GENETIC MARKERS AND VACCINE RESPONSE TO IMMUNOLOGICALLY DEFINED FOOT ROT (*Dichelobacter nodosus*) ANTIGENS IN SHEEP

H.W. Raadsma[1], P.A. MacDonald[2], G. Attard[1], C.S. Wright[3], K.J. Knowler[4], A.E. Beattie[2], K.G. Dodds[3], J.C. McEwan[3] and A.M. Crawford[2]

[1]Centre for Sheep Research & Extension, University of Sydney, Camden, NSW, Australia
[2]AgResearch, Molecular Biology Unit, University of Otago, Box 56, Dunedin, New Zealand
[3]AgResearch, Invermay Agricultural Centre, PB 50034, Mosgiel, New Zealand
[4]Agresearch, Woodlands, RD1, Invercargill, New Zealand

SUMMARY

Variation in antibody responses to 9 defined antigens of *Dichelobacter nodosus* (serogroups A-I) were examined in relation to genetic polymorphism with the ovine MHC, and a genetic linkage map covering 75% of the sheep genome. Within 2 large half sib families, 4 MHC haplotypes were associated with differential antibody responses to defined vaccine antigens presented to the host in a multi-valent vaccine. A genome wide scan suggested additional involvement of genes outside the MHC with an immuno-modulating role in antibody response to most antigens.

Keywords: Antibody-response, footrot-vaccine, genetic-markers, MHC, genome-scan.

INTRODUCTION

Ovine footrot represents a disease of economic significance in most sheep producing countries across the world. In Australia and New Zealand the option to vaccinate with defined antigens of *Dichelobacter nodosus*, an essential organism in the disease, offers an effective management tool (Egerton *et al.* 1987). Such vaccines contain the fimbrial proteins of the organism, which are classified on the basis of 9 distinct serogroups (Designated A-I). No cross-protection exists between the 9 major serogroups, and vaccine response to each serogroup can be measured on the basis of a specific antibody response (K-agglutinating titre) which is indicative of protection, and hence vaccine efficacy (Raadsma *et al.* 1994).

Background. Raadsma *et al.* (1996) demonstrated that antibody response for each major known serogroup (A-I) following vaccination with a multi-valent footrot vaccine was under genetic control. Heritability of vaccine response ranged from 0.26 to 0.58 in 1200 progeny from 129 sire groups. Furthermore genetic correlations among responses to each antigen were generally positive, but ranged from -0.16 to 0.81 and showed considerable heterogeneity indicating that different alleles may regulate specific antibody response to each serogroup. Specific genetic restriction which would favour differential and selective processing of serogroup-specific antigens can occur at several stages of immune effector pathway. Involvement of genes within the ovine major histocompatability (MHC) region, and in particular class II immune response genes has received little attention. Furthermore in other species, genes outside the MHC have also been implicated in control and regulation of antibody production Puel *et al.* (1996).

Aim of current investigation. This investigation examined the possible involvement of polymorphism within the ovine MHC as a contributing factor for differential responses in K-agglutinating antibody titre following vaccination with multi-valent footrot vaccine. This preliminary investigation also made an attempt to examine the possible involvement of genes outside the ovine MHC which contribute to genetic variation in vaccine response.

METHODOLOGY

Vaccination. A commercial whole cell *D. nodosus* vaccine with immunogens from all 9 major serogroups (A-I) was used to vaccinate all animals under a standard protocol at primary vaccination (V1) followed by a secondary vaccination three weeks later (V2). Antibody titres determined from blood samples taken at 0, 3, 6 and 14 weeks after initial vaccination, correspond to pre-vaccination titre, response to primary vaccination (V1), secondary vaccination (V2), and persistence of vaccine response (V2+8). K-agglutinating antibody titres measured as described previously (Raadsma *et al.* 1996) were log-transformed prior to statistical analysis.

MHC investigation. Two large half-sib families, (Texel x Coopworth) x Coopworth backcross, consisting of 131 and 143 progeny, were vaccinated at 4 months of age. The 2 sires were half-brothers with the same Texel grandsire, and were also part of the sire families used in the IMF flock (see below). Within the ovine MHC on chromosome 20, sires and progeny were genotyped for the micro-satellite marker OMHC- 1 (Groth and Weatherall 1994), which was previously found to be highly polymorphic, and would be sufficient to distinguish MHC haplotypes of sire within progeny group for the MHC region, which spans approximately 3 centimorgans (cM). Haplotypes were defined on the basis of distinct fragment length (bp) within the Texel grandsire sire-(IMF 001-274/280), sire 1 (IMF 033-274/282), sire 2 (IMF 003, 278/280), i.e. no common grandparental allele was shared by the two rams. Only serogroups A, B, C and D were measured.

Genome wide scan. All 3rd generation progeny alive at 30 months (n=85) from the AgResearch International Mapping Flock (IMF) (Crawford *et al.* 1995) were vaccinated at this time. Thirteen full-sib families from 4 sires contained information on 254 markers with 75% coverage of the genome (2070 cM). Antibody response to all serogroups A-I, except C, was measured.

Statistical analyses. For each serogroup, the 3 measures taken post-vaccination were combined as the average, since previous principal component analyses had shown an equal weighting could be assigned to each response with the majority of information being contained in the first component. Antibody data from both MHC and genome wide scan were subjected to single point Least Squares analyses. For the MHC investigation, the model included the following fixed effects: sex, birth/rearing type, age of dam, and marker allele (MHC haplotype) within sire. Analyses were conducted for each sire separately. For the genome wide scan- fixed effects included: sex, birth/rearing type, age of dam, dam within sire, and allele within sire- across all 4 sires. Nominal probability and F ratio presented as observed are reported for MHC analyses. Or the full genome scan, the experiment–wise within trait, critical F-value was determined by performing the same analysis using a simulated random variable with a normal distribution in place of the observed data. The upper 5^{th} percentile of the maximum F statistic across markers, from 1000 iterations of this simulation, was taken as the critical F-value.

RESULTS and DISCUSSION

MHC investigation. For sire 1 - 121 progeny were informative- 61 inherited haplotype I- indicated by a fragment length of 282bp, and 60 progeny haplotype II- fragment length 274 bp. For sire 2- 105 progeny were informative- 45 haplotype III (fragment length 280bp), and 60 haplotype III (fragment length 278bp). Table 1 shows the effect of MHC haplotype on antibody response for serogroups A-D as a difference between haplotypes within sire for each serogroup Within sire 1, the difference in antibody response between haplotypes varied in magnitude between the 4 serogroups examined. Furthermore the direction of effect was in opposite direction for serogroups A, and D. For sire 2, the MHC showed a differential effect

for serogroup C only. Results indicate a significant effect up to 0.55 SD in specific antibody response within the range of haplotypes examined here. Conversion of the F statistic under single marker analyses to LOD score (Doerge 1995) indicated a maximum LOD score of 1.79 for sire 2, serogroup C.

Table1. Deviations in antibody titre for 4 serogroups of *D nodosus* for 4 MHC haplotypes defined within half-sib progeny groups for 2 sires

Vaccine antigen- Serogroup:	A	B	C	D
Sire 1- Mean antibody tire (transformed)	6.81	7.10	5.32	7.57
Haplotype I (n=61) vs – Haplotype II (n=60) (difference expressed as SD)	+ 0.73 (0.48)	-0.10	+0.34	-0.79 (0.43)
F	7.4	-	-	5.8
P	0.007	-	-	0.018
Sire 2- Mean antibody titre (transformed)	7.41	7.75	6.81	7.90
Haplotype III(n=45) vs – Haplotype IV (n=60) (difference expressed as SD)	-0.25	-0.40	+0.80 (0.55)	+0.14
F	-	-	8.4	-
P	-	-	0.005	-

Genome wide scan. Simulation analysis resulted in a 5% critical value of F=16. This corresponds to a nominal probability of 0.00003 for the genome wide critical test value. P values which were less than 0.005 when analysing single markers from Chromosome 1p for an association with antibody response to 8 serogroups of *D nodosus* in a multi-valent vaccine are shown in Table 2. Although no single markers exceeded the critical value for the genome wide scan, for 2 serogroups (D, G) markers on the p arm of chromosome 1 showed a potential association (P<0.0006). Markers within this region also showed P values <0.005 for 3 other serogroups. The results indicate a region outside the MHC as a possible source of genes, which may regulate antibody response following vaccination with defined footrot antigens.

Table 2. P value for single markers spaced along p arm of chromosome 1 and their possible association with antibody response to 8 serogroups of *D nodosus* in a multi-valent vaccine. Only P <0.005 shown here, critical F value by simulation = P<0.00003

Marker	ILSTS029	BL41	RM065
Map Position Kosambi-cM	0	24	36
Serogroup A	-	0.00111	-
Serogroup B	-	-	-
Serogroup D	0.00047	-	0.00178
Serogroup E	-	-	-
Serogroup F	-	0.00217	0.00342
Serogroup G	-	-	0.00054
Serogroup H	-	0.00326	-
Serogroup I	-	-	-

General comments. Results presented here suggest that genetic variation within the region of the ovine MHC is associated with differential antibody responses to each of 4 serogroups examined here. The MHC of mammals comprises a significant number of loci, which contain immune response genes. In particular the MHC Class II region contains genes which affect specificity of antibody responses (Roitt 1994). Although it was not possible to distinguish the importance of specific loci within the MHC in the sheep examined here, a potential role of Class II loci in the differential recognition and processing of antigens associated with K-agglutinating epitopes of distinct serogroups of *D nodosus* is feasible. Results from a genome wide scan with polymorphic markers spanning 75% of the ovine genome also suggest involvement of genes in a region outside the MHC and antibody response to 8 serogroups of *D nodosus*. It is not know if this region contains general 'in vivo' modulating or antigen specific immune-response genes. From comparative genetic maps in man, the region may contain immunoglobulin regulation genes and a MHC Class I-like region.

In accordance with details presented by Lander and Kruglyak (1995), the results are of preliminary nature only, since the highest LOD value in the single marker analyses of the MHC (Table 1), was 1.79. Similarly, based on results of the simulation in the genome wide scan, a large number of false positive associations would be inferred if a nominal F value would have been accepted. The simulation presents a conservative approach under which only a few associations remained significant. However the results are consistent that markers within the same region of chromosome 1 show possible associations with all serogroups examined here.

The results presented here also may have implications for current and future vaccines in the control of disease and production. As the antigens in such vaccines become better defined, differential response and in particular sub-optimal responses may well be due to genetic restriction within the host. Finally the approach taken here shows the utility of using a model where defined antigens matched with specific antibody response in a multi-valent footrot vaccine is combined with marker development on the ovine map, to examine the genetic control of vaccine response.

ACKNOWLEDGEMENTS

The work was supported in part by a grant from the NewZealand Foundation for Research, Science and Technology. The support of a travel grant provided by the Department of Industry, Science, and Tourism to HWR is gratefully acknowledged.

REFERENCES

Crawford, A.M., Dodds, K.G., Ede, A.J.et al. (1995) *Genetics* **140**:703-724.

Doerge, R.W. (1995) *Theor.App. Genet.* **90:** 980-981.

Egerton, J.R., Cox, P.T., Anderson, B.J., Kristo, C., Norman, M. and Mattick, J.S. (1987) *Vet. Microbiol.* **14:**393-409.

Groth, D.M. and Weatherall, J.D. (1994) *Animal Genetics* **25**:61.

Lander, E. and Kruglyak, L. (1995) *Nature Genetics* **11**:241-247.

Roitt, I. M. (1994) 'Essential Immunology', Blackwell Scientific Pub, Oxford.

Puel, A., Bouthillier, Y., Mevel, J.C. and Mouton, D. (1996) Proc. ISAG, p275.

Raadsma, H.W., O'Meara, T.J., Egerton, J.R., Lehrbach, P.R. and Schwartzkoff, C.L. (1994) *Vet. Immunol. Immunopath.* **40:** 253-274.

Raadsma, H.W., Attard, G.A., Nicholas, F.W. and Egerton, J.R. (1996) *J. Anim. Breed. Genet.***113:** 181-199.