sires on the disease traits could be estimated, and only 39 bands were of interest (Table 1.), including 5 bands which showed a significant association with fleece rot, 4 with body strike, up to 10 with footrot, 6 with antibody response prior to vaccination, and 2 following vaccination (Table 1). Scaling of the P value for each band to take account of the number of comparisons which were made, after fitting sires, resulted in only 3 bands which had a significant effect on the expression of diseases monitored in this study (Table 1). This included 2 bands (TaqI/DR2.6 and PvuII/DRB1.9) which were associated with footrot following vaccination, a time at

which the majority of sheep had showed healing of the footrot lesions. This group of sheep had a poor curative response to vaccination, even though their antibody titres were similar to those sheep which showed healing. These results suggest that this susceptibility may in part be associated with Class II region of the MHC. Cluster analysis revealed that these 2 bands could not be grouped together, possibly reflecting different "haplotypes".

One band (PvuII/DRB1.4) showed a possible association with body strike. Sheep without the band had 8% body strike (n=30), compared with 32% body strike in sheep for which the band was present (n=40). No single band was associated with all three diseases recorded here, after adjusting for sires and the number of comparisons made.

At this stage it should be stressed that these results are preliminary ones. Although the number of animals for which Class II MHC RFLP results are reported here is reasonable, considerably more animals will need to be analysed before any firm recommendation can be made on the use of DNA RFLPs as indirect selection criteria. In particular, the effect of individual bands or groups of bands needs to be estimated for progeny within sire groups. The identification of Class II haplotypes based on RFLP results also remains a high priority. In due course, with the use of ovine Class II probes instead of equivalent human probes, and a better characterisation of the sheep MHC, it might be possible to identify DNA markers for resistance to important production diseases in sheep.

Our results also show that molecular genetic techniques can readily be incorporated in designed quantitative genetic experiments. The storage of DNA or whole blood or tissue samples from animals for which pedigree and experimental data are available, is strongly recommended, particularly in the case where information is available on traits, such as resistance to disease, which are difficult or expensive to measure.

ACKNOWLEDGMENTS

Work reported here was supported by a grant from the Wool Research Trust Fund on recommendation of the Australian Wool Corporation. Technical support by D.Wood, C.Kristo, G.Attard, A.Crowe, A.Scherer and D.Palmer is gratefully acknowledged.

REFERENCES

DEVERSON,E.V., WRIGHT,H., WATSON,S., BALLINGGALL,K., HUSKISSON,N., DIAMOND,A.G., and HOWARD,J.C. Animal Genetics (In press)

EGERTON,J.R., and RAADSMA,H.W.(1991).In"Breeding Farm Animals for Disease Resistance".Proc. Int. Symp., Bangor (J.B.Owen, and R.F.E.Axford eds).(In press)

GUSTAFSSON,K., WIMAN,K., LARHAMMER,D., RASK,L., and PETERSON,P.A.(1984). Scand. J. Immunol. 19:91.

PICKBOURNE,P., RICHARD,S., BODMER,J.G., and BODMER,W.F.(1978). Histocompatibility Testing 1977., Munksgaard, Copenhagen, pp295.

RAADSMA, H.W. (1991). In "Breeding Farm Animals for Disease Resistance". Proc. Int. Symp., Bangor.(J.B.Owen, and R.F.E.Axford Eds), (In press).

RAADSMA,H.W., EGERTON,J.R., OUTTERIDGE,P.M., NICHOLAS,F.W., BROWN,S.C., CURTIS,M., and LITCHFIELD,A.M.(1991).AAABG, 8:179.

ROBINSON,D.M., HOLBECK,S., SEYFRIED,C., BYERS,P., PALMER,J., and NEPOM,G.T. (1989). Genet. Epidem.6:27.

SCHENNING,L., LARHAMMER,D., BILL,P., WIMAN,K., JONSSON, A., RASK,L., and PETERSON,P.A. (1984).EMBO J. 3:447.

SCOTT, P.C.,CHOI,C.L., and BRANDON, M.R.(1987). J.Immunogenet. 25:133.