FINE MAPPING OF TRYPANOSOMOSIS RESISTANCE LOCI *TIR2* AND *3* USING ADVANCED INTERCROSS LINES WITH MAJOR LOCUS *TIR1* ELIMINATED

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INTRODUCTION

Trypanosome infection (Trypanosomosis) is the most economically important disease constraint to livestock productivity in Africa (WHO 1997). It has been known for many years that certain breeds of cattle show a remarkable resistance to the effects of trypanosomosis. This phenomenon has been termed 'trypanotolerance' because the host tolerates the presence of the parasites, while apparently not showing the severe anaemia and production loss which are characteristic of infection in susceptible breeds. Farming of trypanotolerance livestock provides a partial solution to livestock based agriculture in tsetse-infested areas (Murray and Dexter, 1988). Trypanotolerance is a polygenic trait, and QTL detection and estimation is being undertaken in our lab. The mouse model is a powerful animal model for studying the genetics of disease resistance (Moore and Nagle, 2000). Different inbred mouse strains show dramatically difference in their response to trypanosomosis (Morrison *et al*., 1978), with C57BL/6J being the most resistant, while A/J and BALB/c strains are among the most susceptible. Three trypanosomosis resistance QTL designated as *Tir1*, *Tir2* and *Tir3* were mapped to chromosome 17, 5 and 1 respectively in two F_2 crosses between the susceptible A/J and BALB/c strains and the resistant C57BL/6J mice (Kemp *et al*., 1997). Subsequently, using F_6 C57BL/6 x A/J and C57BL/6 x BALB/c advanced intercross lines (AIL), *Tir1* was fine mapped to a confidence interval (CI) of less than 1cM, while *Tir2*, and *Tir3*, were mapped to a larger CIs (Iraqi *et al*., 2000). *Tir1* represents the major trypanotolerance QTL. The aim of the project reported here is to fine map *Tir2* and *Tir3* in an F_{12} AIL fixed for the susceptible allele at the *Tir1* QTL. The hypothesis is that fixation of the *Tir1* QTL might lead to stronger expression of *Tir2* and *Tir3*, and that use of an F_{12} AIL will increase recombination events facilitating finer mapping of *Tir2* and *3*.

MATERIAL AND METHODS

Development and selection of *Tir1* QTL fixed lines. These lines were generated by genotyping 200 of each males and females of F_9 C57BL/6J x A/J advanced intercross lines with a panel of 12 microsatellite markers which spanned *Tir1* QTL from 15.9 cM (*D17Mit29*) to 21.95 cM (*D17Mit11*) from the centromeric end of the linkage group. Males and females of the F_9 AIL population were selected which were homozygous for either susceptible alleles or resistance alleles at *Tir1* and these animals were intermated to produce two lines ; one homozygous for the susceptibility (D17AA) and one homozygous for the resistance (D17CC) allele. These lines were then bred for two further generations and expanded in size to produce 600 and 100 D17AA and D17CC F_{12} mice. The F_{12} AIL populations of D17AA and D17CC,

and the parental lines were then challenged with *T. congolense* at the age of 12 weeks and survival time recorded over a period of 180 days. Mice surviving beyond 180 days were given a survival time of 180 days.

Microsatellite typing. Two hundred mice representing 33 % of the 600 *T. congolense*-inoculated F_{12} D17AA mice were selected from the phenotypic extremes for genotyping. Thus the first 100 and the last 100 mice to succumb were genotyped. Selective genotyping has been shown to reduce genotyping load with little reduction in power of QTL detection (Darvasi and Soller, 1992). Genomic DNA from the selection was extracted from mouse-tails by conventional phenol chloroform method. Twenty-two markers on chromosome 1 and nineteen on chromosome 5 were selected for genotyping at the previously mapped *Tir2* and *3* regions at a mean interval of 2.36 cM and 2.79 cM respectively. Markers were selected from the mouse genome database at the website http://www.informatics.jax.org/ .

Statistical analyses. Loci having an effect on mean survival time following *T. congolense* inoculation were mapped on chromosomes 1 and 5, and their effects estimated, using the web based QTL Express program (http://qtl.cap.ed.ac.uk/), based on a simple regression method for mapping QTL in line crosses (Haley and Knott, 1992). Permutation tests were repeated 1000 times in order to determine the statistical significant thresholds of linked QTL. The QTL position and significance was confirmed by maximum likelihood estimation method using Mapmaker/Exp and Mapmaker/QTL programs (Lincoln *et al*., 1994).

RESULTS AND DISCUSSION

The survival time of the two F_{12} AIL populations and the parental lines are presented in figure 1. The mean survival times of D17CC, D17AA, C57BL/6 and A/J were 125, 95, 80 and 60. The heterosis for survival times exhibited by D17AA and D17CC are explained by polygenic background effects, and such heterosis has been observed in all previous crossbred populations between C57BL/6J and A/J. The difference in survival between D17AA and D17CC gives and estimate of *Tir1* QTL effect of 30 days. This result is in agreement with the estimate reported by Kemp *et al*. (1997).

The least square and maximum likelihood methods gave equally informative and similar results. QTLs on chromosome 5 and 1 were confirmed in D17AA AILs. The chromosome 1 QTL region resolved into three loci at 67, 86 and 99cM with LOD scores ranging from 3.5-4.5 (Table 1). The loci were mapped to 95 % confidence intervals (CI) ranging from 3-6cM. The most prominent locus had a maximum LOD score of 4.5 with an additive effect on the phenotype. These results confirm previous F6 mapping results (Iraqi *et al*., 2000).

The chromosome 5 QTL resolved into two loci with LOD score above 2.59 (table 1), located at 41cM and 67cM on the MGD map. The 95 % CI of the locus at 41cM obtained by bootstrapping was about 1cM. At this first QTL allele on chromosome 5, the allele from the resistant parent had a positive effect on survival. At the second QTL on chromosome 5 the resistant parent allele had a negative effect on survival.

A number of candidate genes for trypanotolerance can be identified based on QTL locations (http://www.informatics.jax.org/). Among the most interesting candidate genes on chromosome 5 are glucose-6-phosphate dehydrogenase 2 (G6PD2), hexose-6-dehydrogenase (H6PD) and several kinases, while on chromosome 1 the most interesting candidate gene encodes

apolipoprotein A2 (Apoa2), a component associated with high density lipoprotein (HDL), which was shown to have lytic activity against *T. brucei brucei* in human serum (Rifkin, 1978)

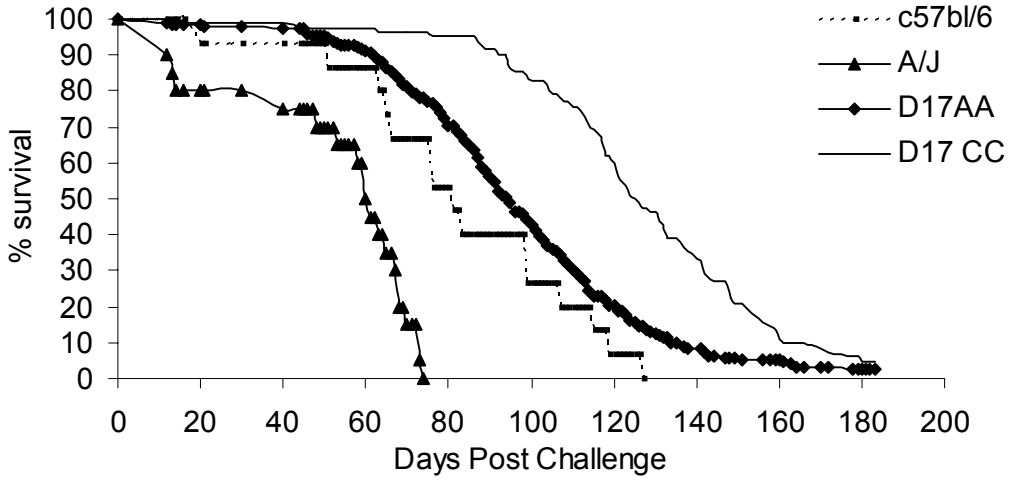

Figure 1. Percentage survival of c57/bl6, AJ, D17AA and D17CC over time after *T. congolense* challenge

Table 1. Trypanotolerance QTL in mice advanced intercross lines fixed for the chromosome 17 A/J allele

QTL	Peak LOD score	95 % CI {LOD score}	Genetic effect additive (SE)/dominance (SE)		MGD location (cM)	Nearest marker	95 % CI (cM)
1a	4.494	2.27	25.98 (5.74)	14.39 (9.02)	67cM	D1-286	5
1c	3.517	2.12	17.80 (4.36)	-4.24 (6.68)	86cM	D1-425	6
1c	4.28	2.04	7.11 (4.80)	24.28 (6.40)	99cM	D1-165	3
5a	3.20	2.04	15.67 (4.40)	-2.29 (6.00)	40cM	D5-158	1
5b	3.53	2.36	-15.33 (4.16)	-13.82 (7.13)	67cM	D5-95	2

CONCLUSION

Knowledge on the position and interactions between QTL, especially if major genes are involved, will help in the identification of positional candidate genes and in the understanding of the complex genetic regulation of trypanotolerance in mice. The 1cM, *Tir2* interval is also sufficient to make positional cloning possible. These results provide essential mapping

information for the identification of candidate trypanotolerance genes in mice, and may be useful for the identification of the homologous genes in livestock.

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