QUANTITATIVE TRAIT LOCI FOR RESISTANCE TO GASTRO-INTESTINAL NEMATODE INFECTIONS IN MICE

D.M. Menge[1,3], J.M. Behnke[2], F. Iraqi[3], A. Lowe[2], A.J. Teale[3,4], J.P. Gibson[3], D. Wakelin[2] and R.L. Baker[3]

[1] Jomo Kenyatta University of Agriculture P. O. Box 62000, Nairobi Kenya
[2] School of Biological Sciences, University of Nottingham, NG7 2RD, UK
[3] International Livestock Research Institute (ILRI), P.O. Box 30709, Nairobi, Kenya
[4] Present address, Institute of Aquaculture, University of Sterling, FK9 4LA, UK

INTRODUCTION

Gastro-intestinal nematodes are arguably the most important disease restricting domestic ruminant production (McLeod, 1995). Control rests heavily on chemotherapy but resistance to anthelmintic drugs by the parasites poses a major threat to livestock industries worldwide (Waller, 1997). An alternative is to produce through selective breeding or genetic modification stock with enhanced resistance to infection (Woolaston and Baker, 1996). Rodent infections provide valuable models to analyse factors controlling resistance to economically important gastro-intestinal nematode parasites (Wakelin, 2000 ; Behnke *et al*., 2000). Resistance and susceptibility in mice are genetically determined making it possible to identify genetic markers and then look for homologues in target ruminants (Kemp *et al*., 1997 ; Kemp and Teale, 1998) or humans (Blackwell, 1996). We report here mapping of quantitative trait loci (QTL) for resistance to *Helgmosomides polygyrus* in mice, a strongyloid worm whose biology is similar to the trichostrongyloid nematodes of economic importance in domestic ruminants.

MATERIALS AND METHODS

Mouse strains and breeding. SWR and CBA mice strains which are resistant and susceptible respectively to *H. polygyrus* (Behnke *et al.,* 2000) were used to produce 514 F_2 mice for phenotyping and genotyping for QTL mapping.

Phenotyping and analysis of phenotypic data. Thirteen batches of 60 mice (40 F_2, 10 SWR and 10 CBA) were infected orally by trickle infections of 125 infective larvae of *H. polygyrus* given weekly for a period of 6 weeks. Faecal egg count (FEC) was determined at 2, 4 and 6 weeks. The mice were necropsied at the end of week 6 and at *post-mortem* examination adult and larval worms were counted to give total worm count (TWC). Sera collected at the end of week 6 of infection was tested by ELISA against adult worm antigen for parasite specific IgG1 titres and against L4 antigen for IgE. Granuloma score (GS) on the intestinal mucosa was recorded at *post-mortem* on a scale of 0 to 4 (low to high). Because of skewed distributions both FEC and TWC measurements were logarithm transformed to normalize the variance. Initially, all traits were analyzed by fitting a least squares model including line (CBA, SWR and F_2), batch (13) and sex (male and female). Prior to selection of F2 mice for genotyping the data were adjusted for batch (all traits) and sex (TWC, GS, IgE and IgG1) and each trait standardized by dividing by its standard deviation (s.d.).

Genotyping and QTL analysis. Selective genotyping (Darvasi and Soller, 1992) was used to type mice from both extremes of resistance and susceptibility using 175 polymorphic microsatellite markers obtained from Research Genetics (Huntsville, USA) and selected to cover close to 100% of the mouse genome. The resistant mice were chosen as those with zero or close to zero TWC of the same sex (where possible) and in the same litter from which the susceptible mice with the highest TWC were chosen. DNA was extracted from tails using standard phenol chloroform procedures. The PCR amplification was performed according to the supplier's recommendations. PCR products were resolved on an automated sequencer (ABI, Perkin Elmer, USA). Fragment length of the PCR products was determined with GENESCAN® software (ABI Perkin Elmer, USA) and marker genotypes were assigned to the mice using GENOTYPER® software (ABI Perkin Elmer, USA). QTL analysis was based on least square (LS) regression analysis using QTL express (Web site: http://qtl.cap.ed.ac.uk), with thresholds for statistical significance determined by a permutation test for each chromosome separately and by maximum likelihood (ML) interval mapping (Lander and Botstein, 1989) using Mapmaker/EXP and Mapmaker/QTL (Lincoln et al., 1994) and including in these analyses the phenotypes of all the F_2 mice. In the ML analyses a QTL was deemed to be significant ($P<0.05$) when the LOD score was greater than 2.0. The X chromosome analysis was for female mice only.

RESULTS AND DISCUSSION

Relative to the resistant SWR mice the susceptible CBA mice had significantly higher logarithm transformed FEC (LFEC), logarithm transformed TWC (LTWC) and IgG1, but significantly lower GS and IgE. For LFEC and LTWC the F2 mice were intermediate to the parental lines but much closer to the resistant SWR mice suggesting a high degree of heterosis for resistance.

Five significant QTL were detected for LTWC, eleven for LFEC, four for GS, two for IgE and three for IgG1 (Table 1). For all QTL for LTWC the resistant allele originated from the resistant SWR parent (as indicated by the negative additive effect), except for chromosome 2 where the additive effect was not significantly different from zero ($P>0.05$). The combined additive effects for the five QTL for LTWC accounted for about 60% of the difference between the parental lines. All the dominance effects for LTWC were in the direction of resistance as indicated by their negative sign. The additive QTL effects for LFEC were not so consistent as the resistant allele came from the CBA parent for the QTL on chromosomes 8, 17 and X (positive effect) and from the SWR parent for the QTL on chromosomes 1, 4, 5, 9, 13 and 19 (negative effect). The dominance effect for QTL for LFEC was in the direction of resistance in all cases except for the QTL on chromosomes 9, 17 and X. The QTL for GS on chromosomes 11, 13 and 17 had positive additive effects indicating that the resistant allele originated from the SWR resistant parent (i.e. with high GS) and the dominance effects were all in the direction of resistance (i.e. positive values for this trait). In contrast the QTL for GS on chromosome 4 had negative additive and dominance effects. For IgG1, the negative additive effects indicate that the resistant alleles originated from SWR for the QTL on chromosome 10, 17 and 18 and all the dominance effects were in the direction of resistance. The additive effects for the two QTL for IgE on chromosomes 12 and 17 were both negative and therefore these resistant alleles originated from the CBA as it is the parental line with the lower IgE titre.

Table 1. Estimated location and effects of Quantitative Trait Loci for the parasitological and immunological correlates of resistance[A]

Chr	Trait[B]	LOD Score	Position (cM)	Additive effect (s.d.)	Dominance effect (s.d.)	% variance explained
1	LTWC	12.52**	21	-0.53	-0.33	16.0
	LFEC4	5.03**	18	-0.39	-0.08	7.8
2	LTWC	3.18**	30	0.03	-0.42	4.5
4	LFEC2	2.42*	70	-0.10	-0.44	5.4
	GS	2.02*	71	-0.23	-0.26	4.6
5	LFEC4	2.32*	57	-0.29	-0.04	4.4
8	LFEC2	3.34*	43	0.15	-0.29	3.2
	LFEC4	2.36*	43	0.13	-0.41	5.1
9	LFEC6	2.00*	40	-0.04	0.35	3.2
10	IgG1	2.30*	36	-0.09	-0.43	5.2
11	GS	2.10*	35	0.22	0.23	3.8
12	IgE	4.13**	44	-0.36	-0.17	7.0
13	LTWC	2.79*	36	-0.18	-0.28	3.4
	LFEC6	3.64**	45	-0.33	-0.17	5.8
	GS	1.91*	23	0.14	0.36	4.3
17	LTWC	4.28**	18	-0.29	-0.14	4.6
	LFEC2	3.95**	50	0.32	-0.19	6.8
	LFEC6	2.18*	57	-0.06	0.31	2.5
	GS	5.02**	17	0.41	0.06	8.5
	IgE	12.85**	21	-0.53	0.39	18.3
	IgG1	6.15**	32	-0.48	-0.01	11.1
18	IgG1	2.29*	32	-0.30	-0.35	7.6
19	LTWC	2.80*	34	-0.24	-0.19	3.7
	LFEC6	2.13*	34	-0.26	-0.12	3.8
X	LFEC4	2.10*	35	0.12	0.48	6.4

[A] QTL parameters for each chromosome (Chr) were derived from maximum likelihood analyses. The significance levels attached to the LOD scores (* = P<0.05; ** = P<0.01) were derived from least squares analyses with thresholds for the F tests established from permutation tests. Location (cM = centimorgans) estimated at the position of the maximum LOD score and the confidence interval for each QTL was 10-30 cM. The additive effect is estimated for the allele originating from the SWR line. The "% variance explained" is the proportion of the total phenotypic variance accounted for by each QTL.

[B] Logarithm transformed faecal egg count at week 2 (LFEC2), week 4 (LFEC4), week 6 (LFEC6), total worm count (LTWC), Granuloma score (GS), immunoglobulin E (IgE) and immunogobulin G1 (IgG1).

Considering the position and effects of the detected QTL, those located on chromosomes 1, 4, 8, and 19 may be single QTL with pleiotropic effects on several resistance parameters. There appear to be two QTL on chromsome 13; one at around 20 cM controlling GS and one around 40 cM controlling LTWC and LFEC6. There is no simple interpretation for chromosome 17 but there are clearly at least two separate QTL and possibly three or more segregating.

Reference to the mouse Jackson Laboratory mouse genome database has identified a number of candidate genes within the significant QTL that may regulate immunity to *H. polygyrus* (Web site: http://www.informatics.jax.org/). The response generated by infection is complex in terms of the components involved, their effects on the host and the time-scale of their activity. The outcome of infection is determined by the balance between mechanisms that promote and those that down-regulate resistance. Resistance is T cell dependent (Finkelman *et al*., 1997) and the QTL for LTWC on chromosomes 1, 2 and 17 include genes known to influence T cell activity. The QTL for LTWC on chromosomes 13 and 17 also contain genes which control important aspects of mast cell production. The relative importance of these candidate genes and others mapping to the QTL identified in this study remains to be determined but it is possible to see an overall role in regulating T cell and antibody responses and controlling the functionally important mast cell response.

CONCLUSION

These results show a complex quantitative disease resistance trait that is in large part controlled by 16 or more QTL on fourteen chromosomes, which appear to have differing mechanisms of action against the parasite. Finer resolution gene mapping followed by positional cloning is now possible and is being pursued. This should provide valuable insights into the molecular basis of host resistance to gastrointestinal nematode parasites, which will have important implications for the development of novel disease control strategies for livestock and humans.

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